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**Sexual selection and the evolution of female ornaments :
an examination of female plumage colouration using
comparative analyses and long-term data sets collected
in blue tit (*Cyanistes caeruleus*) populations**

Amélie Fargevieille

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THÈSE

Pour obtenir le grade de
Docteur

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Fonctionnelle et Evolutive » à Montpellier**

**Spécialité : Écologie, Évolution, Ressources
Génétiques, Paléobiologie**

Présentée par Amélie FARGEVIELLE

Sélection sexuelle et

évolution des ornements femelles

**Une étude de la coloration du plumage femelle utilisant des
analyses comparatives et des jeux de données à long terme
issus de populations de mésange bleue (*Cyanistes caeruleus*).**

Soutenue le 13 décembre 2016 devant le jury composé de

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Pour qu'il y ait un changement évolutif par sélection chez une espèce, il faut que certains individus soient avantagés de manière à être capables de transmettre leurs caractères à leur descendance (Darwin 1871). Les traits associés au changement évolutif doivent être variables au sein de l'espèce, certaines valeurs de traits doivent avantager celui qui les porte et les niveaux d'expression (variation) des traits doivent être transmissibles à leur descendance. Au sein d'une espèce, certains individus peuvent être avantagés dans une propension particulière - l'accès à la reproduction - et par ce biais produire plus de descendance. Les traits qui leur donnent cet avantage sont sélectionnés par le processus de sélection sexuelle (Darwin 1871). Ces traits sont traditionnellement nommés caractères sexuels secondaires. La plupart des caractères sexuels secondaires sont associés aux mâles des espèces animales. Les travaux de Bateman (1948) et Trivers (1972) ont permis d'avancer une théorie expliquant la présence plus marquée des caractères sexuels secondaires chez les mâles. Leurs concepts se basent sur l'anisogamie, c'est-à-dire l'asymétrie dans la production des gamètes entre mâles et femelles. Les femelles produisent un faible nombre de gros gamètes et investissent dans les soins parentaux afin de maximiser les chances de survie de leurs descendants. D'un autre côté, les mâles produisent un grand nombre de gamètes de petite taille et maximisent le nombre de leurs descendants en multipliant les événements de reproduction. Le nombre réduit de gamètes et l'investissement dans les soins parentaux par les femelles limitent leur possibilité de se reproduire avec de nombreux partenaires. Les femelles deviennent ainsi le sexe limitant et ont intérêt à choisir un bon partenaire afin de maximiser les chances de survie de leurs descendants. De leur côté les mâles entrent en compétition pour avoir accès aux femelles. Par cette vision, les caractères sexuels secondaires sont attendus comme plus développés chez les mâles qui sont limités dans leur accès à la reproduction. Il existe deux formes de compétition et de caractères sexuels secondaires associés. Les mâles peuvent entrer en compétition entre eux par le biais de combats ou en arborant des traits qui signalent leur dominance. On parle alors d'armements ou de badges de statut et de sélection intra-sexuelle. Ces armements ou badges de statut peuvent parfois permettre d'éviter des combats coûteux en indiquant le niveau de dominance des individus qui les possèdent. Les mâles peuvent aussi être en compétition par leur attractivité auprès des femelles. Dans ce cas-là, on parle d'ornements arborés par les mâles et qui peuvent être préférés par les femelles par le biais de la sélection intersexuelle.

Si les ornements sont répandus chez les mâles, il existe également de nombreuses espèces chez lesquelles les femelles peuvent aussi arborer des ornements, armements ou

badges de statut. Historiquement les chercheurs, tel Darwin, avaient noté la présence de tels traits chez les femelles mais leur attention s'était portée sur le développement d'un cadre théorique pour expliquer l'extravagance marquée des traits mâles, extravagance qui constituait en elle-même un bon paradoxe évolutif. Lorsque les idées de Darwin furent reprises entre les années 1980 à 2000, les chercheurs s'intéressèrent également à tester empiriquement et théoriquement l'évolution des ornements chez les mâles, notamment chez des espèces où la compétition pour l'accès aux femelles est très forte (espèces polygynes, espèces à système de lek) et présentant ce qui est appelé un fort dimorphisme sexuel (différence entre les sexes pour la valeur d'un trait ou de plusieurs traits). L'absence de dimorphisme sexuel, appelé monomorphisme, a été par contraste souvent reliée aux espèces monogames à soins biparentaux. L'hypothèse alors majoritairement acceptée pour expliquer la présence d'ornements chez les femelles était reliée au principe de corrélation génétique. Lande (1980) par le biais d'un modèle théorique a expliqué ce principe de la sorte : le caractère ancestral de l'espèce est monomorphique avec absence de traits ornementaux. Par le processus de sélection sexuelle, des traits ornementaux évoluent en premier lieu chez les mâles. Ils évoluent par la suite chez les femelles par le partage du matériel génétique transmis aux générations suivantes. Comme les femelles investissent plus dans la reproduction, ont leur fitness plus associée au nombre de descendants produits, peuvent être plus vulnérables à la prédation quand elles sont gravides, incubent ou s'occupent seules des jeunes, le processus de sélection naturelle réduit la valeur des traits ornementaux portés par les femelles. Dans le cas d'espèces monogames à soins biparentaux, une compétition réduite chez les mâles du fait d'une variance dans l'accès aux femelles plus faible devrait entraîner une plus faible évolution des traits ornementaux chez les mâles et ces espèces devraient garder un monomorphisme sans traits extravagants. Aussi comme les mâles s'occupent des descendants, leur survie pendant la phase de soins parentaux est essentielle à la production de descendants et tout trait diminuant la vulnérabilité à la prédation pendant cette phase peut être favorisé. Pour expliquer la présence d'espèces avec un monomorphisme présentant des traits ornementaux développés chez les deux sexes, Lande propose que ces espèces seraient moins soumises à des pressions de sélection naturelle sur les femelles, en lien par exemple avec une prédation moins forte ou une apparition évolutive plus récente des traits ornementaux.

De nombreuses recherches ont ainsi été menées visant à mieux comprendre les traits ornementaux chez les mâles. Des méthodes comparatives à l'échelle interspécifique ont également été menées pour valider les hypothèses précédemment avancées. Souvent basées

sur le dichromatisme sexuel (la différence de coloration entre les sexes), elles ont trouvé cependant plusieurs résultats contraires aux prédictions de départ (Amundsen 2000). Concernant le système d'appariement, il est apparu que chez huit des neuf espèces de colibris polygynes testées, les femelles arboraient des colorations aussi importantes que les mâles (Bleiweiss 1992). Une autre étude a cependant permis de mettre en évidence qu'il n'y avait pas plus d'espèces dichromatiques dans le système polygyne que dans le système monogame (Møller 1986). Par ailleurs, l'incorporation des phylogénies dans les méthodes comparatives modernes a permis de réaliser que contrairement à ce que l'on pensait le dichromatisme sexuel était souvent un état dérivé plutôt qu'un état ancestral. Ces études ont aussi montré que les traits ornementaux portés par les femelles sont plus labiles que les traits mâles avec de nombreux évènements de perte et de gain de coloration chez les femelles (Irwin 1994; Martin and Badyaev 1996; Burns 1998). Ces résultats ont encouragé à considérer les processus qui pouvaient expliquer l'évolution de la coloration chez les femelles. Dans le même temps, des études empiriques chez différentes espèces portaient sur la présence d'ornements chez les femelles et les liaient à des processus similaires à ceux présents chez les mâles. Parmi elles, une étude expérimentale chez la Starique cristatelle (*Aethia cristatella*) a montré que les mâles semblaient préférer des femelles arborant une plus grande crête (Jones and Hunter 1993). En 2000, Amundsen a alors publié un récapitulatif sur les oiseaux afin de rassembler les différents processus qui pouvaient expliquer la présence de l'ornementation chez les femelles tels que la corrélation génétique, la compétition pour l'accès aux mâles (sélection intra-sexuelle) ou pour l'accès à des ressources ou un territoire (sélection sociale), le choix de partenaire par le mâle (sélection sexuelle), l'appariement par homogamie (Amundsen 2000). D'autres hypothèses ont également été suggérées, notamment en lien avec la reconnaissance de l'espèce ou l'évitement du harcèlement.

Par la suite, de nombreuses études empiriques ont été réalisées afin de mieux comprendre l'évolution des traits ornementaux chez les femelles et ont montré l'importance de ces facteurs. Par exemple, chez les oiseaux, des badges de statut ont été confirmés chez deux espèces par la brillance du bec des femelles du chardonneret triste (*Carduelis tristis*) (Murphy et al. 2009) et la couronne bleue des femelles de mésange bleue (*Cyanistes caeruleus*) (Midamegbe et al. 2011). Le choix de partenaire par le mâle a également été décrit chez plusieurs espèces, notamment chez le coq domestique (*Gallus domesticus*) ; chez cette espèce polygyne, Pizzari et ses collaborateurs ont montré que les mâles transféraient plus de sperme aux femelles arborant une plus large crête (Pizzari et al. 2003). Par ailleurs plusieurs

études expérimentales ont porté sur la fonction des ornements femelles et ont montré des associations entre l'ornementation des femelles et leur qualité (Roulin et al. 2001) ou entre les ornements des femelles et leur capacité à investir dans la reproduction (Doutrelant et al. 2008, 2012; Midamegbe et al. 2013). De même, des récapitulatifs ont été publiés pour valoriser l'évolution des ornements femelles au sein de différents taxons (Clutton-Brock 2007, 2009) ou l'évolution de l'ornementation mutuelle (Kraaijeveld et al. 2007).

En revanche, alors que les études comparatives avaient été déterminantes pour expliquer les forces évolutives expliquant la variation des traits chez les mâles et l'intérêt à se pencher sur l'évolution des ornements femelles, elles sont restées jusqu'à peu uniquement centrées sur les traits mâles ou le dimorphisme sexuel. Récemment, cependant, une première étude comparative a été réalisée sur les espèces d'étourneaux africains et a mis en relation l'évolution des ornements chez les femelles avec la coopération (Rubenstein and Lovette 2009). En utilisant le dichromatisme sexuel, cette étude a montré qu'il y avait plus de monochromatisme parmi les espèces coopératives d'étourneaux africains que parmi les espèces solitaires, et a relié ce résultat à la compétition accrue entre les femelles pour l'accès à la reproduction chez ces espèces chez qui très peu de couples ont accès à la reproduction. Deux études comparatives plus récentes encore ont également inclus la coloration femelle dans leurs analyses (Dale et al. 2015; Dunn et al. 2015). Ces études conduites à très large échelle ont testé des facteurs très généraux tels que la migration, la taille du corps, le lieu de vie (milieu tropical, milieu tempéré) (pour l'étude de Dale et al. 2015) et des indices de sélection sexuelle mixant le système d'appariement (monogame, polygame) et l'investissement du mâle dans les soins parentaux comme une valeur dichotomique (présence/absence). Ainsi, à la lumière de ces travaux il manquait toujours une étude comparative centrée sur les ornements femelles et qui prendrait en compte de manière spécifique les processus propres à leur évolution. Elle pourrait en particulier tester de façon plus fine le rôle du choix mâle dans l'évolution des ornements femelles tout en contrôlant d'autres processus comme la corrélation génétique ou la sélection naturelle. Cet aspect représente une facette de mon travail de thèse et la première partie du travail présenté.

A l'échelle intraspécifique, en population naturelle, comme dit plus haut, des études expérimentales ont démontré un choix mâle, une forte compétition entre femelles et des liens entre traits femelles et qualité ou investissement maternel. En populations naturelles, les processus de sélection sexuelle ainsi que la plupart des traits sont associés à une grande complexité (Kuijper et al. 2012). Les traits ornementaux et plus particulièrement la coloration

sont associés à une grande labilité due à des variations environnementales et développementales (Zahavi 1975; Cotton et al. 2006). La variation spatiotemporelle observée sur les traits ornementaux peut également être issue d'une variation des préférences pour les traits. Par exemple, une étude travaillant sur une espèce de demoiselle (*Ischnura elegans*) a mis en avant des variations spatiales dans la préférence émise par les femelles pour la taille du mâle. L'originalité de ces résultats repose sur la distance qui séparait les populations montrant des variations de préférence (Svensson and Gosden 2007) ; ces populations étaient situées dans un périmètre de 25 km. De même, en travaillant sur la sélection sur plusieurs traits susceptibles d'être impliqués dans le choix de partenaire par les femelles du bruant noir et blanc (*Calamospiza melanocorys*), Chainé et Lyon (2008) ont constaté que la sélection sur les différents traits fluctuait beaucoup d'une année sur l'autre, ce qui les a conduits à l'incapacité à déceler quel trait était choisi. Cette variation spatiotemporelle est donc un phénomène indispensable à documenter en population naturelle. Or, la majorité des études corrélatives travaillant sur l'ornementation, que ce soit chez les mâles ou les femelles, se basent sur un faible échantillon, une seule population et rarement sur plus de trois années successives de données. Le deuxième axe de ma thèse consistait à étudier cette variation spatiotemporelle chez une espèce modèle utilisée dans un programme à long terme et chez qui des traits ornementaux ont été liés à la sélection sexuelle et sociale chez les femelles, la Mésange bleue (*Cyanistes caeruleus*).

Ma thèse s'est donc axée sur l'évolution des ornements femelles avec un intérêt particulier pour le rôle de la sélection intersexuelle. En utilisant comme caractère, au cœur de cette évolution, la coloration du plumage des oiseaux, mon approche a été divisée en deux parties distinctes. D'une part j'ai conduit une approche interspécifique qui visait à confronter des traits d'histoire de vie liés à l'évolution du choix de partenaire chez le mâle, avec l'évolution de la coloration du plumage des femelles. Les traits d'histoire de vie utilisés ont été choisis car identifiés comme clés dans les modèles théoriques (Johnstone 1996; Kokko and Johnstone 2002). La deuxième partie consistait en une approche intraspécifique chez la Mésange bleue (*Cyanistes caeruleus*), une espèce socialement monogame à soins biparentaux. Chez cette espèce deux patches de coloration sont liés à la sélection sexuelle et sociale, à la fois chez les mâles et les femelles. Pour ces deux zones, des études empiriques ont montré des phénomènes liés à la sélection sexuelle et sociale chez les deux sexes (voir Kingma et al. 2009; Remy et al. 2010; Vedder et al. 2010; Midamegbe et al. 2011; Limbourg et al. 2013 pour la calotte bleue; Peters et al. 2011; Doutrelant et al. 2008, 2012 pour le patch jaune de la

bavette, mais voir Parker 2013 pour les deux patchs chez les mâles). Ils représentent donc un intérêt dans l'étude de la coloration femelle liée à la sélection intersexuelle. Dans l'approche intraspécifique, dans un premier temps, les conditions d'évolution de la coloration (variation, héritabilité et sélection) ont été testées en tenant compte de la potentielle variation spatiotemporelle. Ensuite, l'appariement par homogamie, comme hypothèse de choix mutuel de partenaire, a été recherché, toujours dans un contexte de potentielle variation spatiotemporelle, ainsi que les facteurs qui pouvaient expliquer le patron trouvé.

1. Approche interspécifique

Afin de pouvoir mener une analyse comparative à l'échelle interspécifique, il a fallu d'abord définir les traits d'histoire de vie permettant de tester l'impact du choix de partenaire par les mâles. Des modèles théoriques ont été développés entre les années 1990 et 2000 pour définir les paramètres susceptibles de faire évoluer de manière stable le choix de partenaire par les mâles et le choix mutuel de partenaires (Johnstone 1996; Kokko and Johnstone 2002). Trois paramètres-clés sont apparus comme essentiels à l'évolution du choix de partenaire. Le premier concerne le temps investi dans des processus liés à la reproduction (e.g. les soins parentaux) ; s'investir dans des processus liés à la reproduction ne permet pas de consacrer ce temps à initier une reproduction avec un autre partenaire, limitant la maximisation du nombre d'évènements de reproduction. Ainsi, plus ce temps d'investissement est long, plus les individus ont intérêt à choisir leur partenaire. Le deuxième paramètre concerne la probabilité de rencontre d'un partenaire potentiel de reproduction. Lorsque la probabilité de rencontrer un potentiel partenaire est faible, il s'avère coûteux de rejeter celui rencontré. Ainsi ce paramètre définit que le choix de partenaire évolue plus facilement quand le taux de rencontre de partenaires potentiels est élevé. Enfin, le troisième paramètre concerne la variation dans la qualité des potentiels partenaires. Si la qualité intrinsèque des individus de l'autre sexe est équivalente, choisir un partenaire représente un coût inutile. Une variation dans la qualité des partenaires est donc nécessaire à l'évolution du comportement de choix de partenaire. Outre les traits d'histoire de vie liés au choix de partenaire par les mâles, il était aussi nécessaire de prendre en considération les coûts associés à la reproduction et à la production d'ornements. Plus précisément, un modèle théorique s'est intéressé aux limitations dans l'expression de la coloration chez les femelles (Fitzpatrick et al. 1995). Ce modèle se base sur le partage de l'allocation des ressources et montre que la production d'ornements coûteux peut limiter l'investissement en énergie dédié à la reproduction. Produire des ornements baisserait la fertilité et pourrait être contre-sélectionné. Il existerait ainsi des compromis entre

l'investissement dans les ornements et l'investissement dans la reproduction pour les femelles.

A la lueur de ces paramètres, j'ai donc pu définir des métriques permettant de représenter ces facteurs dans mes analyses. Concernant l'évolution du choix de partenaire par les mâles chez les oiseaux, le critère le plus important à définir était l'investissement du mâle dans les soins parentaux. Traditionnellement, et en raison d'une littérature souvent difficile à exploiter, ce critère est défini de manière dichotomique par l'absence ou la présence de soins paternels (Dale et al. 2015; Dunn et al. 2015 pour des références récentes). Cependant, cette dichotomie est peu réaliste puisque l'investissement du mâle peut être très variable d'une espèce à l'autre, allant du partage de l'incubation jusqu'à un investissement superficiel dans le nourrissage des jeunes. Pour aller au-delà de cette dichotomie et tester des conditions un peu plus réalistes, je me suis basée sur le travail d'une étude qui a défini un calcul permettant de prendre en compte l'investissement relatif des mâles dans les soins parentaux (Webb et al. 2010). Cette étude a identifié les différentes étapes des soins parentaux (de la construction du nid au soin au nid) et considère également dans son calcul l'investissement des femelles (voir Méthodes MS1). J'ai donc repris cette formule comme mesure relative à l'investissement des mâles dans les soins parentaux. Le deuxième critère essentiel à définir concernait les coûts de la reproduction pour les femelles. Ces coûts sont liés à leur investissement dans la reproduction et une mesure liée à la ponte semblait évidente. Cependant, il existe deux stratégies majeures de reproduction : certaines femelles misent sur une large ponte, d'autres sur des œufs plus gros. Afin de considérer ces deux aspects, j'ai utilisé la mesure développée par Watson et ses collaborateurs en 2015 et qui consiste à utiliser le volume de ponte, correspondant au produit entre le volume d'un œuf et la taille de ponte (Watson et al. 2015). Des coûts de reproduction peuvent également être associés à la vulnérabilité à la prédation en période de reproduction. J'ai également défini un score pour prendre en compte cette donnée, englobant des scores liés à l'ouverture de l'habitat, à la position du nid, à l'ouverture du nid et au statut migratoire de l'espèce. Enfin, il semblait également important de prendre en compte le taux de rencontre, un autre facteur-clé développé par les modèles théoriques, sachant que la densité est très variable entre les espèces d'oiseaux. Pour cela, j'ai utilisé un score repris des travaux de Bennett et Owens en 2002, basé sur le maximum de densité en période de reproduction (Bennett and Owens 2002). D'autres facteurs ont également été pris en compte par l'influence qu'ils pouvaient avoir sur l'évolution des couleurs ou sur des facteurs

précédemment définis (notamment la masse corporelle des femelles qui est à la base de nombreux compromis entre traits).

Dans un second temps, j'ai cherché les espèces sur lesquelles travailler. Les passereaux semblaient représenter un taxon modèle pour les analyses comparatives. En effet ce taxon a été très étudié et les données accumulées dans la littérature montrent une forte variabilité dans les traits d'histoire de vie sélectionnés, ainsi que qu'une forte variabilité dans les colorations arborées, que ce soit chez les mâles ou chez les femelles. J'ai décidé de restreindre mon analyse à 17 familles proches phylogénétiquement et de travailler sur des espèces du Paléarctique occidental et du Néoarctique. Limiter les espèces aux zones tempérées de l'hémisphère nord permettait de s'affranchir d'un facteur pouvant influencer l'évolution de la coloration : la vie en milieu tropical qui semble associée à des femelles plus colorées (Dale et al. 2015). De plus, les espèces de passereaux de l'hémisphère nord ont été bien étudiées et la collecte des données sur les traits d'histoire de vie était facilitée. La coopération étant un autre facteur affectant la coloration (Rubenstein and Lovette 2009; Dale et al. 2015), j'ai décidé de ne pas utiliser les rares espèces coopératives présentes. Enfin, les espèces insulaires ont également été évincées du jeu de données final, suite aux récents résultats trouvés par Doutrelant et al en 2016, reliant le syndrome insulaire à l'évolution de la coloration (Doutrelant et al. 2016). Les spécimens ont été sélectionnés au musée national d'Histoire Naturelle de Paris, et ont ensuite été mesurés à l'aide d'un spectromètre. Mâle et femelle de chaque espèce ont été pris en compte, et l'entièreté du corps a été mesurée, par la définition de 21 zones topographiques. Par la suite, un modèle visuel a été utilisé (Vorobyev and Osorio 1998) afin d'obtenir des mesures de l'intensité de la coloration du plumage femelle tout en prenant en compte le point de vue du récepteur. Pour chaque spécimen, deux variables de coloration ont été extraites de ce modèle visuel. D'une part j'ai calculé le volume de couleur qui permet entre autre d'exprimer la visibilité de la coloration en tenant compte des contrastes de coloration entre les différents patches. D'autre part j'ai calculé l'intensité chromatique, qui correspond à la moyenne des distances euclidiennes entre chaque point et le centre achromatique (qui correspond au point stimulant de manière égale les quatre cônes simples et situé au barycentre du tétraèdre visuel) (Maia et al. 2016). Une couleur plus chromatique est ainsi une couleur plus visible (voir Encart 1 pour plus de détails)

La méthode des moindres carrés avec correction phylogénétique (PGLS) a été utilisée pour faire tourner les modèles statistiques (Felsenstein 1985; Harvey and Pagel 1991). Dans ces modèles, les variables de couleur femelles représentaient la variable à expliquer, et les

autres facteurs définis plus haut (soins parentaux, densité, investissement femelle dans la ponte, prédation) ont été mis en facteurs fixes. Ces modèles ont également inclus la variable de couleur mâle pour prendre en compte un effet potentiel de la corrélation génétique. En accord avec nos prédictions, les résultats ont montré un important rôle de l'investissement du mâle dans les soins parentaux pour l'évolution de la coloration du plumage des femelles (voir Chapitre II-MS1). Les résultats ont aussi soulevé les limitations dans l'évolution de la coloration du plumage des femelles par les coûts liés à la reproduction. En effet, pour la variable « intensité chromatique », on trouve que les femelles les plus colorées sont chez les espèces où les mâles investissent plus dans les soins paternels, mais que cet effet est surtout prononcé quand la femelle elle-même investit peu dans la reproduction. Lorsque l'investissement initial de la femelle augmente, la pente de la corrélation entre traits femelles et investissement male diminue, ce qui peut correspondre à une limite de la coloration femelle imposée aux femelles par le compromis avec l'investissement dans la reproduction. Ces résultats confirment donc l'importance de considérer l'investissement du mâle mais aussi les coûts liés à la reproduction pour les femelles et s'accordent avec les travaux des modèles théoriques. Pour la variable « volume de couleur », l'investissement du mâle dans les soins parentaux est positivement corrélé à la coloration femelle sous une condition particulière et fortement exprimée : une importante vulnérabilité à la prédation. Il semble qu'un investissement fort du mâle dans des conditions qui peuvent lui être coûteuses favorise l'évolution du choix de partenaire par le mâle, ce qui entraîne l'évolution de la coloration femelle. Enfin, il est à noter qu'il existe une forte corrélation positive entre la coloration des mâles et celle des femelles. Cette forte corrélation phénotypique permet de confirmer une forte corrélation génétique, même si d'autres facteurs écologiques, tels que le régime alimentaire partagé par les mâles et les femelles d'une même espèce, peuvent renforcer la corrélation phénotypique trouvée.

Ainsi, à l'échelle interspécifique, cette thèse a permis d'avancer dans la connaissance sur les mécanismes conduisant à l'évolution des ornements femelles. En appuyant sur l'importance de la corrélation génétique, elle a mis en évidence pour la première fois au niveau interspécifique un effet majeur de l'investissement du mâle dans les soins parentaux. Ce facteur étant lié au choix de partenaire par les mâles, il montre ainsi que la sélection intersexuelle peut favoriser l'évolution des ornements femelles. Cette analyse a aussi fourni pour la première fois au niveau interspécifique des résultats qui insistent sur la nécessité de considérer les coûts liés à la reproduction quand on travaille sur les ornements femelles. Par

ailleurs, les résultats obtenus ouvrent de plus larges pistes, exploitables avec le jeu de données récolté, pour mieux comprendre l'évolution de la coloration chez les femelles (voir discussion du chapitre IV).

2. Approche intraspécifique

Le second axe de ma thèse s'est intéressé à déterminer la force de la sélection sur les ornements femelles en populations naturelles dans le but de mieux comprendre l'importance de la variabilité spatiotemporelle et ses conséquences sur le rôle potentiel de la sélection intersexuelle à l'échelle d'une espèce. La Mésange bleue (*Cyanistes caeruleus*) représente un bon modèle pour ce type d'étude (voir Encart 2). Il s'agit d'une espèce qui niche facilement dans les nichoirs installés par l'Homme et qui peut donc être suivie sur le long terme dans plusieurs populations. Elle est socialement monogame, les mâles et femelles arborent des plumages colorés et partagent les soins parentaux. Cette espèce est particulièrement intéressante à étudier sur le long terme car si chez cette espèce un certain nombre d'études ont visé à évaluer le rôle de ces ornements dans le cadre de la sélection sexuelle (e.g. Hunt et al. 1999; Sheldon et al. 1999; Alonso-Alvarez et al. 2004; Delhey et al. 2007; Korsten et al. 2007; Parker et al. 2011; Peters et al. 2011; Doutrelant et al. 2012), de manière surprenante très peu d'études se sont intéressées à l'évaluation des patrons d'appariement dans les populations (mais voir Andersson et al. 1998; Garcia-Navas et al. 2009). Ces patrons peuvent pourtant apporter de l'information sur la possibilité de favoriser l'adaptation locale voire la spéciation dans certains cas (Jiang et al. 2013). Par ailleurs, malgré les nombreux processus liés à la coloration étudiés chez cette espèce, une méta-analyse récente a montré que le seul résultat fort était la présence de dichromatisme sexuel pour la couleur bleue de la calotte (Parker 2013). Parker a relié l'absence de résultats forts dans la méta-analyse au fait que les études sur la coloration des mésanges bleues utilisent en général de faibles échantillonnages, répartis sur un court laps de temps et très peu répliqués. Afin d'avoir des résultats robustes, il est nécessaire de travailler sur un large échantillonnage, plusieurs années successives et répliquer les expériences dans plusieurs populations.

Le suivi annuel réalisé au Centre d'Ecologie Fonctionnelle et Evolutive (CEFE) de Montpellier sur plusieurs populations de Mésange bleue m'a permis d'accéder à de telles données. Le programme, initié en 1975 par Jacques Blondel, suit actuellement quatre populations annuellement. La population continentale, nommée D-ROUVIERE, se situe dans une forêt de chênes blancs à 18 km au nord-ouest de Montpellier. Les trois autres populations sont situées dans le nord de la Corse. La population d'E-PIRIO, située dans une forêt de

chênes verts, se trouve en altitude dans la vallée du Fango. Les deux autres populations sont localisées à 25 km de Pirio, dans la vallée du Regino, et sont distantes l'une de l'autre de 5 km au maximum (voir Insert 2 Figure 2). L'une se situe dans une forêt de chênes blancs et est dénommée D-MURO, l'autre dans une forêt de chênes verts et est appelée E-MURO (à noter que « D » correspond à « deciduous » et caractérise une couverture de chênes blancs, tandis que le « E » correspond à « evergreen » et caractérise une couverture de chênes verts). Malgré leur proximité, les deux populations MURO montrent des caractéristiques propres et de premiers éléments permettent de penser qu'il existe de l'adaptation locale dans ces deux populations (voir MS A1 Charmantier et al. 2016). Il existe également des différences à plusieurs points de vue entre les quatre populations, notamment sur des traits morphologiques ou sur la taille de ponte (entre la population continentale et les populations corses) ou sur des traits phénologiques (entre la population d'E-PIRIO et les autres populations, il existe jusqu'à un mois de différence dans l'initiation de la ponte). Le suivi annuel de ces populations s'accompagne depuis 2005 de la récolte de plumes issues de deux patchs pour des mesures de coloration. Initié par Claire Doutrelant, ce suivi à long terme de la coloration des individus reproducteurs sur les quatre stations étudiées m'a permis de constituer une base de données couleurs comprenant plus de 6000 individus sur plus de dix ans. L'utilisation de ce jeu de données permet donc de prendre en compte la variation spatiotemporelle et d'évaluer la complexité qu'elle génère dans la compréhension générale de l'évolution de la coloration.

Je me suis tout d'abord intéressée aux trois conditions-clés liées à l'évolution des traits - variation, héritabilité, sélection - tout en tenant compte de la variation spatiotemporelle (Chapitre III – A). La base de données constituée m'a d'abord permis de réaliser une description simple de la variation phénotypique (voir Chapitre III – A partie I, extraits du MS A1), chez les mâles et les femelles, dans les quatre populations et pour cinq variables de couleurs (trois sur la couronne bleue, deux sur la bavette jaune). Ensuite, la base de données constituée a permis l'utilisation des outils de génétique quantitative pour évaluer l'héritabilité des traits de coloration entre population continentale et population corse (les trois populations corses ont été regroupées pour augmenter la robustesse des analyses – voir Chapitre III – A - MS2). Enfin, l'estimation de gradients de sélection liés aux deux colorations a été réalisée, en se basant sur des mesures de succès de reproduction (voir Chapitre III – A -MS3). Une étudiante de Master 2, Aurore Aguetz, a tout d'abord calculé des gradients de sélection, repris par la suite et incorporée dans une méta-analyse intraspécifique (Nakagawa and Santos 2012). Pour l'instant, seules des analyses sur la date de ponte et la taille de ponte ont été faites

mais des analyses postérieures à la rédaction de ce manuscrit devraient être réalisées sur d'autres mesures telles que le taux d'éclosion, le succès d'envol des jeunes ou le recrutement des jeunes dans la population (voir discussion du Chapitre IV).

La première constatation qui sort de ces trois analyses confirme l'importance de la variation spatiotemporelle. Si l'étude sur la variation phénotypique se base essentiellement sur les différences entre les populations, elle indique néanmoins une large variation spatiale. Elle met aussi en évidence qu'il existe le même niveau de variation chez les femelles que chez les mâles (voir Chapitre III – A partie I, extraits du MS A1) pour toutes les variables. L'étude sur l'héritabilité met également en exergue la variation entre populations. Si elle confirme l'héritabilité de certaines variables (notamment les variables chromatiques), elle montre que les valeurs d'héritabilité varient d'une population à l'autre, avec notamment les variables liées à la calotte bleue qui ont une plus forte héritabilité chez les femelles dans la population continentale. Cette étude confirme également une forte corrélation génétique entre les sexes, évaluée pour les traits héréditaires. Là encore, notamment pour la variable chromatique de la bavette jaune, il peut exister une forte différence dans la corrélation génétique entre population corse et population continentale. Enfin, l'étude sur les gradients de sélection met tout d'abord en évidence la variation spatiotemporelle existante et va dans le même sens que l'étude publiée par Chainé et Lyon en 2008 sur le bruant noir et blanc (Chainé and Lyon 2008). La fluctuation dans les gradients de sélection est importante entre années et ne semble pas fluctuer de la même façon entre les populations. La première approche classique proposée par Aurore Aguetaz, étudiante de Master 2, a donné des résultats très complexes et difficilement interprétables. Nous avons donc décidé de calculer de simples gradients de sélection directionnelle pour chaque variable, chaque année et chaque population et de les analyser ensuite grâce à des outils de méta-analyses intraspécifiques (Nakagawa and Santos 2012). Ces résultats confirment la variation spatiale détectée auparavant : les variables sont associées à des gradients de sélection moyens différents selon les populations. Ce phénomène semble plus fort chez les femelles. Par ailleurs, et de manière très intéressante pour notre étude, la très grande majorité des gradients de sélection décelés dans cette méta-analyse concerne la coloration des femelles. De même, et en accord avec les résultats sur l'héritabilité, ce sont les variables chromatiques qui sont le plus souvent décelées. L'utilisation d'autres mesures de succès de reproduction semble importante pour la suite. En effet, date de ponte et taille de ponte sont des indices fortement liés aux femelles ; les autres métriques qui

impliquent plus le mâle peuvent offrir des résultats différents avec des gradients de sélection plus fortement décelés chez les mâles.

Au final cette partie de l'étude chez la mésange bleue a montré de la variation, de l'héritabilité et de la sélection, malgré une très forte variabilité spatiotemporelle ; elle a aussi suggéré que la coloration des femelles pouvait être considérée comme un trait sujet à la sélection sexuelle. Les analyses d'héritabilité mettent aussi en avant l'importance de la corrélation génétique entre les sexes, facteur permettant aussi l'évolution des ornements femelles (ou mâles). Ces facteurs ont donc indiqué la possibilité d'évolution de l'ornementation mutuelle chez cette espèce. Le phénomène d'appariement par homogamie a donc été évalué dans une dernière partie de thèse (Chapitre III – B).

L'évaluation de l'appariement par homogamie a été effectuée sur les deux variables de couleur, dans les quatre populations sur les dix années. Si la coloration peut montrer une forte variation spatiotemporelle, la préférence pour les traits peut également varier entre populations et entre années (voir Svensson and Gosden 2007 pour la variation spatiale; Chaine and Lyon 2008 pour la variation temporelle). Dans un premier temps, j'ai donc calculé un coefficient de corrélation de Pearson (outil traditionnellement utilisé pour calculer l'appariement par homogamie) pour chacune des cinq variables, pour les dix années et dans chaque population. Tout comme dans le cas de la sélection, les résultats ont montré une très forte variation spatiotemporelle avec des patrons d'homogamie qui fluctuaient entre des valeurs négatives, nulles ou positives (voir Chapitre III – B –MS4). Néanmoins, contrairement aux résultats trouvés dans les mesures de sélection, les patrons de variation temporelle liés à l'appariement par homogamie semblent être identiques d'une population à l'autre. Une analyse plus approfondie, plus longue et mettant en relation ces variations à des facteurs climatiques, sera nécessaire pour confirmer et comprendre ce patron. Pour pouvoir tirer des conclusions sur le patron d'appariement par homogamie malgré la complexité liée à la variation spatiotemporelle mise en évidence, j'ai à nouveau utilisé les outils méta-analytiques. Pour chaque population et chaque couleur (pas de variation entre variables au sein des couleurs), le patron d'appariement par homogamie trouvé est apparu significativement positif et compris entre 0.10 et 0.20 (voir Chapitre III – B –MS4). De l'homogamie pour la coloration existe donc bien chez la Mésange bleue. Pour chaque population, l'appariement était légèrement plus fort sur le jaune que sur le bleu. Enfin, de façon très surprenante et intéressante, les populations situées à 5 km de distance montraient un appariement plus fort

que les deux autres populations, permettant de spéculer légèrement sur la possibilité d'une adaptation locale liée à l'appariement par homogamie.

L'appariement par homogamie n'est pas forcément le reflet d'un choix de partenaire. Partager un même habitat, attaquer la saison de reproduction au même moment ou avoir le même âge peut entraîner des similarités dans la valeur des traits entre les individus. De même, une même paire qui reste liée plusieurs saisons de reproduction peut partager des conditions écologiques communes, ce qui peut entraîner une augmentation de la similarité entre les individus de la paire au fil des saisons. Tous ces facteurs peuvent indirectement conduire à des patrons d'appariement par homogamie (Crespi 1989; Kraaijeveld et al. 2007). Ainsi afin de comprendre les facteurs à l'origine de l'appariement par homogamie dans nos populations, des études complémentaires ont été réalisées (voir Chapitre III –B- MS5). Pour cette étude, la variation spatiotemporelle a été mise de côté et les données ont été centrées et réduites par population et par année, afin de se détacher de cette variation. Plusieurs prédictions ont été testées ; la coloration individuelle (mâle et femelle) a été utilisée pour tester l'hypothèse de choix de partenaires basé sur la qualité. La date de ponte a permis de voir si l'appariement observé était surtout dû à une plus grande possibilité de choix pour les individus les plus précoces à initier la reproduction. La qualité de l'habitat a permis de tester si l'appariement était le résultat d'une compétition pour l'acquisition du territoire et / ou était plus grande dans les habitats de meilleure qualité. Enfin, des tests liés à l'âge des individus, ou à la proportion d'individus adultes dans la population, ont également été réalisés pour déterminer si un appariement plus fort pouvait être observé chez les oiseaux les plus expérimentés. Le résultat principal obtenu a montré que pour chaque variable et chaque sexe les individus les plus extrêmes (très colorés ou très ternes) étaient moins bien appariés que les individus plus intermédiaires, ce qui suggère que les individus de coloration extrême pourraient avoir plus de difficulté à trouver un partenaire qui leur ressemble. Les analyses estimant les effets liés à l'initiation de la reproduction, à la qualité de l'habitat et à l'âge n'ont donné aucun résultat significatif, on peut donc penser que l'appariement est bien lié au choix de partenaires. Cependant, les analyses méritent d'être approfondies notamment en testant des proxys plus précis sur la qualité de l'habitat ou un indice d'initiation de la reproduction antérieure à la date de ponte. De plus des protocoles expérimentaux effectués en populations naturelles qui permettraient de tester l'importance de la territorialité et les limites du choix de partenaire doivent être envisagés avant de pouvoir aboutir à cette conclusion.

Ainsi, l'approche intraspécifique donne des éléments suggérant que la sélection intersexuelle, par le choix de partenaire, pourrait favoriser l'évolution de l'ornementation femelle chez la Mésange bleue. Les résultats sur l'héritabilité indiquent également que la corrélation génétique peut également fortement influencer cette ornementation. La force des résultats obtenus repose dans l'utilisation d'un jeu de données rassemblant dix ans de données sur quatre populations, permettant ainsi de prendre en compte un facteur majeur : la variation spatiotemporelle.

Les deux approches abordées lors de ma thèse offrent donc au final trois résultats forts. Le premier confirme l'importance de la corrélation génétique et la nécessité d'en tenir compte dans les analyses, notamment à l'échelle interspécifique. Le deuxième résultat fort montre l'effet de la sélection intersexuelle (choix de partenaire mâle ou choix mutuel) pour l'évolution et le maintien des ornements femelles. Enfin, au-delà de cette prédiction confirmée, le troisième résultat fort concerne la complexité associée à l'évolution des ornements chez les deux sexes. Les résultats montrent la nécessité absolue de prendre en compte les fortes fluctuations spatiotemporelles associées à l'évolution des ornements et des préférences pour décrire des patrons plus généralisateurs.

CHAPTER I
INTRODUCTION

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1. Traits implied in mate acquisition: historical view and sexual dimorphism

1.1. *Sexual, social selection and secondary sexual characters*

When Darwin proposed his theory of evolution (1859), he defined three conditions required for evolutionary changes: variation in the focal trait, relation with fitness, and inheritance of the value of the trait. Under these conditions, a value of the trait (variation) permits an individual to have a better chance to produce surviving offspring (fitness gain), which may inherit (heritability) the value from its ancestor, leading to evolutionary change. The main difference between natural selection and sexual selection lies in the fitness proxy affecting the phenotypic trait (Darwin 1871) (Figure 1). If natural selection acts on the traits affecting fecundity and the survival chance of an individual, sexual selection acts on the traits affecting an individual chance to obtain a mate, thus on its ability to produce offspring. Under sexual selection, individuals are not competing to survive or to produce more offspring but to succeed in mating.

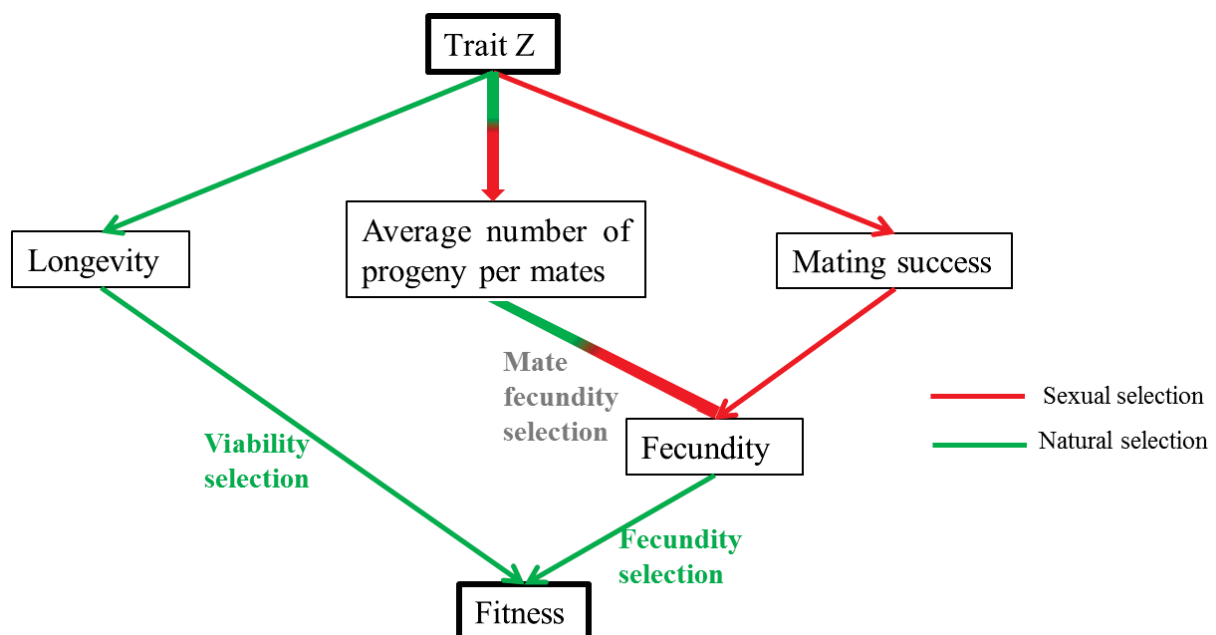


Figure 1-Illustration of various pathways to an individual fitness; arrows indicate relation among processes which may induce variation in trait Z. Adapted from figure 1 in Delph et al. published in *Integrative and Comparative Biology* in 2006.

Following Andersson (1994) definition, differences in mating success arise mostly through competition over mates (intra-sexual selection) and/or mate choice (intersexual selection). Sexual selection is mediated by traits, secondary characters or behaviour, which

vary among individuals. These differences in access to reproduction may be expressed directly by the number of breeding mates an individual acquires or indirectly by the quality of the individuals it has access to. There are different types of secondary sexual characters depending on sexual selection process underlying its evolution.

- Armaments (Figure 2A) such as antlers or horns in ungulates or badges of status (coloured patches (Rohwer 1977), psychological armaments (Senar 2006)) evolve under intra-sexual selection and are used by opponents to fight or assess competitor quality. They sometimes permit to avoid conflicts.
- Ornaments (Figure 2B) evolve under intersexual selection via mate choice and may express the quality or the physical condition of its bearer.

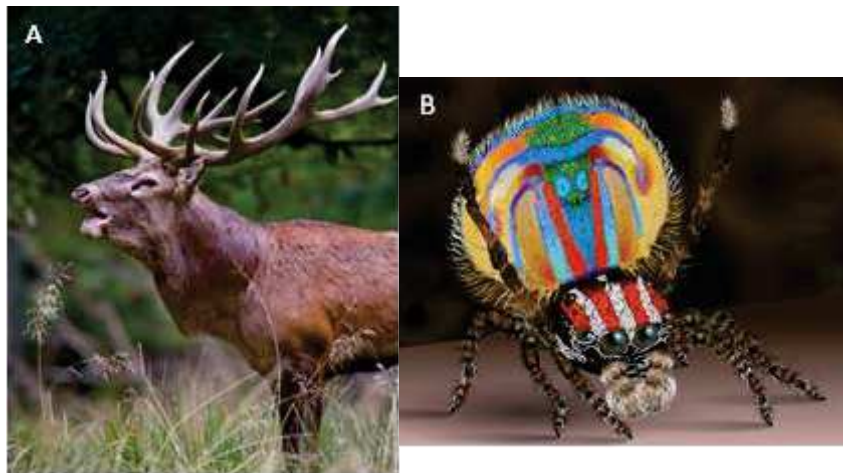


Figure 2A/ antlers of a male red deer (*Cervus elaphus*) as an illustration of armaments; **B/** conspicuous colour patterns of a male peacock spider (*Maratus volans*) as an illustration of ornaments. Licensed-free pictures from *Wikipedia*

Armaments and badges of status may also evolve under social selection (Tibbetts and Safran 2009). Social selection arises when individuals are competing for access to resources such as food or territory (Lyon and Montgomerie 2012). Of course, both type of sexual/social characters are not mutually exclusive and may be expressed by the same signal (Berglund et al. 1996).

1.2. Asymmetric investment in reproduction and sexual dimorphism

Numerous studies and observations seem to establish a different role between sexes in reproduction. It is often admitted that females, which invest more in reproduction than males, choose males whereas males are competing for access to females. Bateman's experiment with

Drosophila (1948) provided one of the first insights for such an asymmetric sex role in reproduction. He showed that male relative fertility (proxy of reproductive success) increased with the number of mates whereas increasing the number of mates did not provide any increase in relative fertility for females (Figure 3). He deduced females were limited by their ability to produce eggs whereas males were limited by the number of mates they could get.

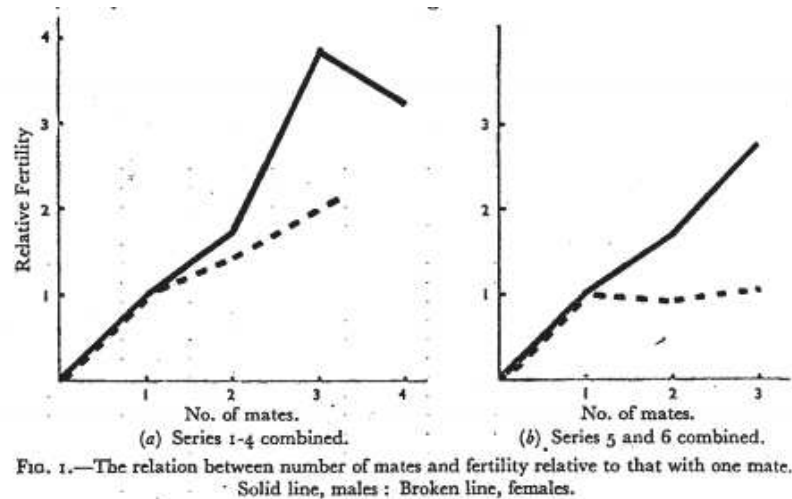


Figure 3. Illustration from figure 1 in Bateman published in *Heredity* in 1948

Trivers (1972) developed a hypothesis focusing on anisogamy but also parental care to explain this phenomenon. At the gamete level, in multicellular organisms with separated sexes, there is frequently an important size asymmetry between males and females, leading to an asymmetry in reproductive strategies, to the extent of sexual conflict. On the one hand, males are producing numerous small gametes. Under Trivers' hypothesis, males gain by maximising the number of breeding mates to increase their chance of obtaining offspring. On the other hand, females are producing a small amount of large gametes. Their investment is thus concentrated in a very little amount of potential offspring. Under Trivers' hypothesis, females gain by finding a mate which may maximise the survival chance of their offspring and may thus choose their mate. Therefore, with competitive males and choosy females, the development of secondary sexual characters may also be asymmetric. Bateman's hypothesis, referred as the Darwinian-Bateman paradigm has been commonly used in researches on conventional sex roles (Dewsbury 2005) but recently his hypothesis has been challenged statistically and experimentally (Snyder and Gowaty 2007; Gowaty et al. 2012). However a very recent meta-analysis tested Bateman's principles and confirmed the generality of conventional sex roles in polygamous species of animals (Janicke et al. 2016).

The development of secondary sexual traits in males offers the possibility to signal their intrinsic quality, either to potential mates (via intersexual selection) or to potential opponents (via intra-sexual selection). To be considered honest, many signals have to be condition-dependent and thus costly to produce, maintain or display (Zahavi 1975; Grafen 1990; Cotton et al. 2004). Selection should not favour the evolution of secondary sexual traits in females. If females are not competing and males do not choose them, developing secondary sexual traits would be a useless cost. In order to maximise the survival of females and their offspring, selection should favour the most cryptic females (Darwin 1871; Wallace 1889; Fisher 1930). This strong asymmetry in the development of secondary sexual traits between sexes is known as sexual dimorphism.

Sexual dimorphism is more or less pronounced depending on the species. Secondary sexual characters are only present in males in some species (armaments, coloured patches, extravagant ornamental traits, etc.); they may also be displayed by females in a more attenuated way. Sexual dimorphism should be more present in polygynous species, in which variance in reproductive success is strongly skewed in one sex (Andersson 1994; Badyaev and Hill 2003) and less present in socially monogamous species with biparental care (Kraaijeveld 2003).

1.3. Variation in sexual dimorphism

1.3.1. The strength of sexual selection

For a long time, sexual dimorphism has been considered as an evolutionary derived state with dull monomorphism as the ancestral state. Under this view, sexual selection was seen as the primary force shaping sexual dimorphism; sexual selection would lead to evolutionary changes going towards greater ornamentations in males, limited by natural selection pressures (Darwin 1871; Fisher 1930). Several hypotheses have been proposed to understand this increased ornamentation in males through sexual selection. The “runaway process” developed by Fisher added the notion of innate preference in female for more elaborate ornaments (Figure 4A), therefore including the idea of a higher reproductive success associated with female preference (Fisher 1930). One possibility for this innate preference could be a sensory bias (Endler and Basolo 1998) or a mere preference for a trait, which does not encode information on the bearer’s quality (Prum 2012). The handicap hypothesis (Zahavi 1975; Hamilton and Zuk 1982; Grafen 1990) was influential in carrying the idea that traits chosen by females are not arbitrary but are costly traits related to the condition of individuals and providing either direct or indirect benefits to females (Figure 4B). The “sexy sons”

hypothesis (Weatherhead and Robertson 1979) is another hypothesis which was developed in order to understand polygynous system (Figure 4C) where males provide no parental care. It represents a classical example of indirect benefits of female choice. Under this hypothesis, females prefer mating with attractive males despite their low parental care involvement and fidelity. Females gain compensation by bearing “sexy sons” with higher future mating success, which represents an indirect benefit for females (Kokko et al. 2002).

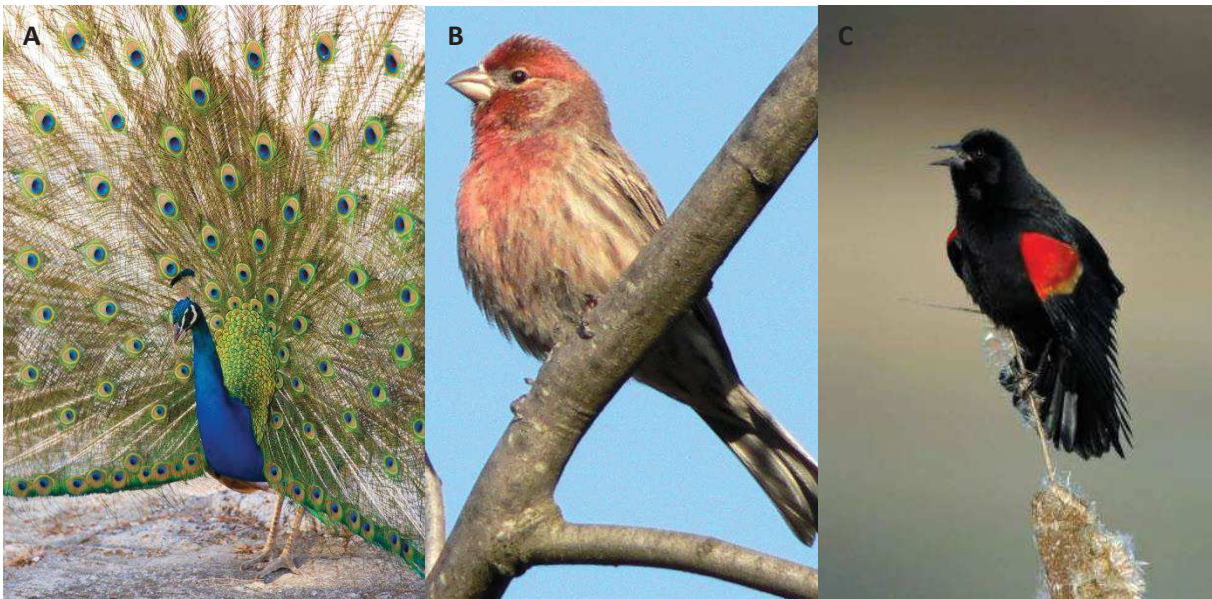


Figure 4 Illustrations of three theories for the evolution of male conspicuous ornaments in birds. A/ Male peacock (*Pavo cristatus*) extravagant tail illustrates Fisher's Runaway process; B/ redness in male house finch (*Carpodacus mexicanus*) illustrates the theory of handicap; C/ epaulets in male red-winged blackbird (*Agelaius phoeniceus*) illustrate the “sexy sons” theory. Licensed-free pictures.

1.3.2. Alternative explanations

Alternative explanations for variation in sexual dimorphism in birds included selection for cryptic females due to their vulnerability to predation during nest attendance (Wallace 1889). These hypotheses and their derivatives have been subjected to numerous debates and to theoretical and empirical tests including the use of modern phylogenetic comparative methods (Felsenstein 1985; Harvey and Pagel 1991) especially regarding sexual dichromatism (i.e. difference in colouration between males and females) in birds (Badyaev and Hill 2003). These studies revealed the evolution of sexual dichromatism was most probably involving other complex factors maintaining dichromatism such as genetic drift and indirect selection. Comparative studies on monogamous and polygynous species also showed

sexual dimorphism was not strongly associated with polygynous species (Møller 1986). Finally, contrary to expectation, modern phylogenetic comparative methods revealed sexual dichromatism was rather an ancestral state than a derived state (Wiens 2001; Badyaev and Hill 2003 for birds; Ord and Stuart-Fox 2006 for lizards). For example, Bleiweiss (1992) found that most variation in the degree of dichromatism among species of hummingbirds (Trochilidae) was due to female variation. Also, two major comparative analyses on dichromatism in songbird families Parulinae and Cardinalinae concluded on the necessity to consider the evolution of female colouration through male mate choice or female competition (Irwin 1994; Martin and Badyaev 1996; Badyaev and Hill 2003). But so far, this has rarely been done.

2. Origins and limits of female ornaments

Researchers documented the degree of variation in sexual dimorphism and the existence of female ornamentation in numerous species of birds (Amundsen 2000) as well as in other taxa (see Amundsen and Forsgren 2001 for fishes; see Harrison and Poe 2012 for lizards; see review in Clutton-Brock 2009 for other taxa). Several factors have been advanced to explain the evolution and maintenance of female ornaments.

2.1. *Genetic correlation*

One of the first hypotheses historically emitted to understand female ornamentation was based on genetic correlation between female and male traits. Under this hypothesis, secondary sexual traits in females are a by-product of the presence of traits in males. The original hypothesis stated that traits are expressed in males, through sexual selection. Then, due to strong genetic correlation between males and females, traits are expressed and maintained in females. Theoretical models developed by Lande (1980) reinforced this principle of genetic correlation. They also highlighted the importance of variation in selection pressures among species which can explain the interspecific variation in degrees of dimorphism (Figure 5). Secondary sexual traits appearing more recently in males or a lower pressure of natural selection affecting females would explain sexual monomorphism or low sexual dimorphism (Price 1996). Despite the importance of the correlated response hypothesis, the effect of genetic correlation on ornaments has rarely been studied (but see Price and Burley 1993; Price 1996; Chenoweth and Blows 2003; Roulin and Jensen 2015).

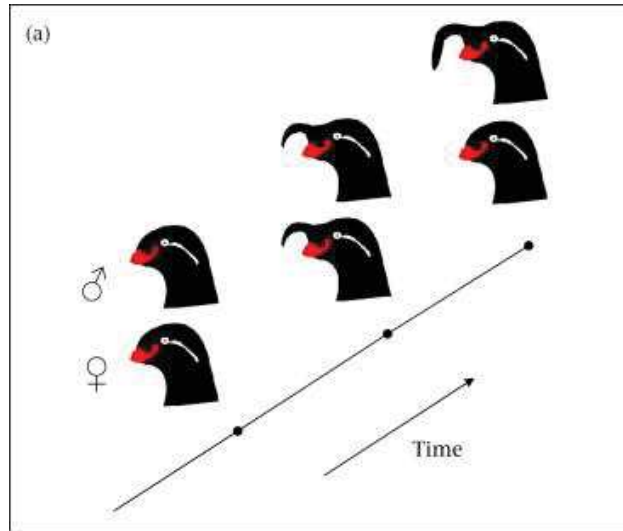


Figure 5. Illustration of Lande's hypothesis (1980) explaining the evolution of male ornaments. Dull monomorphism is the ancestral state; sexual selection promotes the evolution of ornaments in males in a first step; with genetic correlation, ornaments then appear also in females; in the final step, female ornaments fade away under natural selection pressure. Illustration from Figure 1a in Kraaijeveld et al. published in *Animal Behaviour* in 2007.

This hypothesis was largely admitted as the main hypothesis explaining female ornamentation among evolutionary biologists working on sexual selection. However it relied on the assumption of dimorphism as an evolutionary derived state when dimorphism appeared to be an ancestral state (Badyaev and Hill 2003). Furthermore, modern phylogenetic comparative methods revealed higher lability in female ornamentation evolution with a higher frequency in gain and loss of ornaments in females compare to males (Wiens 2001; Johnson et al. 2013). If genetic correlation may partly explain the evolution of female ornaments, other factors must be considered (Amundsen and Pärn 2006).

2.2. Male mate choice, mutual mate choice

Male mate choice is expected to be one important factor explaining the evolution of female ornamentation. Based on empirical studies several key factors have been found to be associated with the evolution of male mate choice.

2.2.1. The operational sex ratio

Classically, it is admitted that operational sex ratio – the ratio of sexually active males to females (Edward and Chapman 2011) – affects sex roles. The limiting sex chooses whereas the limited sex competes for access to the choosy limiting sex. And indeed, inversed-sex-role species are mainly species with male-only parental care. In those species, males are fully

invested in reproduction with a limited possibility to mate with other individuals. They then become the limiting choosy sex whereas females are available to mate with other individuals and become the limited competing sex. Male mate choice has been described in species with reversal sex roles. For instance, in pipefish *Nerophis ophidion*, female reproductive success is limited by male parental care and females have been observed to be more active during courtship (Rosenqvist 1990). Rosenqvist performed mate choice experiments and found males were discriminating among females on the basis of female size and female skin folds, which are ornamental.

Nonetheless, there is also a large evidence that sex roles are more variable and dynamic than classically expected (Clutton-Brock and Vincent 1991), even when operational sex ratio seems skewed towards males. Male mate choice has been observed in many species thought to have conventional sex roles among different taxa (review in chapter 6 in Andersson 1994).

2.2.2. Female fecundity

Variation in female fecundity is one of the other key factors favouring male mate choice. In different species from different taxa, female size is positively correlated to fecundity and males seem to prefer larger females (see Gwynne 1981 for an example in insects; Cote and Hunte 1989 for fishes; Olsson 1993 for lizards). In two-spotted gobies (*Gobiusculus flavescens*), variation in female yellow-orange belly colouration has been correlated to variation in fecundity; mate choice experiments revealed male preference for females displaying a brighter coloured-belly compared to drabber females (Amundsen and Forsgren 2001).

2.2.3. Sperm depletion

Sperm depletion is another factor favouring male mate choice. In *Spermophilus tridecemlineatus* ground-squirrel, males discriminate among females and avoid transferring sperm to some already mated females. This discrimination was related to second-male fertilization which declines with time spent since first-male fertilization (Schwagmeyer and Parker 1994). In the polygynous feral fowl (*Gallus gallus*), males were also found to transfer a larger amount of sperm towards females displaying a larger comb (Pizzari et al. 2003).

2.2.4. Mutual mate choice and assortative mating

The occurrence of male mate choice in species supposed to have conventional sex roles does not preclude female mate choice. Mutual mate choice occurs when males and

females both show preference for traits in mates. In a monogamous species of birds, the Crested auklet (*Aethia cristatella*), an experiment described the presence of male preference for larger crest in females (Jones and Hunter 1993) as well as female preference for larger crest in males, showing an example of mutual mate choice based on the same ornament.

One of the explanations for the occurrence of mutual mate choice is related to the process of assortative mating (Trivers 1972; Kraaijeveld et al. 2007). Assortative mating is defined in behavioural ecology as a process of non-random mate choice based on similarity with oneself (Jiang et al. 2013). In the case of mutual mate choice, individuals of a pair are assessing their mate on the same trait or array of traits. Assortative mating can thus reinforce genetic correlation between the signal and the preference for this signal. However, assortative mating can also occur from other processes (Kraaijeveld et al. 2007). Mate choice from only one member of a pair can also lead to assortative mating if the individual is choosing its mate based on similarity to itself. Directional mate preference can also favour assortative mating; for instance, if individuals with the highest value of a trait are better competitors and prefer highest values of the same trait in their mates, it will form a pair of individuals of highest values. Individuals with lower values will only have access to potential mates of lower values, creating then assortative mating (McLain and Boromisa 1987). Convergence of the degree of ornaments within a pair can also lead to assortative mating if pairs remain together across different breeding seasons, age together and/or share the same habitat. This may reinforce resemblance within a pair, favouring assortative mating. Finally, if ornaments are correlated with age and/or arrival on breeding site, individuals can also happen to pair together, sharing incidentally the same ornaments (Gimelfarb 1988). When assessing assortative mating as a driver of the evolution of mutual ornamentation within a population, it is thus important to test factors which may drive assortative mating before concluding it is due to mutual mate choice. However, few studies have attempted to do so, and most of them are related to size-assortative mating (Montiglio et al. 2016).

2.2.5. The evolution of male mate choice

The amount of empirical evidence led theoretical models to test the factors leading the most competing sex to choose, despite a classical operational sex ratio biased towards males. These models defined conditions leading to female mate choice, male mate choice (Figure 6) or mutual mate choice (Johnstone et al. 1996; Johnstone 1997; Kokko and Johnstone 2002). Based on the hypothesis that the competing sex may also develop preferences, these models

defined a combination of three key-parameters expected to lead to the evolution of choice in the competing sex, despite effects of operational sex ratio.

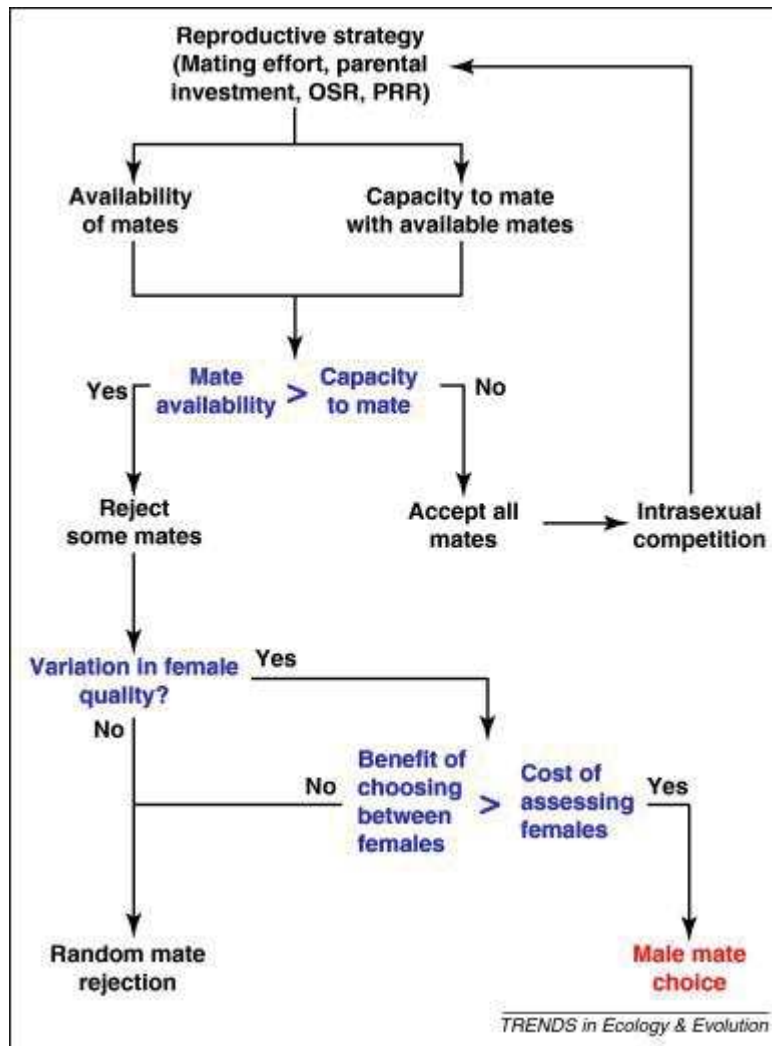


Figure 6. Flowchart summing up factors affecting the evolution of male mate choice, from figure 1 in Edward & Chapman published in *TRENDS in Ecology & Evolution* in 2011. OSR refers to operational sex ratio, PRR to potential reproductive rate.

2.2.5.1. Encountering rate

Choosing a mate includes the idea of rejecting potential mates. In some cases such as lower density or higher viscosity of habitat, it can be costly to reject a potential mate since probability to encounter another mate is low. Choosing would thus be favoured in higher densities with simultaneous encounter of potential mates and would be less likely to happen with sequential encounter of mates (Barry and Kokko 2010).

2.2.5.2. Time out of reproduction

This term refers to the time invested in reproductive processes which are not mating or attempting to mate (time in). “Time out” includes time spent competing for one mate, attracting one mate, as well as time being invested in parental care. Investing time in these processes reduces time invested in attempting to mate with other individuals; it reduces thus the total number of mates. Mate choice would be favoured when time invested in reproductive processes other than mate attempts is high.

2.2.5.3. Variation in mate quality

As shown earlier based on field studies on variation in female fecundity, mate choice should be favoured when there is variation in mate quality. Assessing mate quality needs also to be reliable to favour mate choice.

So far, no studies have tested whether these predicted factors could explain the large interspecific variation we observed in female ornamentation. Such data are crucially missing to advance our understanding of the effect of male mate choice on the diversity of female colouration.

2.3. *Female-female competition: intra-sexual selection and social selection*

Inversed-sex-roles were first defined in species with females being the most competitive sex. Observations of female-female competition were a major step in understanding the plasticity in sex roles among species and two forms of female-female competition were defined (Lebas 2006). Competition for access to mates is classified as intra-sexual selection whereas competition for access to resources or territory is referred as social selection (Clutton-Brock 2009; Lyon and Montgomerie 2012). Intra-sexual selection and social selection pressures on females can lead to the development of weaponry or badges of status in females (Tobias et al. 2012).

The Eurasian Dotterel (*Charadrius morinellus*) is a polyandrous bird with sexual dichromatism biased toward females. In this species, females bearing a brighter plumage were found to be more successful at winning access to males (Owens et al. 1994). Female weaponry developed through intra-sexual selection can also occur in promiscuous species. For instance, receptive females in the African Topi antelopes (*Damaliscus lunatus*) fight with their horns for access to males (Bro-Jørgensen 2007). Cooperative species in birds have been recently used to

test relations between intra-sexual selection and secondary sexual traits in females. In cooperative species of birds, only few females have access to reproduction, increasing potentially competition among females. At the interspecific level, Rubenstein & Lovette (2009) used comparative methods in African starlings (*Sturnidae*) and found a more frequent presence of female ornaments in cooperative species.

At the interspecific level, the development of ornaments and weaponry in relation to female-female competition is well documented in terms of social selection. At the edge between intra-sexual and social selection are the competitions for access to resources to feed offspring or access to better breeding territories. For instance, female dung beetles (*Onthophagus sagittarius*) with larger horns have a better access to resources to raise offspring (Watson and Simmons 2010). The facial patterns of queens in social paper wasps (*Polistes exclamans*) are related to their access to suitable nest sites (Tibbetts and Sheehan 2011). Tropical species of birds were also found to be more monochromatic than temperate species (Friedman et al. 2009; Johnson et al. 2013; Dale et al. 2015). Among hypotheses emitted to explain this phenomenon, female participation to year-longed occupied territory in tropical species could favour the evolution of weaponry in females. Finally, experimental tests showed the occurrence of badges of status in females for two species of birds, which defend territories during breeding period. Beak brightness of female American goldfinches (*Spinus tristis*) (Murphy et al. 2009) or ultraviolet reflectance of the blue crown in blue tits (*Cyanistes caeruleus*) (Midamegbe et al. 2011) were found to be used as badges of status in females.

2.4. *Alternative hypotheses*

Genetic correlation, male mate choice and female-female competition are important hypotheses to understand and justify the evolution and maintenance of female ornaments. Alternative hypotheses may also help understand female ornaments (Amundsen and Pärn 2006).

2.4.1. Species recognition

Female and male ornaments could be used to avoid heterospecific reproduction and hybridization (McNaught and Owens 2002; Doutrelant et al. 2016).

2.4.2. Sexual concealment

Mimicking males by displaying male-like ornaments could be an alternative strategy to increase female survival. Costs related to the expression of these traits would largely be compensated by risks related to male sexual harassment (Langmore and Bennett 1999).

2.4.3. Hormonal regulation

At a proximal level, female ornamentation could be due to a higher expression of male hormones (Kimball and Ligon 1999; Eens et al. 2000). This hypothesis is not mutually exclusive with former hypotheses. For instance, a higher production of testosterone may increase female aggressiveness and territory-defence behaviours; this could also lead to the production of coloured patches related to resource defence.

2.5. *Limits of female ornamentation*

Although female ornament is widespread among taxa with some examples of extravagant traits, expression of ornaments and weaponry in males seems still more widespread and extravagant than in females (Edward and Chapman 2011; Janicke et al. 2016). Besides all explanations developed above, remains the possibility of lower expression of ornaments in females due to different factors.

The first factor related to a lower expression in females was related to female reproductive behaviour associated with an increased risk of predation (Wallace 1889). For instance in birds, behaviour related to nest attendance can lead to a stronger natural selection on females (Soler and Moreno 2012).

Female ornaments could also be limited by constraints in resource allocations and trade-off with fecundity. Due to anisogamy in the number of gametes produced, females invest additional allocation to eggs to insure offspring survival (Trivers 1972; Chenoweth et al. 2006). Resources and energy expended in eggs should be more important than male resource allocation in sperm, at least per single offspring. Thus allocating a large amount of resources to the production of ornaments may affect egg quality and fecundity in females and lead to a trade-off between fecundity and expression of ornaments (Figure 7) (Fitzpatrick et al. 1995). Sexual selection for the evolution of female ornaments should thus be self-limited. However, despite this cost, theoretical works suggested female ornaments may be maintained through stabilizing

mating preferences (Nordeide et al. 2013) with males avoiding to choose females which invest too many or too few resources in ornaments (Chenoweth et al. 2006).

In the case of an operational sex ratio biased towards males, females rejected by males should eventually find a mate (Long et al. 2009; box 4 in Edward and Chapman 2011), limiting the impact of male mate choice on female sexual traits.

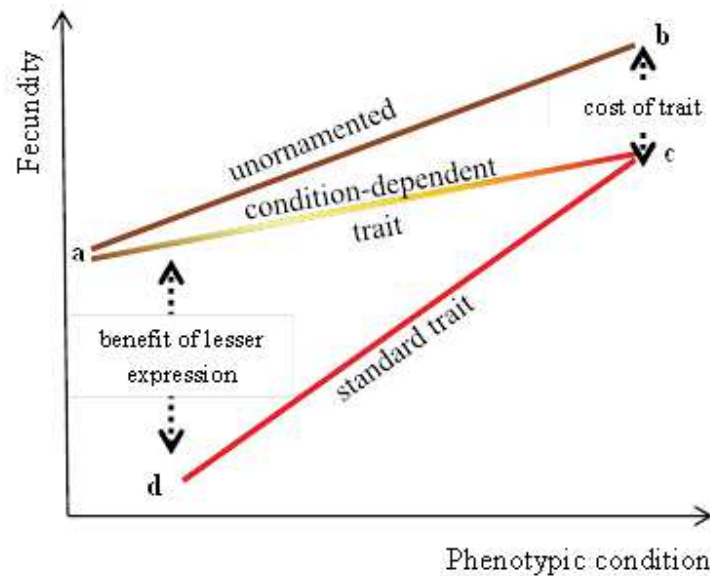


Figure 7. Relations between female phenotypic condition and fecundity depending on the type of trait expressed. Line “ab” represents the relation between phenotypic condition and fecundity when no ornament is expressed (no expression of trait permits a larger allocation to fecundity, explaining why line “ab” is the line showing the highest values for fecundity). Line “dc” represents the relation between phenotypic condition and fecundity when the ornament is expressed at the same level in every female (females in higher phenotypic conditions would have less costs of producing the trait compare to females in lower phenotypic conditions, although fecundity decreases for all females). Line “ac” represents the relation between phenotypic condition and fecundity when the ornament can be expressed with different values (represented here with colour gradation); females of low phenotypic condition would gain fecundity by reducing trait expression but would still have a lower fecundity than females of high phenotypic condition expressing the highest value of the trait. Illustration adapted from figure 1C. in Fitzpatrick et al. published in *Biological Journal of the Linnean Society* in 1995.

3. Sexual/social selection in the wild: the necessity to consider spatiotemporal variation

The study of sexual selection can be rather complex because of the intertwinement of many parameters: coevolution of preferences and ornament production, interactions among fitness components (viability, fecundity, etc.), sex-specific differences in selection pressures and their outcome on evolution, etc. (Kuijper et al. 2012). Another issue largely complicating the evolution of sexually and socially selected traits is related to environmental dynamics (Wiens 2001; Bro-Jørgensen 2010).

For instance, the search for gradients of selection for multiple traits in a population of lark buntings (*Calamospiza melanocorys*) revealed strong temporal variation (Chaine and Lyon 2008) over years, leading to the inability to detect which trait was under sexual selection in this population. Experiences on female mate choice for male size in close Swedish populations of the blue-tailed damselfly (*Ischnura elegans*) revealed spatial variation in female preferences (Svensson and Gosden 2007). Depending on the population, females preferred larger or smaller males. Spatial and temporal environmental heterogeneity can thus affect the outcomes of selection (Cornwallis and Uller 2010) through the expression of the sexually selected trait or through the preference for this trait.

Wiens and collaborators (2001) reviewed environmental factors which may affect sexually selected traits and preferences. Difference in signal transmission in various environments can affect sexually selected traits. For instance, the opacity of water in which inhabit threespine sticklebacks (*Gasterosteus aculeatus*) may render their red colouration inconspicuous, affecting mate choice, thus sexual selection for colouration (Reimchen 1989). Similarly, noises associated with urbanisation make low-frequencies signals used for acoustic communication in Great tits (*Parus major*) inefficient (Halfwerk et al. 2011). The evolution of ornaments can also be affected by constraints in resources. Hill has shown that variation in the belly redness (from pale yellow to bright red) of male house finches (*Carpodacus mexicanus*) was positively correlated to their intake of carotenoid-pigment in their diet (Hill 1994). The lack of red individuals in some populations was found to be due to the paucity of carotenoid in food leading to an inability for males to express red colouration. Resources can as well vary among years affecting expression of traits (Linville and Breitwisch 1997). Increased predation risk is another factor which can affect both ornaments and choosiness. Colouration of male guppies (*Poecilia reticulata*) was found to be negatively correlated to predation rate among different populations (Endler 1983). Concerning mate choice, an experimental approach in sand goby (*Pomatoschistus minutus*) showed females reduced their time allocated to choice with an increase of predation risk (Forsgren 1992).

Temporal variation related to change in environmental factors as well as spatial variation among populations can be widespread. They can affect both ornamentations and preferences. When focusing on the evolution of ornaments in wild populations, it is therefore necessary to

consider potential variation due to environmental heterogeneity and to conduct research on different populations in numerous successive years (Cockburn 2014).

4. For a better understanding of female ornamentation: the case of female plumage colouration

As highlighted with numerous examples above, many questions related to the evolution of sexually selected traits were studied using plumage colouration (reviewed in Badyaev and Hill 2003; Amundsen and Pärn 2006).

4.1. *At the interspecific level*

Sexual dichromatism - the difference between male and female plumage colouration - was used as the main proxy in understanding the evolution of male ornamentation. Female plumage colouration was mainly considered as the drab counterpart to male plumage colouration. But major comparative studies in the 1990s revealed the importance of female plumage colouration, its labile evolutionary changes and the necessity to consider processes underlying its evolution (Irwin 1994; Martin and Badyaev 1996; Burns 1998). In 2000, Amundsen reviewed empirical evidence that ornamentation in female birds might be under sexual selection, including several examples of female plumage colouration related to female quality and/or to male mate choice (Amundsen 2000).

Females of many species of birds are colourful and plumage colouration remains a useful trait for the study of female ornaments, especially with the development of spectrophotometry and visual models (see Insert 1). Although more developed in the past years as empirical evidence (Kraaijeveld et al. 2007; Clutton-Brock 2009), tests of hypotheses related to the evolution of female plumage colouration are still scarce. The necessity to consider processes favouring the evolution of female ornaments was emphasized in a major comparative study using plumage colouration (Irwin 1994). Nonetheless, and despite major progress in the study of plumage colouration, female plumage colouration was still assessed in comparative studies embedded in the classical proxy of sexual dichromatism. Even in recent literature, the use of comparative studies to understand the evolution of plumage colouration relied mostly on male colouration (Eaton 2006; Mason et al. 2014) or on the difference between male and female plumage (Figuerola and Green 2000; Friedman et al. 2009; Simpson et al. 2015). One pioneer study focused on female colouration and tested the effect of social selection on the evolution of female ornaments (Rubenstein and Lovette 2009). They showed that the occurrence of sexual

monochromatism was more frequent in cooperative species of African starlings compared to territorial species. Although centred on female selective pressures, the study still used dichromatism assessed with human vision as a proxy for the evolution of female colouration (but see Maia et al. 2016).

Very recently, two published studies answered to the claim (Burns 1998) of analysing male and female colouration separately (Dale et al. 2015; Dunn et al. 2015). They both focused on testing ecological and life-history variables which may affect male and female colouration independently. To test the effect of sexual selection, they both used mating system and a dichotomous factor of male investment in parental care (absence/presence); Dunn and collaborators also added a measure of testes size which can affect male or female colouration. These studies can be considered as pioneers as they included the possibility for sex-specific evolution. However, as any study, they have limits and offer space for further research. Their proxies of sexual selection are too large to test the processes described above as drivers of female ornamentation. Furthermore, when Dunn and collaborators used spectrophotometry on six different patches, they still used their variables in a context of monochromatism or dichromatism (by separating their sample species and testing some factors on dichromatic species and others on monochromatic species) (Dunn et al. 2015). Dale and colleagues did not separate monochromatic species from dichromatic species: they tested their predictions on male colouration, female colouration and dichromatism (Dale et al. 2015). However, they did not use spectrophotometry to assess colouration in males and females and created a score based on pictures from plates in the Handbooks of the Birds of the World (Figure 8a) (Hoyo et al. 1992). Their method has two potential flaws. First, it could not account for ultraviolet colouration which has been proven to be important in avian vision (Odeen et al. 2011). Second, due to the representation of the birds on plates, analysis of colouration was restricted to parts of the head and the throat of each bird (Figure 8b) when sexually selected colouration could be expressed anywhere on female body. For instance, a study on the American redstart (*Setophaga ruticilla*) analysed multiple carotenoid-based patches in female; coloured patches on the flanks and the tail in females were varying and were related to female and male investment in parental care (Osmond et al. 2013). Similarly, in the Tawny owl (*Strix aluco*), female tail colouration seemed to reflect female quality (Roulin et al. 2003). Another problem can emerge from working on book plates. Despite the incredible quality of drawings, different authors have participated, using different techniques, which may

increase heterogeneity unrelated to proper interspecific variation. It is thus essential to go further and test detailed factors leading to the evolution of female colouration, while working on a more homogenous and complete data set.

Theoretical studies defined parameters likely to affect the evolution of female plumage colouration via the evolution of male mate choice (Johnstone et al. 1996; Kokko and Johnstone 2002; Edward and Chapman 2011) and comparative studies provide a powerful tool to test the predictions. In the context of mutual mate choice, female plumage colouration is affected by parameters related to male mate choice. Therefore, to determine the importance of male mate choice in the evolution of female plumage colouration, I focused on the parameters related more specifically to male choice, while considering parameters which may limit them.

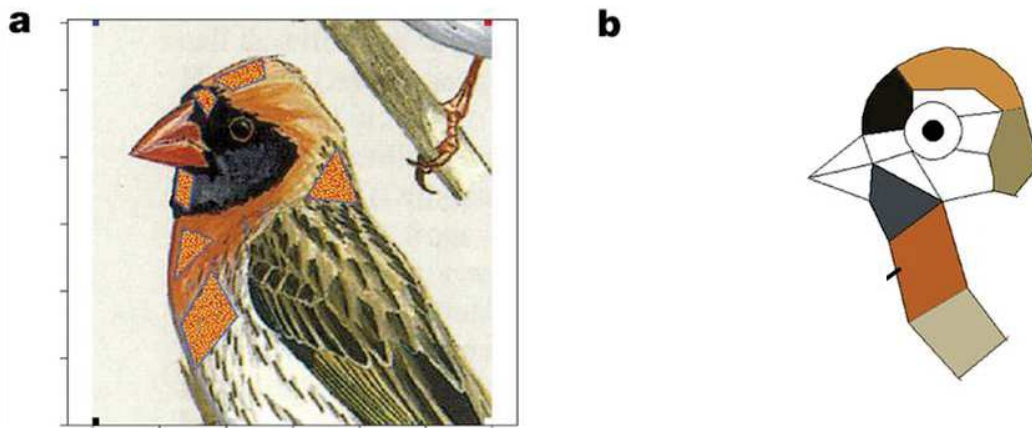


Figure 8 Illustrations of the score used by Dale et al. 2015 to assess male and female plumage colouration. a/ Example of plate from the Handbooks of the Birds of the World (Del Hoyo 1992) used to assess colouration in male red-billed quelea (*Quelea quelea*). b/ Schema representing the six patches used to assess colouration, restricted to areas on the head and throat of the bird. Illustration adapted from figure 2 from Dale et al. published in *Nature* in 2015.

4.2. At the intraspecific level

Several empirical studies have related the development of female plumage colouration to sexual and/or social selection. Amundsen (2000) reviewed five studies relating male preference for female plumage colouration (Burley 1977 for the Feral pigeon; Muma and Weatherhead 1989 for the Red-winged blackbird; Hill 1993 for the House finch; Amundsen et al. 1997 for the Bluethroat; Hunt et al. 1999 for the Blue tit) and three studies relating female plumage colouration to female quality (Amundsen et al. 1997 for the Bluethroat; Linville et al. 1998 for

the Northern cardinal; Johnsen et al. 2005 for the Red-winged blackbird). More recently a meta-analysis (Hegyí et al. 2015) listed eight other studies relating female plumage colouration to their own or their mate feeding rate (see Rohde et al. 1999 for the Bluethroat; Roulin et al. 2001 for the Barn owl; Jawor et al. 2004 for the Northern cardinal; Johnsen et al. 2005, Garcia-Navas et al. 2012 and Limbourg et al. 2013 for the Blue tit; Siefferman and Hill 2005 for the Eastern bluebird; Balenger et al. 2007 for the Mountain bluebird).

West-Eberhard (1983) suggested that female conspicuousness could also be related to competitive function. Several empirical studies found a relation between female plumage colouration and competition for mates or resources (see Johnson 1988 for the Pinyon jay; Holberton et al. 1989 for the Dark-eyed junco; Wilson 1992 for the Great tit; Owens et al. 1994 for the Eurasian dotterel; Swaddle and Witter 1995 for the European starling; Jones and Hunter 1999 for the Crested auklet; Jawor et al. 2004 for the Northern cardinal; Midamegbe et al. 2011 for the Blue tit). When there are many examples of sexually and/or socially selected traits of plumage colouration in females, most of these studies were performed on a low sample size, within a small amount of time (one to three breeding seasons) and were not replicated. It is necessary to take into account the variation associated with plumage colouration development and/or preferences for traits, to see the extent of the evolution of plumage colouration in females.

Assortative mating on colouration can also favour the evolution of female plumage colouration. Again, several empirical studies have assessed the degree of assortative mating on colouration in birds (e.g. Andersson et al. 1998 and Garcia-Navas et al. 2009 for Blue tits; Jawor et al. 2003 for Northern cardinals; Silva et al. 2008 for European rollers; Grunst and Grunst 2014 for Yellow warblers; Harris and Siefferman 2014 for Eastern bluebirds). Similarly to studies relating sexual and/or social selection to female plumage colouration, most of these studies were performed on a low sample size, within a single population in a small amount of time. The case of blue tits typically expressed the lack of consideration of spatiotemporal variation. There was inconsistency in the results on assortative mating in blue tits, in two different studies assessing the degree of assortative mating on blue crown colouration. A first study on a Swedish population found a positive value of assortative mating on the colouration of the blue crown (Andersson et al. 1998) whereas the study on a Spanish population failed to find any pattern on the same patch (Garcia-Navas et al. 2009). Those two studies were realised on a low sample size, in only one

population and over a single breeding season. There is a necessity to enlarge sample size, to work on different successive years and to replicate the study in different populations. This would enable to consider spatiotemporal variation and to see if there is an overall assortative mating. Furthermore, assortative mating cannot solely be explained by assortative mate preference in one sex or both (Kraaijeveld et al. 2007). Despite empirical work defining factors explaining assortative mating (Crespi 1989), only few studies have attempted to test these factors and to my knowledge none of them worked on ornaments (see Taborsky et al. 2014; Montiglio et al. 2016 for examples on size-assortative mating in fishes). It is thus important to assess which factors drive assortative mating on plumage colouration, based on a solid data set.

5. Thesis main axes

My thesis focused on the importance of male mate choice to explain the evolution of female ornamentation. I assessed female plumage colouration in species with conventional sex roles. I used correlational approaches from macro-evolutionary scale to micro-evolutionary scale to fulfil the lack of knowledge about the evolution of female ornaments. The development of new statistical tools offers the possibility to provide complementary tests to the traditional methods used to test male mate choice. Many studies used experimental work to prove male mate choice on one single species and assessed mating patterns on a single year with a limited sample size. Here I used two main approaches to depart from these traditional methods.

In chapter II (Chapter II - MS1), using phylogenetic comparative methods, I tested whether predictors identified by theoretical models as drivers of male mate choice led to the evolution of female plumage colouration in species of songbirds. In these analyses, I also tested the effects of factors predicted to limit the expression of female ornamentation and I controlled for the phenotypic correlation with male ornamentation.

In chapter III, given spatial and temporal variations needed to be considered when assessing colouration and the strength of selection in wild populations, I conducted analyses on a large data set including several years and populations. I focused on a model species of passerine with biparental care, low survival, and low dichromatism, the Blue tit (*Cyanistes caeruleus*) (see Insert 2). In this species, previous researches have shown that colouration might be sexually or socially selected in both sexes (Vedder et al. 2008; Midamegbe et al. 2011; Doutrelant et al. 2012; Limbourg et al. 2013) but these results have been criticised and refuted for male

colouration (Parker 2013). It is thus necessary to use large data sets to understand the processes behind the evolution of colouration in this species. I worked on two colour patches, the structure-based colouration of the blue crown and the carotenoid-based colouration of the yellow chest patch (see Insert 1) measured in both males and females every year since 2005 in four populations in the South of France and in Corsica (representing around 6000 individuals) (see Figure 1 Insert 2). I intended to test a special case of mutual mate choice, assortative mating. Before testing assortative mating patterns among populations and years, I first checked the three conditions for sexually selected traits to evolve; I measured inter-individual variation (Chapter II-A – Part 1 as extracts from MS A1), I built the database for a study aiming at determining the heritability of ornaments and the strength of genetic correlation between male and female ornamentation (Chapter II-A – MS2) and I conducted the first selection analyses related to reproductive success (Chapter II-A – MS3). Then I assessed the pattern of assortative mating (Chapter II-B – MS4) and tested several predictors which may explain assortative mating in the model species (Chapter II-B – MS5).

INSERT 1: PLUMAGE COLOURATION AND ITS QUANTIFICATION

Feather is a specific tissue in birds, composed of barbs and barbules assembled differently depending on the function of the feather. Since feathers are dead structures, colour cannot change radically between moults, although mechanisms such as fading or abrasion can alter plumage colouration (Gill 1995). Plumage colouration can be well conserved and can be assessed on stuff specimens or on feathers sampled from the field if they have been kept in the dark at cool temperature (Doucet and Hill 2009). There are two main processes to produce feather colourations in birds.

1. Chemical and physical colourations of plumage

Pigment-based colourations depend on the selective absorption of certain wavelengths of light by the pigments included in the structure (Eliason et al. 2015). The two main types of pigments deposited in feathers are melanins and carotenoids. Carotenoids have been the focus of studies investigating the role of sexual selection on colour evolution (McGraw 2006a). First, carotenoids produce quite conspicuous colourations ranging from yellow to red. Then, carotenoids are not endogenously produced by animals and need to be metabolized from nutrients. Finally, their chemical properties are related to health as they can be used as antioxidants (Burton and Ingold 1984) and immunomodulators (Lozano 1994). Carotenoid-based colourations have therefore been a stepping stone in understanding condition-dependent traits (Faivre et al. 2003). By contrast, melanins are synthesized from amino acid precursors and can be directly produced by birds. They are responsible for black, brown, grey, rufous, buff shades of plumage colouration (McGraw 2006b). The cost of producing melanins and their role in sexual selection processes are not as straightforward as for carotenoids. However, honesty of melanin-based signals can be explained by pleiotropy (Ducrest et al. 2008; Roulin 2016). The production of melanin is related to physiological pathways also controlling the production of testosterone and corticosterone, two hormones known to be associated respectively to high level of aggressiveness and stress behaviours. Social control can also insure honesty from melanin-based signals, which have been shown to be often involved in intra-sexual and social selection as badges of status (Badyaev and Hill 2000; Senar 2006; Tibbetts and Safran 2009). Pigmented-based colouration of feathers may also involve other pigments such as porphyrins, pterins or psittacofulvins. The codeposition of different pigments can also produce colouration, limiting the ability to discriminate among the

type of pigmented-colourations as recommended by Badyaev and Hill (Badyaev and Hill 2000). For instance, the olive-coloured plumage of European greenfinches (*Carduelis chloris*) (Figure 1A) is the result of codeposition of yellow xanthophyll (carotenoid) pigment and brown melanin (McGraw 2006a).



Figure 1: Illustrations of plumage colouration. A/ The olive-coloured plumage of the European goldfinch (*Carduelis chloris*) is due to the codeposition of carotenoid and melanin. B/The blue plumage of the Eastern bluebird (*Sialia sialis*) is due to structural colouration. C/ The yellow plumage of the American goldfinch (*Carduelis tristis*) is a combination of structural and carotenoid-based colouration. Licenced-free pictures

Structural colouration is a mechanism depending on the selective scattering of certain wavelengths of light through barb and barbule nanostructures (Eliason et al. 2015). In the case of non-iridescent structural colouration, blue (Figure 1B), violet, green and ultraviolet hues are produced by the coherent scattering of light through the spongy medullary keratin of barb feathers (Prum 2006). Structural colourations are thought to be condition-dependent due to the variation in the development of nanostructures. Several studies in different species have established a link between mate choice and structural colouration (Hill 2006) and few as a badge of status (Midamegbe et al. 2011). However, to date the condition-dependence of structural colouration has been rarely tested experimentally (but see Shawkey et al. 2003; Doutrelant et al. 2012). Pigment-based colouration and structural colouration can also interact to produce plumage colouration. For instance, in the American goldfinch (*Carduelis tristis*) (Figure 1C), yellow is obtained by the interaction between carotenoid deposition and structural colouration (acting in the yellow and the UV-peak) (Shawkey and Hill 2005). Then again, working on the different types of colouration as recommended by Badyaev and Hill (2000) may be difficult and biased.

2. Quantifying colourations

Easiness for humans to discriminate among colouration has made plumage colouration a classical model for the evolution of ornaments (Badyaev and Hill 2003). Historically, brightness, colour or sexual dichromatism were assessed with human vision (Figuerola and Green 2000; Dunn et al. 2001; Biard et al. 2009; Rubenstein and Lovette 2009); colouration was sometimes also assessed based on Munsell colour chips (Jawor et al. 2003). Even recently, some key studies relied on human perception of bird colouration (Dale et al. 2015) or encouraged the use of human vision to assess sexual dichromatism (Seddon et al. 2010). Nonetheless, a better understanding of avian vision and the use of spectrophotometry in the 1990s revealed birds have four single-cone photoreceptors when humans have only three. This discovery led to two major differences between avian and human visual performances: birds can see in ultraviolet wavelengths and their rate of discriminating among subtle colour variation is different from humans (Cuthill et al. 2000; Hastad and Odeen 2008) (Figure 2). The use of spectrometer with light enabling the measure of reflectance including ultraviolet wavelengths (300-400 nm) became widespread, offering opportunities to quantify plumage colouration more correctly.

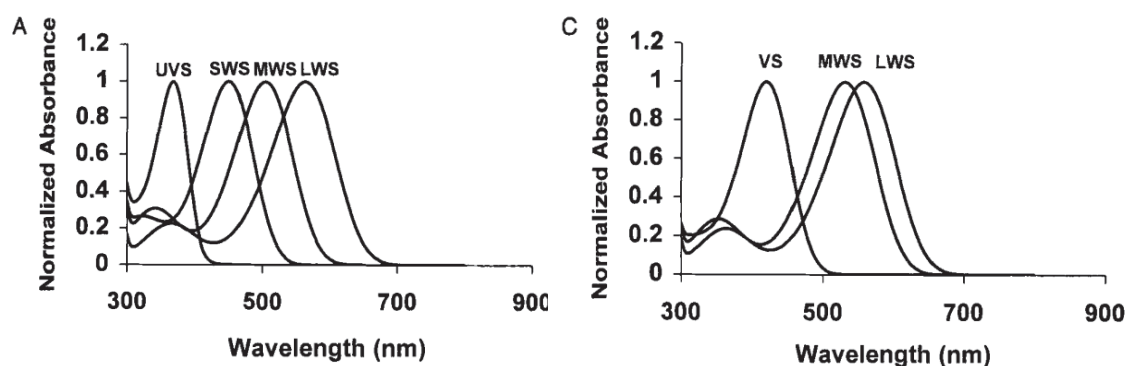


Figure 2 Absorbance spectrum template representing visual pigments in the single-cone photoreceptors compared in A/ European starling (*Sturnus vulgaris*) and C/ Human (*Homo sapiens sapiens*). UVS= ultraviolet sensitive; SWS= short wavelength sensitive; MWS= medium wavelength sensitive; LWS= long wavelength sensitive, VS= Violet sensitive. Range of wavelength perception in birds for each single-cone is different compared to humans, in which the medium-wavelength-sensitive single cone is largely overlapping the long-wavelength-sensitive single-cone to enable subtle discrimination between green and red. Adapted from figure 1 in Cuthill et al. published in *Advances in the Study of Behaviour* in 2000.

The use of complete reflectance spectrum in the bird-visible range offers many ways and indices to quantify colouration. The main indices used are brightness, hue and saturation (also

called chroma) (Figure 3). A standardized index of brightness, comparable among species, is the sum of all the percentage reflectance values (one value for each wavelength) in the spectral region of interest (i.e. 300 nm – 700 nm covering ultraviolet to long wavelengths) divided by the number of values summed. A common index of hue is calculated as the wavelength at which reflectance is the highest; this index can be difficult to use with relatively flat spectra, because index of hue can largely fluctuate over the spectral region. Finally an index of saturation can be the ratio between total reflectance in a region of interest (for instance 300-400 nm if ultraviolet is the region of interest) and total reflectance over the spectral region (Montgomerie 2006).

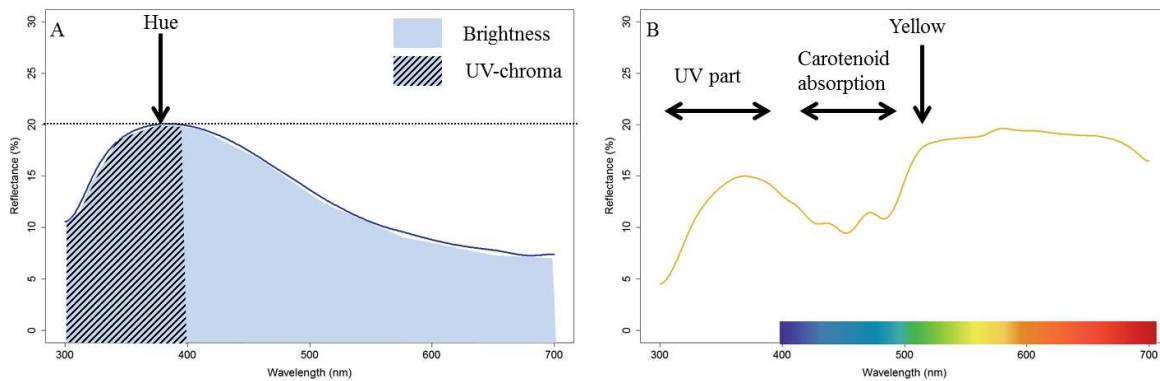


Figure 3: Representation of spectra and indexes of colouration. A/ Mean spectrum from blue colouration of the blue crown in Blue tit (*Cyanistes caeruleus*). The blue area (including the hatched area) represents the index of brightness. The hatched area represents the index of chroma in the ultraviolet region. The arrow represents the value where reflectance is maximal (Hue). B/ Mean spectrum from yellow colouration of the yellow chest in Blue tit. The arrows represent the different parts of plumage colouration (UV+yellow). Spectra realised with package *pavo* (Maia et al. 2013) in R

Since colouration can act as a signal aimed at a receiver, it is also very interesting to consider the way the receiver perceives the signal. From emission to reception, four filters need to be included: the effect of the ambient light (type of light, filters before light reaches the signal such as canopy), the reflectance of the signal (i.e. the way it is emitted), the transmittance to the receiver (filters between the emitter and the receiver, for instance fog can be a strong filter for the transmittance to the receiver) and the receiver sensitivity (for instance, if the signal emits partly in ultraviolet and the receiver is human, the receiver is not able to perceive the ultraviolet part of the signal) (Endler 1990). Visual models have been proposed as a tool to consider these different filters. They are usually drawn in a tetrahedral colour space (Goldsmith 1990) in the case of the four single-cone sensitivity of birds (Vorobyev and Osorio 1998; Endler and Mielke 2005; Hastad and Odeen 2008). The development of software and packages in R (Maia et al. 2013)

have made visual models more widespread nowadays (Doutrelant et al. 2016; Maia et al. 2016 for recent examples) and several indexes of colouration can be extracted. For instance, average plumage chromaticity (Figure 4A) refers to the calculation of the mean Euclidian distance from the achromatic centre (colour space area where the four cones receive equal stimulation) (Maia et al. 2016) and expressed the intensity of colouration. Colour volume (figure 4B) refers to a calculation of the space used in the tetrahedral colour space, expresses colour diversity in bird plumage and gives an idea of contrasts; different colours stimulate differently the four cones leading to higher dispersion in the colour space, increasing colour volume value (Renoult et al. 2015).

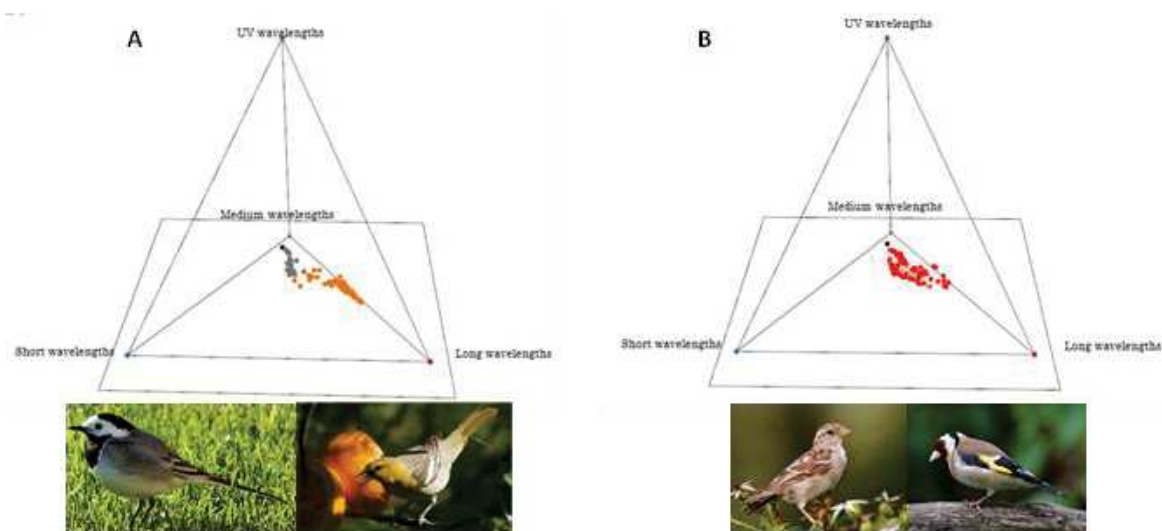


Figure 4: Tetrahedral visual colour space with each colour dot representing each spectrometric measure in a given bird and its place in the colour space. Black dot represents the achromatic centre, where every single-cone photoreceptor is equally stimulated. A/ representation of “plumage chromaticity” with measures taken in two species of birds. Grey dots represent measurements in the female Grey wagtail (*Motacilla alba*) (on the left) while orange dots represent measurements in the female Bullock’s oriole (*Icterus bullockii*) (on the right). All points measured in the orange and brown Bullock’s oriole are further from the achromatic centre compared to points measured in the black and white Grey wagtail. B/ representation of “colour volume” with measures taken in two species of birds. Brown dots represent measurements in the female House sparrow (*Passer domesticus*) (on the left) while red dots represent measurements in the female European goldfinch (*Carduelis carduelis*) (on the right). The volume occupied by brown dots from brownish and rather uniform House sparrow is less important than the volume occupied by red dots from varied-coloured European goldfinch. Tetrahedral colour space realised with package *pavo* in R (Maia et al. 2013). Licenced-free pictures.

CHAPTER II

INTERSPECIFIC LEVEL: THE USE OF THEORETICAL MODELS TO TEST THE IMPACT OF MALE MATE CHOICE ON FEMALE ORNAMENTS



Pictures of female birds used in the comparative study. Female Western bluebird (*Sialia mexicana*) on top left; female Red-breasted Nuthatch (*Sitta canadensis*) on top right; female European bullfinch (*Pyrrhula pyrrhula*) on bottom left; female Varied thrush (*Ixoreus naevius*). Free-licenced pictures.

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MS1: Paternal care and reproductive costs drive the evolution of female ornamentation. Fargevieille Amélie, Arnaud Grégoire, Doris Gomez and Claire Doutrelant. *Last corrections before submission in Nature Letters*

1 **Paternal care and reproductive costs drive the evolution of female ornamentation**

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5 6 **Summary**

7 Female ornamentation has long been considered as a by-product of the genetic correlation with
8 male ornamentation, restrained by the strength of natural selection on female conspicuousness¹.
9 Recent comparative analyses however showed a clear role for social selection in females² and
10 tested a possible role for intersexual selection^{3,4}. To determine how direct sexual selection could
11 favour the evolution of female ornamentation, we tested the effects of factors identified by
12 theoretical models as drivers for the evolution of male mate choice^{5,6}. Our comparative analyses -
13 based on the spectrophotometric measurements of 133 songbird species of 17 close-related
14 families - shows that paternal care favours the occurrence of female ornamentation and,
15 importantly, this effect is found to interact with reproductive costs. First, concerning colour
16 intensity, direct female colour evolution is possible in presence of increasing paternal care when
17 females do not invest too much in egg production. Second the effect of parental care on female
18 colour contrast evolution is also positive when both sexes are very vulnerable to predation.
19 Overall our study clearly validates the predictions of theoretical models showing that paternal
20 care explains the evolution of female ornamentation through male mate choice and points a
21 central role of the cost of reproduction for both sexes.

22

23 **Letter**

24 Sexual selection has wide consequences on the evolution of phenotypes and the adaptability of
25 species⁷. Late twentieth-century researches showed that intra-sexual selection (competition for
26 access to females) and intersexual selection (female mate choice) are major evolutionary
27 processes favouring the evolution of male ornamentation⁸. By contrast the factors at the origin of
28 female ornamentation still need to be confidently assessed to fully understand the action of sexual
29 selection and its consequences on adaptation. While the potential effect of genetic correlation is
30 central³, the use of modern phylogenetic comparative methods reveals a larger lability in the
31 evolution of female ornaments pointing the need to consider the effect of other factors than
32 genetic correlation¹⁰ and suggesting the evolution of ornaments may be more complex in females
33 than in males⁶.

34 Two recent comparative studies have tested the potential role of intersexual selection^{3,4} on the
35 evolution of female ornamentation using broad proxies mixing several processes such as
36 qualitative estimates of paternal care, mating system and sexual dimorphism. To determine the
37 importance of male mate choice¹² shown to occur in nature^{11, 14}, precise proxies testing one
38 process at a time are needed.

39 We can assess the role of male mate choice on the evolution of female ornaments with proxies of
40 some key factors predicted by theoretical models to favour and maintain the evolution of male
41 mate choice^{5,6}. Two central factors are “time out of mating” (i.e. time spent in processes related
42 to mating) and “cost of reproduction for females”. “Time out of mating” reduces male
43 opportunity to breed with several mates and favours the evolution of male mate choice⁵. “Cost of
44 reproduction for females” reduces female probability to invest too much in signalling because
45 their initial investment in reproduction is higher and determinant for reproductive success¹³.
46 These factors can be captured respectively by male investment in parental care¹⁴ and female

47 initial investment in reproduction¹⁵. A last, the “encountering rate”¹⁶ is also an important factor
48 even more difficult to quantify. When this rate is high, the cost of rejecting a potential mate
49 without finding a new one rapidly decreases favouring the evolution of male mate choice.
50 Maximal breeding density¹⁷ can be used as a proxy for the “encountering rate” but this proxy can
51 also be related to more intense intra-sexual competition¹⁸ conducting female ornamentation
52 towards a limit when cost of competition due to high densities exceeds benefits of
53 ornamentation^{19,20}.

54 We ran phylogenetic generalised least squares (PGLS) models²¹ to test whether these proxies are
55 predictors of interspecific female colour variation. To capture the complexity of the evolution of
56 female ornamentation, we also included in our analyses two-way interactions between the main
57 proxies defined above and covariables outlining the effect of other factors already known to
58 affect the evolution of ornaments: genetic correlation, vulnerability to predation (a cost of
59 reproduction in both sexes), body size and breeding duration (see Methods). We excluded the
60 potential effects related to tropical life and enhanced female competition associated with
61 cooperative breeding^{2,3} by restricting our species sample (see Methods). We used a spectrometer
62 to measure the whole adult breeding plumage colouration of male and female specimens selected
63 in museum^{4,20}; considering that conspicuous colour patches could be located anywhere on the
64 body. Bird vision of colour was also considered as we defined a visual model based on avian
65 vision and extracted two colour components: average plumage chromaticity (called “chroma”
66 afterwards) and colour volume, which reflected the two sides of colour conspicuousness,
67 respectively colour intensity and colour contrast²² (see Methods).

68 For both female colour variables, we first found a strong positive effect of male colouration
69 highlighting the prevailing role of phenotypic correlation between female and male colouration
70 (Figure 1a-b; Figure 2a-b). This confirms correlation between sexes is a key factor to consider

71 when working on plumage colouration in one sex, highlighting genetic correlation between sexes
72 can limit sex-specific ornamentation evolution. However, other factors independent of genetic
73 correlation can also reinforced the phenotypic correlation, such as common diet or mutual mate
74 choice²³.

75 An interaction between male contribution to parental care and female initial reproductive
76 investment in reproduction was the other model term explaining female chroma variation (Figure
77 1a). As predicted by theoretical models⁵ for low female investment, female chroma increased
78 when male contribution to parental care increased (Figure 1c). However, when female initial
79 investment was very high, there was on the contrary a decrease of female chroma when male
80 contribution increased. Producing ornaments is often costly in both sexes²⁴ and trade-offs
81 between female investment in offspring and female investment in ornaments have been predicted
82 by theoretical models^{6,25} and found by some empirical studies¹⁰. Our results confirm for the first
83 time this trade-off at the interspecific level, favouring the potential for intensity of colouration to
84 be expressed in species where females invest little at egg stage and males offer a larger
85 contribution to parental care.

86 Besides the strong positive effect of male colouration on female colour volume, male
87 contribution to parental care interacting with vulnerability to predation (Figure 2a) was the
88 second model term explaining female colour volume variation. The result showed that the effect
89 of paternal care was overridden by vulnerability in most conditions with female colour volume
90 decreasing when vulnerability to predation increased. This case confirms former comparative
91 analyses testing only the role of this variable²⁶, highlighting the predominate effect of natural
92 selection on female colouration. However, when male investment in reproduction was high
93 (Figure 2c), i.e. when male contribution to parental care nearly equalled female contribution,
94 female colour volume on the contrary increased with vulnerability to predation. Sexual selection

95 may then counter-balance the effect of natural selection when male invests a lot in reproduction
96 and has extra cost due to predation.

97 The model also retained two other less pronounced predictors affecting female colour volume
98 (Figure 2a). There was a negative quadratic effect of breeding density and a positive effect of
99 female initial investment in reproduction (a). The effect of breeding density was in line with our
100 prediction on “encountering rate” for the lower score of maximal breeding density; female colour
101 volume increased when maximal breeding density increased (Figure 2d). By contrast, for the
102 highest scores of breeding density, female colour volume decreased when maximal breeding
103 density increased. Maximal breeding density may express the risk of rejecting a potential mate as
104 well as the effects of sociality and we cannot dissociate these effects here. The effect of female
105 initial investment in reproduction was contrary to our prediction and showed an increase in
106 female colour volume when initial investment in reproduction was higher (Figure 2e).

107 To sum up, by testing for the first time the predictions of theoretical models on male mate choice
108 at the interspecific level with comparative methods, we showed the crucial role of both paternal
109 care and costs of reproduction on direct female colour evolution. These complex interactions of
110 factors might explain the higher lability in ornament evolution observed in females compared to
111 males⁹. Our results contribute altogether to confirm the necessity to progress towards a change in
112 the paradigm on the origin and evolution of female ornaments^{11,12} despite the predominant role of
113 male colouration. They also contribute to a more balanced and more realistic view of sexual
114 selection. As sexual selection is intrinsically related to individual fitness and the adaptability of
115 populations²⁷, this claims for the need of new research integrating these dimensions of mutual
116 mate choice and female signalling in theoretical models and empirical studies looking at the
117 population consequences of sexual selection.

118

119 **Data accessibility**

120 This section will provide the Dryad DOI if the manuscript is accepted.

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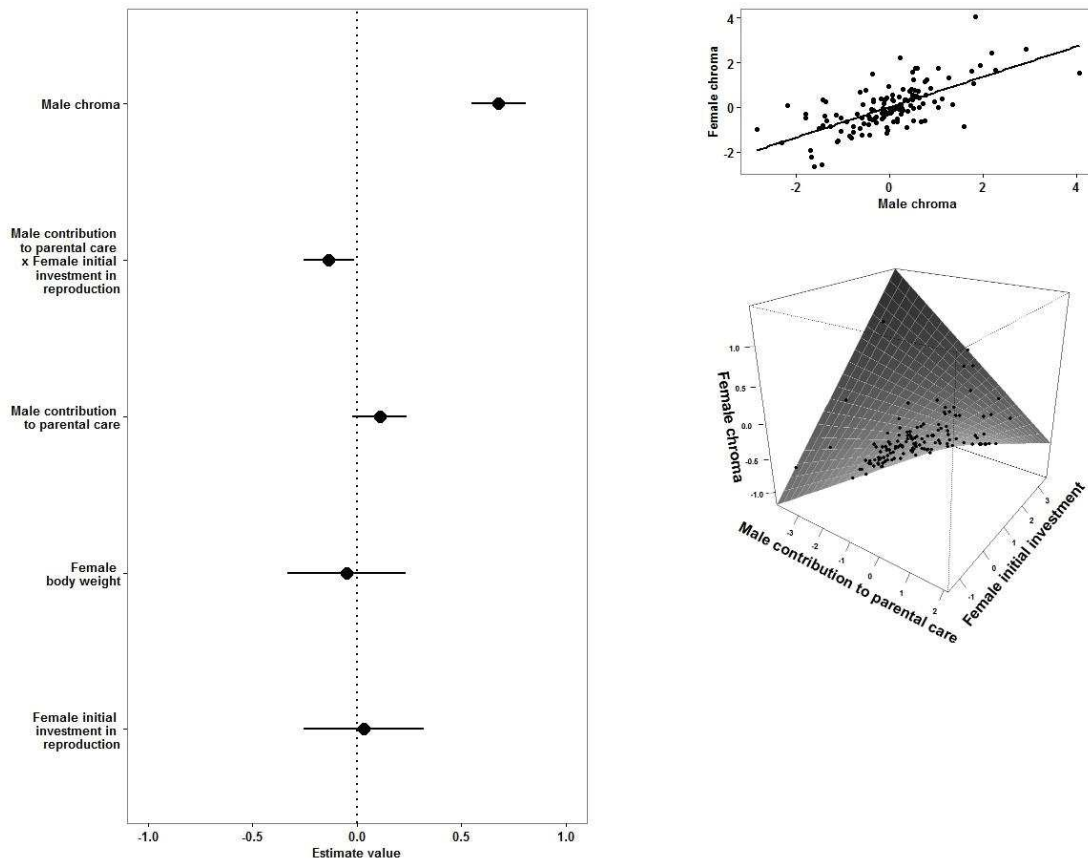


Figure 1 Female chroma model. 1a- represents a forest plot of the mean estimates with their 95% confidence interval for each predictor retained in the female chroma final model, the dotted line representing an estimate value of zero. 1b represents the correlation between male chroma and female chroma with dots representing raw values and the black line representing mean estimate of the correlation. 1c represents the surface of the two-way interaction between male contribution to parental care and female initial investment, dots representing individual projections.

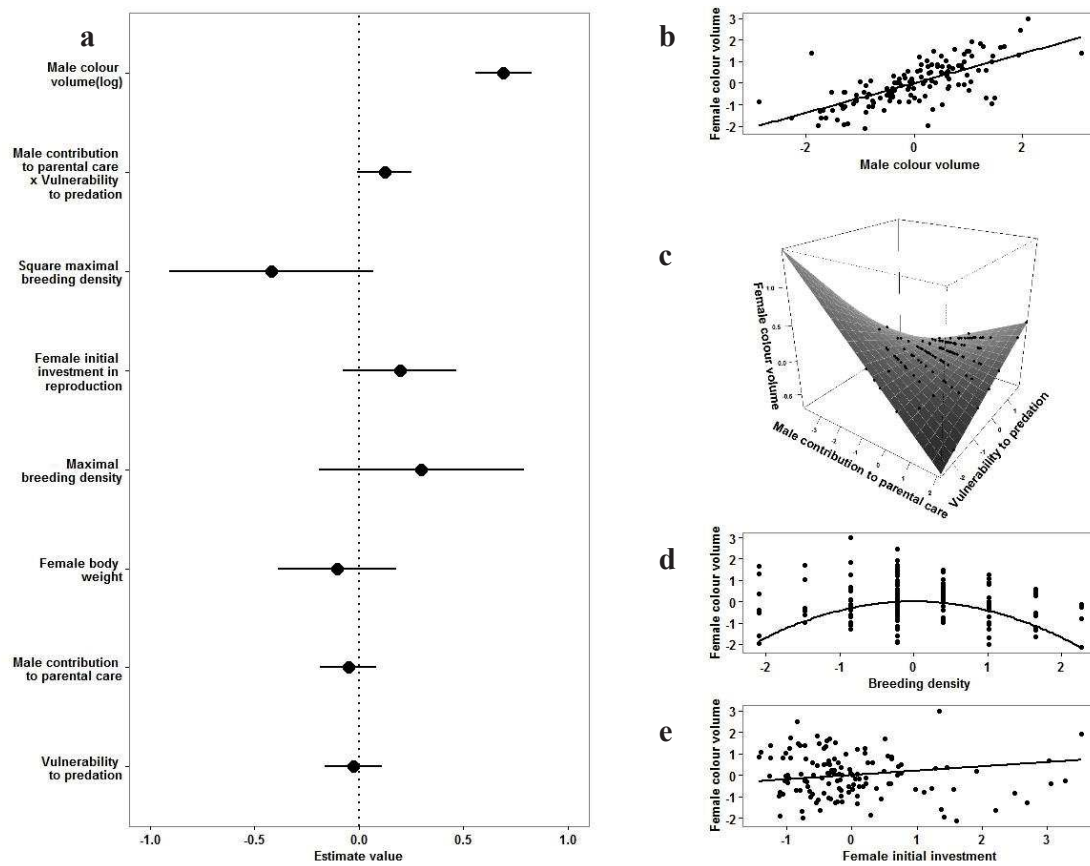


Figure 2. Female colour volume model. 1a- represents a forest plot of the mean estimates with their 95% confidence interval for each predictor retained in the female colour volume final model, the dotted line representing an estimate value of zero. 1b represents the correlation between male colour volume (log) and female colour volume (log) with dots representing raw values and the black line representing mean estimate of the correlation. 1c represents the surface of the two-way interaction between male contribution to parental care and vulnerability to predation, dots representing individual projections. 1d represents the negative quadratic effect between female colour volume (log) and maximal breeding density with dots representing raw data and the black line representing the quadratic effect. 1e represents the positive correlation between female initial investment in reproduction and female colour volume (log) with dots representing raw data and the black line representing the linear correlation.

Methods

We worked on songbirds from close-related families (see ESM Table S1 for complete list). Our choice was motivated by 1) the amount of variation in male and female colouration in those families visible in the Handbooks of the Birds of the Worlds²⁸ (see ESM Figure S1), 2) a visual system close to blue tit visual system^{29,30}, limiting potential bias in colouration estimation, 3) the presence of variation in the focal life-history traits. We restricted our sample to 1) species breeding in temperate zones of North America and Europe to avoid latitudinal effect on plumage colouration³¹, 2) continental species to avoid an effect related to island syndrome²⁰ and 3) species showing no cooperative breeding to avoid the effects of increased social competition and reproductive skew^{2,3}.

Life-history traits were extracted from literature^{32,33}. Male contribution to parental care (“time out of mating” proxy) was computed for three different stages of parental care: incubation (I), chick rearing (brooding and feeding) (R) and post-fledgling (F). Due to the lack of information in many species, male feeding female during incubation and brooding was not considered. We used the method developed by Webb et al. 2010¹⁴ to calculate the proportion of contribution and thus computed it as follow

$$P_{I\text{male}} = \frac{\sum \text{male contribution to incubation}}{\sum \text{male contribution to incubation} + \sum \text{female contribution to incubation}}$$

$$P_{T\text{male}} = \frac{P_{I\text{male}} * t_I + P_{R\text{male}} * t_R + P_{F\text{male}} * t_F}{t_T}$$

Where $P_{T\text{male}}$, $P_{I\text{male}}$, $P_{R\text{male}}$, $P_{F\text{male}}$ refer respectively to proportion of male contribution to “total parental care” (T), “incubation” (I), “chick-rearing” (R) (brooding and chick feeding) and “post-fledgling” (F) and t_T , t_I , t_R , t_F refer respectively to duration (in days) of “total parental care”

(T), “incubation” (I), “chick-rearing” (R) and “post-fledgling” (F) with $t_T = t_I + t_R + t_F$. When available, we used actual proportion of contribution; when described with words, we used a rule of thumb to score the proportion of contribution by rating adverbs and equivalent qualifying male and female contribution (rarely, often, female-only, etc., see details in ESM table S2). Median activity duration was used when mean activity duration was not available. When possible we used information corresponding to the subspecies used, otherwise we used information for main subspecies. In all species used, female total contribution to parental care was equal or higher than male total contribution. There was no species with male-only parental care behaviour, avoiding this particular case of reverse sexual selection which already received large attention.

Clutch volume (mm^3) was used to assess female initial investment in reproduction (“female cost of reproduction” proxy) as the product of egg volume by clutch size¹⁵. This offers the possibility to consider both strategies of female investment in reproduction: large clutch size or large eggs. Mean clutch size was used only when median clutch size was not available. Egg volume (mm^3) was calculated as Hoyt’s formula³⁴ as $\text{egg volume} = 0.509 * (\text{egg length}) * (\text{egg width})^2$. Since we used close-related species of songbirds, we assumed no problem related to a potential asymmetric shape of eggs.

To assess maximal breeding density (“encountering rate” proxy), we established scores based on Bennett and Owens work¹⁷; since they were working at larger taxonomic scales, we rearrange scores to have a better-fitted distribution. Minimal distance (m) between nests was used when maximal number of nests per hectare was not available (see ESM Table S3). Breeding density was also squared to test a quadratic relation with female colouration, allowing the possibility to test the negative effect of competition on female colouration when density is very high.

We set up a score to account for vulnerability to predation. Three main ecological factors were considered to affect species vulnerability to predation: migratory behaviour, openness of habitat, and two characteristics describing nest factor: the height of nest placement and the openness of the nest. For each characteristic, we assigned a score from 0 to 2 from lower to higher vulnerability³⁵. We then summed the four scores and obtained a general score varying between 0 and 8 (see ESM Table S4). Since there were two characteristics for nest component and one for each of the two other factors, nest component had a larger weight in the final score of vulnerability, accounting for the higher risk of predation related to nest attendance.

Total breeding period duration (in days) was also added as a covariable since it may influence investment in reproduction, time out of reproduction, encountering rate and thus plumage colouration³⁶. It was calculated as the product between single-brood duration and the mean number of broods per breeding period. Total breeding period duration was log-transformed to fit a normal distribution.

Stuff specimens of adults in breeding plumage were selected (by AF and DG) from the National Museum of Natural History collections in Paris. We only selected individuals where sex, adult status, subspecies and region were specified. If there was no mention of subspecies but region was specified, we verified in the Handbooks of the Birds of the World²⁸ subspecies correspondence to the region; if there were more than one subspecies present in the region leading to doubts on subspecies, we did not select the specimen. When possible, we selected males and females from the same locality (see ESM Table S1). When not possible, we selected males and females of the same subspecies coming from the closest localities as possible. We also restricted our sample to individuals collected on the continent because populations from islands may be affected by island syndrome²⁰.

We selected 267 specimens (134 males and 133 females, see ESM Table S1) for 133 species. We selected one male and one female from each species, focusing on best-conserved birds. Since humans and birds class colouration differently, it is highly recommended to use spectrometry³⁷. Former analyses have shown a good reliability of spectrometry analyses of plumage colouration on well-conserved stuff specimens compared to fresh specimens³⁸. Plumage colourations were measured in males and females by the same person (AF) using Jaz spectrometer (Ocean Optics, Dunedin FL, USA) with Jaz light source covering 300 nm to 700 nm and a 200 μm fibre conducting light at a 45° angle to feathers and held at a fixed distance of 2 mm from plumage. The reflectance of white standard was controlled between specimen measurements.

We allowed the possibility for colouration to be expressed anywhere on the plumage. We thus defined 21 topographic zones, which were measured in each bird to have an estimate of colouration on whole body (see ESM figure S2). When a bird presented different colours in one of these 21 zones (i.e. if it presents different colour patches or a patch composed of different colours; based on human vision and verified with spectrum shape) we took a measure for each colour (up to four different colours for one zone were found in some species); we always took the same amount of measures in the other sex, even when there were less colours. This leads to a number of measures varying from 22 to 45 depending on the species (see ESM Table S1) with an average number of 33 measures per bird. We tested the repeatability by measuring three times each colour of some specimens (see ESM Table S1).

Colour measurements were extracted using R³⁹ package *pavo*⁴⁰. For specimens where three measures were taken for each colour patch, we calculated mean spectra after controlling for concordance in the shape of each spectrum. We used ideal Goldsmith's tetrahedral colour space and blue tit visual system^{41,42} to build our visual model. Average plumage chromaticity was

calculated as the mean Euclidian distance from the achromatic centre (colour space area where the four cones receive equal stimulation)⁴³. Average plumage chromaticity (“chroma”) expressed the intensity of colouration. We also calculated colour volume, which expressed colour diversity in bird plumage; different colours stimulate differently the four cones leading to higher dispersion in the colour space, increasing colour volume value²². We finally calculated mean distance between all colour pairs⁴² as indication of contrasts but since it was highly correlated with colour volume ($r_{\text{Pearson}}=0.77$ with [0.68-0.83] 95% confidence intervals for correlation between female colour volume and female contrasts), we omitted it from further analyses. Chroma and colour volume were mildly correlated ($r_{\text{Pearson}}=0.40$ with [0.25-0.54] 95% confidence intervals for correlation between female chroma and female colour volume). Male and female colour volumes were log-transformed to fit a normal distribution.

All predictors were standardised to a mean of zero and a unit variance. We tested multicollinearity between predictors using R package *yhat*⁴⁵. Apart from a high correlation between female body weight and female initial investment in reproduction, there was very low multicollinearity among our predictors (see ESM Table S5). Since female body weight was retained as a proxy for body size effect on female plumage colouration³, we decided to keep it as an independent predictor in our model; to account for their collinearity, we kept female body weight predictor in the model selection whenever female initial investment predictor was retained.

When performing comparative methods, it is important to consider potential non-independence among phylogenetically-related species⁴⁶. With our species list (see ESM Table S1), we extracted 500 random phylogenetic trees (dryad source tree 2481), using Hackett Backbones from birdtree.org website^{47,48}. Phylogenetic trees were extracted using R package *ape*⁴⁹. We first ran a backward stepwise model selection using phylogenetic general least-squared analysis (PGLS)²¹

with R packages *phytools*⁵⁰ and *nlme*⁵¹ on a consensus tree, estimating Pagel's lambda phylogenetic signal in the residual error simultaneously to running the models⁵². From the complete model, we only retain predictors with a p-value inferior to 10% (see ESM table S6). Since female body weight and female initial investment in reproduction were correlated, we retained “female body weight” predictor in our final model although it was non-significant. We ran PGLS analyses on both final models (female chroma and female colour volume) on each of the 500 phylogenetic trees and estimated simultaneously Pagel's lambda associated to residual error for each of the 500 trees and extracted Pagel's lambda mean value and the 95% confidence intervals associated (average lambda of 0.338 with [0.330-0.347] 95% confidence interval for female chroma model and average lambda of 0.545 with [0.543-0.547] 95% confidence interval for female colour volume model). Using R package *caper*⁵³ we ran again PGLS analyses on the 500 trees for both final models, defining Pagel's lambda associated to residual error as the average value obtained before. The latter analysis was performed to account for phylogenetic uncertainty⁵⁴; we used model-averaging procedure⁵⁴ based on AIC weight associated with each tree and calculated the estimate value and 95% confidence intervals associated with each of the predictors retained in our final models (see ESM Table S7).

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Table S1. List of bird species used in analyses. Scientific names correspond to names used in birdtree.org. “Collection locality” refers to the locality where the bird was collected (Locality, State and Country). “Number of measurements” refers to the total number of measures taken on the bird with “*3” indicating measures have been taken three times. (1) Two specimens of male *Myadestes townsendi* were selected; the second one was measured on the zones stained on the first specimen.

Scientific name	Male		Female	
	Collection locality	Number of measurements	Collection locality	Number of measurements
<i>Agelaius phoeniceus</i>	Auburn, NY USA	38	Rochester, NY USA	38
<i>Aimophila ruficeps</i>	Dulzura, CA USA	33	Dulzura, CA USA	33
<i>Amphispiza bilineata deserticola</i>	Tucson, AZ USA	28	El Rosario, MX	28*3
<i>Quiscalus mexicanus</i>	Chimalapa, MX	23	Tlatizapan, MX	23
<i>Anthus pratensis pratensis</i>	Plougasnou, FR	36	Plougasnou, FR	36
<i>Anthus spinoletta spinoletta</i>	Urdos, FR	27	Saint-Jean-de-Luz, FR	27
<i>Xanthocephalus xanthocephalus</i>	Silver Lake, MI USA	25	Bixby, MO USA	25*3
<i>Bombycilla garrulous garrulous</i>	Uppsala, SE	28*3	Uppsala, SE	28
<i>Miliaria calandra</i>	Pons, FR	37	Jonzac, FR	37
<i>Cardinalis cardinalis coccineus</i>	MX	27	Chimalapa, MX	27
<i>Carduelis cannabina cannabina</i>	Lion-sur-Mer, FR	38	Le Puy-Notre Dame, FR	38
<i>Carduelis carduelis carduelis</i>	Eure-et-Loir, FR	35	Andeville, FR	35*3
<i>Carduelis chloris chloris</i>	Plougasnou, FR	27*3	Remomeix, FR	27
<i>Carduelis flammea flammea</i>	Armentières-en-Brie, FR	43	Le Mage, FR	43
<i>Carduelis pinus</i>	Auburn, NY USA	39	Auburn, NY USA	39
<i>Carduelis psaltria</i>	Dulzura, CA USA	25	Dulzura, CA USA	25
<i>Carduelis spinus</i>	Vannes, FR	39	Remomeix, FR	39
<i>Carduelis tristis</i>	Auburn, NY USA	24*3	Kamouraska, QC CA	24
<i>Carpodacus mexicanus</i>	Dulzura, CA USA	37	La Paz, MX	37
<i>Carpodacus purpureus purpureus</i>	Ithaca, NY USA	37	Ithaca, NY USA	37
<i>Catharus guttatus</i>	Reading, NY USA	27	Jackson County, MI USA	27
<i>Catharus ustulatus ustulatus</i>	Los Angeles, CA USA	26	Long Beach, CA USA	26
<i>Emberiza citrinella citrinella</i>	Plougasnou, FR	42	Noisiel, FR	42
<i>Certhia familiaris macrodactyla</i>	Saint-Cassin, FR	39	Lucéram, FR	39
<i>Certhia brachydactyla megarhynchos</i>	Le Puy-Notre Dame, FR	38	Le Puy-Notre Dame, FR	38
<i>Cinclus cinclus cinclus</i>	Condat-sur-Vienne, FR	28	Solignac, FR	28
<i>Cistothorus palustris palustris</i>	Auburn, NY USA	34	Cayuga, ON CA	34
<i>Coccothraustes coccothraustes coccothraustes</i>	South-West, FR	32	Tencin, FR	32
<i>Dendroica coronate auduboni</i>	Pasadena, CA USA	33	El Monte, CA USA	33
<i>Dendroica petechia castaneiceps</i>	La Paz, MX	28*3	La Paz, MX	28
<i>Dumetella carolinensis</i>	Ixtacomitan, MX	23	Ixtacomitan, MX	23*3
<i>Luscinia luscinia</i>	Joinville-le-Pont, FR	23	Amani Forest, TZ	23

<i>Emberiza cia cia</i>	Aussois, FR	37	Urdos, FR	37
<i>Emberiza cirius</i>	Trois-Monts, FR	42	Le Puy-Notre Dame, FR	42
<i>Sitta canadensis</i>	Cayuga, OR USA	29	New Haven, CT USA	29
<i>Emberiza hortulana</i>	Cellule, FR	34	Fronsac, FR	34
<i>Emberiza leucocephalos</i>	Baïkal Lake, RU	42	Zaisan-Nor, KZ	42
<i>Emberiza schoeniclus schoeniclus</i>	Saint-Jean-du-Doigt, FR	42	Tonneins, FR	42
<i>Erithacus rubecula</i>	Saix, FR	28	Morsang-sur-Orge, FR	28
<i>Icterus bullockii</i>	California, CA USA	30*3	Dulzura, CA USA	30
<i>Spizella arborea</i>	Auburn, NY USA	35	Rochester, NY USA	35
<i>Ficedula albicollis albicollis</i>	Laconie, GR	29	Laconie, GR	29
<i>Ficedula hypoleuca hypoleuca</i>	Bouillé-Loretz, FR	27	Chaponost, FR	27
<i>Ficedula parva parva</i>	Teheran, IR	24	Teheran, IR	24
<i>Fringilla coelebs coelebs</i>	Brunoy, FR	31	Plougasnou, FR	31
<i>Fringilla montifringilla</i>	Le Crottoy, FR	36	Plougasnou, FR	36
<i>Icterus spurius</i>	Ixtacomitan, MX	29	Ixtacomitan, MX	29*3
<i>Coccothraustes vespertinus vespertinus</i>	Watkins Glen, NY USA	27	Lewis County, NY USA	27
<i>Hylocichla mustelina</i>	Yaxchilan, MX	28	Yaxchilan, MX	28
<i>Sitta carolinensis</i>	Auburn, NY USA	34	Auburn, NY USA	34
<i>Euphagus cyanocephalus</i>	El Monte, CA USA	22	Whittier, CA USA	22
<i>Icterus galbula</i>	Motzorongo, MX	29	Comitan, MX	29
<i>Icterus parisorum</i>	El Triumfo, MX	33*3	El Triumfo, MX	33
<i>Spizella pusilla</i>	Reading, NY USA	29	Wheatland, WY USA	29
<i>Zoothera naevia meruloides</i>	Nicasio, CA USA	30*3	Pasadena, CA USA	30
<i>Junco hyemalis aikeni</i>	Newcastle, WY USA	25	Newcastle, WY USA	25*3
<i>Loxia curvirostra curvirostra</i>	Jurques, FR	37	Pierre-de-Bresse, FR	37
<i>Anthus trivialis trivialis</i>	Remomeix, FR	36	Trois-Monts, FR	36
<i>Luscinia megarhynchos megarhynchos</i>	Saint-Jean-de-Luz, FR	22	Wissous, FR	22
<i>Luscinia svecica cyanecula</i>	Nancy, FR	29*3	Ansauville, FR	29
<i>Melospiza georgiana</i>	Reading, NY USA	40	Reading, NY USA	40
<i>Melospiza lincolnii lincolnii</i>	Chippewa, OH USA	45	Fleming's, OH USA	45
<i>Melospiza melodia</i>	Dulzura, CA USA	40	Dulzura, CA USA	40
<i>Mimus polyglottos leucopterus</i>	MX	25	Los Angeles, CA USA	25
<i>Monticola saxatilis</i>	Tizi n'Taka, MA	39	Mwaktau, KE	39
<i>Monticola solitarius solitarius</i>	Nice, FR	34*3	Marseille, FR	34
<i>Motacilla alba alba</i>	Hendaye, FR	29	Ranville, FR	29
<i>Motacilla cinerea</i>	Rennes-les-Bains, FR	29	Saint-Jean-du-Doigt, FR	29
<i>Icterus cucullatus igneus</i>	Yucatan, MX	29	Mérida, MX	29*3
<i>Oenanthe hispanica hispanica</i>	Douar Zaara, TN	26*3	Redeyef, TN	26
<i>Muscicapa striata striata</i>	Ahetze, FR	27	Trois-Monts, FR	27

<i>Myadestes townsendi</i> (1)	Paradise, AZ USA/ Cochise County, NV USA	25	Silver City, NM USA	25
<i>Chondestes grammacus</i>	Dulzura, CA USA	36	Pasadena, CA USA	36
<i>Oenanthe isabellina</i>	Ito, TD	26	100 km North from Rosso, MR	26
<i>Oenanthe oenanthe oenanthe</i>	Remomeix, FR	34	Biarritz, FR	34*3
<i>Geothlypis trichas</i>	Auburn, NY USA	30	Auburn, NY USA	30
<i>Sitta europaea caesia</i>	Saumur, FR	27	Mouliherne, FR	27*3
<i>Passer montanus montanus</i>	Chamigny, FR	32*3	Saint-Jean-de-Luz, FR	32
<i>Passerculus sandwichensis princeps</i>	South Duxbury, MA USA	38	South Duxbury, MA USA	38
<i>Motacilla flava flava</i>	Saint-Jean-de-Luz, FR	26	Remomeix, FR	26
<i>Passerella iliaca</i>	Auburn, NY USA	36	Reading, NY USA	36
<i>Passerina amoena</i>	Santa Barbara, CA USA	28	CA USA	28
<i>Passerina caerulea</i>	MX	41	MX	41
<i>Passerina ciris</i>	Naha, MX	27*3	Naha, MX	27
<i>Passerina cyanea</i>	Cayuga, TX USA	24	Auburn, NY USA	24
<i>Passerina versicolor</i>	Ixmiquilpan, MX	26	Ixmiquilpan, MX	26
<i>Petronia petronia</i>	Tataouine, TN	37	Tunis, TN	37
<i>Pheucticus ludovicianus</i>	Cayuga, TX USA	37	Naha, MX	37
<i>Pheucticus melanocephalus</i>	CO USA	37	CO USA	37
<i>Phoenicurus ochruros gibraltariensis</i>	Lorraine, FR	26	Le Puy-Notre Dame, FR	26
<i>Phoenicurus phoenicurus phoenicurus</i>	Trois-Monts, FR	28	Lagny-sur-Marne, FR	28
<i>Pipilo maculatus megalonyx</i>	Dulzura, CA USA	27	Dulzura CA USA	27*3
<i>Piranga flava hepatica</i>	La Parada, MX	25*3	Oaxaca, MX	25
<i>Piranga olivacea</i>	Ann Arbor, MI USA	23*3	Lorne Park, ON CA	23
<i>Piranga rubra</i>	Tepic, MX	25	Tepic, MX	25*3
<i>Anthus campestris campestris</i>	Manonville, FR	30	FR	30
<i>Prunella collaris collaris</i>	CH	32	Isgoun Ouagouns, TN	32
<i>Prunella modularis modularis</i>	Trois-Monts, FR	32	Trois-Monts, FR	32
<i>Pipilo crissalis</i>	Pasadena, CA USA	24	Pasadena, CA USA	24
<i>Pyrrhula pyrrhula pyrrhula</i>	Ahetze, FR	26*3	Ahetze, FR	26
<i>Turdus iliacus iliacus</i>	Ahetze, FR	30	Argenton l'Eglise, FR	30
<i>Quiscalus quiscula aeneus</i>	Webster, TX USA	23	Auburn, NY USA	23
<i>Pooecetes gramineus</i>	Rochester, NY USA	38	Rochester, NY USA	38
<i>Regulus ignicapilla ignicapilla</i>	Saint-Pern, FR	33	Saumur, FR	33
<i>Regulus regulus regulus</i>	Remomeix, FR	32	Magny-les-Hameaux, FR	32
<i>Salpinctes obsoletus</i>	Tehuantepec, MX	32	Dulzura, CA USA	32
<i>Saxicola rubetra</i>	Remomeix, FR	40	Solignac, FR	40
<i>Saxicola torquatus rubicola</i>	Meuvaines, FR	39	Meuvaines, FR	39
<i>Seiurus noveboracensis</i>	Auburn, NY USA	28	Rochester, NY USA	28*3
<i>Serinus serinus</i>	Saumur, FR	38	Plougasnou, FR	38

<i>Setophaga ruticilla</i>	Ixtacomitan, MX	26	Ixtacomitan, MX	26*3
<i>Sialia currucoides</i>	Basset, VA USA	25*3	Coachella, CA USA	25
<i>Sialia mexicana</i>	Dulzura, CA USA	27	Dulzura, CA USA	27*3
<i>Sialia sialis fulva</i>	Comitan, MX	28	Comitan, MX	28
<i>Regulus calendula calendula</i>	Kern River, CA USA	28	Pasadena, CA USA	28*3
<i>Erythropygia galactotes galactotes</i>	Rabat, MA	29	Berkane, MA	29
<i>Calamospiza melanocorys</i>	Colorado Springs, CO USA	39	Chihuahua, MX	39
<i>Motacilla citreola</i>	Irkoutsk, RU	27*3	Petchora Valley, RU	27
<i>Sturnella neglecta neglecta</i>	Summer Lake, OR USA	43	Garnsey, OR USA	43
<i>Thryomanes bewickii</i>	Santa Rita Mountains, AZ USA	26	Santa Rita Mountains, AZ USA	26
<i>Toxostoma rufum</i>	Ithaca, NY USA	28	Fort Benning, GA USA	28*3
<i>Troglodytes troglodytes troglodytes</i>	Plougasnou, FR	34	Morlaix, FR	34
<i>Oreoscoptes montanus</i>	Menifee, CA USA	31	Cajon Wash, CA USA	31
<i>Turdus merula merula</i>	Île-de-France, FR	27	Paris, FR	27
<i>Turdus migratorius</i>	Cayuga, TX USA	29	Rochester, NY USA	29
<i>Turdus philomelos philomelos</i>	Bourron-Marlotte, FR	30	Biarritz, FR	30
<i>Turdus pilaris</i>	Le Puy-Notre Dame, FR	32	Orly, FR	32*3
<i>Turdus torquatus torquatus</i>	Pyrénées-Atlantiques, FR	31	Saint-Jean-du-Doigt, FR	31
<i>Turdus viscivorus viscivorus</i>	Aussois, FR	32	Aussois, FR	32
<i>Vermivora celata sordida</i>	Pasadena, CA USA	26	CA USA	26
<i>Passer domesticus domesticus</i>	Signy-Signets, FR	37	Trois-Monts, FR	37
<i>Zonotrichia albicollis</i>	Reading, NY USA	35	CA	35
<i>Zonotrichia leucophrys gambeli</i>	Pasadena, CA USA	30*3	Santa Barbara, CA USA	30

Table S2. Descriptive contribution of male and female in parental care translated into scores

Activity description	Male	Female	Comments
Female only	0	1	
Both	1	1	
Female mostly/mainly/primarily/Greater by female	0.5	1	
Male occasionally/rarely/may helps	0.2	1	
Male higher than female	1	0.5	
Only male if female starts 2d brood	1	1	Female still invested in reproduction even if it is another brood
Both, more male when female broods	1	1	Female still highly invested
Involvement of male varies	0.5	1	
Mainly male at first	1	1	“at first” means female invested in brooding
Some males recorded to cover eggs for considerable periods	0.2	1	Few males, few information on male incubation duration
Female more than male	0.7	1	
Female leaves first	1	0.5	Post-fledgling period
Males may cover eggs briefly	0.2	1	
Observation of male incubating	0.5	1	
More supplies by males	1	0.7	
One observation of male feeding	0.1	1	
Male with small harem; male if fledgling in territory	0.1	1	Very rare situation
<i>Miscellaneous</i>			
"Several weeks" estimated as 21 days			
When information on activity or duration is missing, median value for all species used			

Table S3. Score set up for maximal breeding density with “x” as the maximal number of nests or as the minimal distance (m) for the species

Number nests/ha	Minimal distance between nests (m)	Score
$x < 0.05$ nests/ha	$x > 200$ m	1
$0.05 \leq x < 0.25$	125 m $< x \leq 200$ m	2
$0.25 \leq x < 0.5$	50 m $< x \leq 125$ m	3
$0.5 \leq x < 2.5$	35 m $< x \leq 50$ m	4
$2.5 \leq x < 5$	20 m $< x \leq 35$ m	5
$5 \leq x < 25$	12.5 m $< x \leq 20$ m	6
$25 \leq x < 50$	5 m $< x \leq 12.5$ m	7
$x \geq 50$ nests/ha	$x \leq 5$ m	8

Table S4. Scores set up for vulnerability to predation, from lowest vulnerability to highest vulnerability

Ecological factor	Description	Score	Comment
Habitat	Closed	0	Forests
	Intermediate	1	Edge of forests, riparian habitats, mixed habitats
	Open	2	Prairies, wetlands
Nest position	Tree	0	>2 m
	Shrub	1	50 cm-2 m
	Ground	2	<50 cm
Nest attributes	Closed, hole-nesting	0	
	Semi-open, well concealed	1	
	open	2	
Migratory behaviour	Sedentary	0	
	Partially migrant	1	
	Migrant	2	

Table S5. Coefficients from the commonality analysis performed for each predictor using additive multiple regression models. Each predictor was tested against the six other predictors to explore multicollinearity. “Unique” refers to the variance explained uniquely by the predictor tested, while “common” refers to the variance jointly explained by the association of two or more predictors (multicollinearity). r^2 and r^2_{adj} are the two estimates of the proportion of the variance explained by the full model. Presence of collinearity is highlighted in bold. a/ Multicollinearity exploration for chroma predictor models. b/ Multicollinearity exploration for colour volume predictor models.

a/		Male chroma	Male contribution to parental care	Female initial investment	Maximal breeding density	Vulnerability to predation	Female weight (log)	Total breeding duration (log)
r^2		0.10	0.09	0.79	0.06	0.08	0.78	0.041
r^2_{adj}		0.06	0.04	0.78	0.01	0.04	0.77	-0.005
Male chroma	Unique	-	0.0003	0.012	0.014	0.005	0.003	0.001
	Common	-	0.0005	0.054	-0.008	0.002	0.022	0.004
Male contribution to parental care	Unique	0.0003	-	0.013	0.015	0.004	0.013	0.020
	Common	0.0005	-	-0.010	-0.007	-0.004	-0.009	-0.004
Female initial investment	Unique	0.051	0.058	-	0.023	0.028	0.688	0.005
	Common	0.015	-0.055	-	-0.003	-0.021	0.023	0.007
Maximal breeding density	Unique	0.014	0.014	0.005	-	0.0007	0.001	0.009
	Common	-0.007	-0.006	0.015	-	0.0003	0.010	-0.004
Vulnerability to predation	Unique	0.005	0.004	0.007	0.0007	-	0.015	0.0002
	Common	0.002	-0.004	0.0005	0.0003	-	0.025	0
Female weight (log)	Unique	0.012	0.054	0.672	0.005	0.064	-	0.0004
	Common	0.014	-0.050	0.080	0.006	-0.024	-	0.010
Total breeding duration (log)	Unique	0.001	0.019	0.001	0.009	0.0002	0.0001	-
	Common	0.004	-0.003	0.010	-0.004	0	0.010	-
b/		Male colour volume (log)	Male contribution to parental care	Female initial investment	Maximal breeding density	Vulnerability to predation	Female weight (log)	Total breeding duration (log)
r^2		0.12	0.12	0.79	0.05	0.05	0.78	0.05
r^2_{adj}		0.07	0.08	0.78	0.006	0.006	0.77	0.006
Male colour volume (log)	Unique	-	0.035	0.009	0.008	0.0003	0.002	0.012
	Common	-	0.07	0.045	-0.006	0.0003	0.029	0.015
Male contribution to parental care	Unique	0.035	-	0.018	0.018	0.003	0.014	0.014
	Common	-0.007	-	-0.015	-0.010	-0.003	-0.010	0.002
Female initial investment	Unique	0.037	0.072	-	0.020	0.034	0.691	0.003
	Common	0.017	-0.069	-	-0.003	-0.027	0.059	0.008
Maximal breeding density	Unique	0.007	0.017	0.005	-	0.001	0.001	0.008
	Common	-0.006	-0.009	0.015	-	-0.0001	0.011	-0.003
Vulnerability to predation	Unique	0.0003	0.003	0.008	0.001	-	0.016	0.0002
	Common	0.0003	-0.003	-0.0008	-0.0001	-	0.024	0
Female weight (log)	Unique	0.008	0.058	0.681	0.005	0.068	-	0.0002
	Common	0.023	-0.054	0.070	0.007	-0.028	-	0.010
Total breeding duration (log)	Unique	0.011	0.013	0.0007	0.008	0.0002	0	-
	Common	0.016	0.003	0.011	-0.003	0	0.010	-

Table S6. F-values and p-values associated to predictors retained in the final model. NS means non-significant values that were not retained in the final model. a/ Model to test predictors on female chroma; b/ Model to test predictors on female colour volume (log).

a/ Female chroma	F-value	p-value	b/ Female colour volume (log)	F-value	p-value
Male chroma	105.5	<0.0001	Male colour volume (log)	107.5	<0.0001
Male contribution to parental care	2.82	0.10	Male contribution to parental care	0.010	0.76
Female initial investment	0.13	0.72	Female initial investment	2.94	0.09
Maximal breeding density	NS	NS	Maximal breeding density	4.13	0.04
Square of maximal breeding density	NS	NS	Square of maximal breeding density	3.11	0.08
Vulnerability to predation	NS	NS	Vulnerability to predation	0.02	0.90
Female weight (log)	0.06	0.81	Female weight (log)	0.14	0.71
Total breeding duration (log)	NS	NS	Total breeding duration (log)	NS	NS
Male contribution x Female investment	5.47	0.02	Male contribution x Female investment	NS	NS
Male contribution x Breeding density	NS	NS	Male contribution x Breeding density	NS	NS
Male contribution x Square breeding	NS	NS	Male contribution x Square breeding density	NS	NS
Male contribution x Vulnerability	NS	NS	Male contribution x Vulnerability	3.04	0.08
Male contribution x Total breeding	NS	NS	Male contribution x Total breeding duration	NS	NS
Female investment x Breeding density	NS	NS	Female investment x Breeding density	NS	NS
Female investment x Square breeding	NS	NS	Female investment x Square breeding	NS	NS
Female investment x Vulnerability	NS	NS	Female investment x Vulnerability	NS	NS
Female investment x Total breeding	NS	NS	Female investment x Total breeding duration	NS	NS
Breeding density x Vulnerability	NS	NS	Breeding density x Vulnerability	NS	NS
Breeding density x Total breeding	NS	NS	Breeding density x Total breeding duration	NS	NS
Square breeding density x Vulnerability	NS	NS	Square breeding density x Vulnerability	NS	NS
Square breeding density x Total breeding	NS	NS	Square breeding density x Total breeding	NS	NS

Table S7. Estimate values and 95% confidence intervals associated calculated with multimodel-inference. a/ Values with female chroma as the variable to explain (Pagel's $\lambda=0.339$) and b/ Values with female colour volume as the variable to explain (Pagel's $\lambda=0.545$).

a/ Chroma	Estimate	95% Confidence	Standard-error	b/ Colour volume (log)	Estimate	95% Confidence	Standard-error
Male chroma	0.679	0.551;0.808	0.066	Male colour volume (log)	0.692	0.557;0.826	0.069
Male contribution to parental care	0.109	-0.021;0.239	0.066	Male contribution to parental care	-0.051	-0.187;0.085	0.069
Female initial investment	0.032	-0.257;0.322	0.148	Female initial investment	0.196	-0.077;0.469	0.139
Female weight (log)	-0.051	-0.334;0.231	0.144	Maximal breeding density	0.299	-0.191;0.790	0.250
Male contribution x Female investment	-0.134	-0.256;-0.013	0.062	Squared breeding density	-0.421	-0.909;0.067	0.249
				Vulnerability to predation	-0.026	-0.165;0.112	0.071
				Female weight (log)	-0.106	-0.389;0.178	0.145
				Male contribution x Vulnerability	0.123	-0.009;0.254	0.067

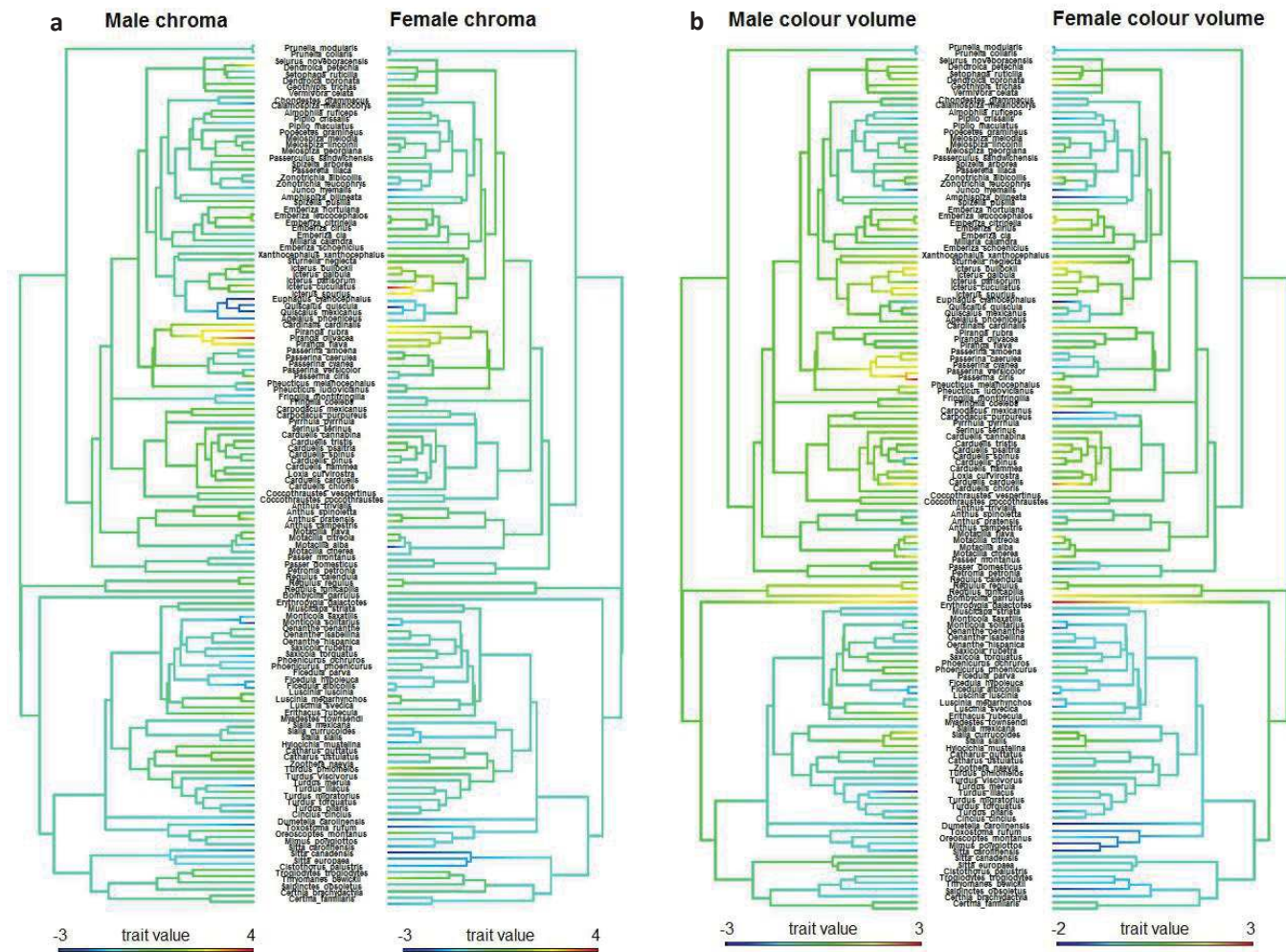


Figure S1. Phylogenetic relations between species used in our analyses. Phylogenetic tree #7833 from birdtree.org, Hackett backbones. Colours refer to trait value of colouration for 1a/ chroma and 1b/ colour volume (log)

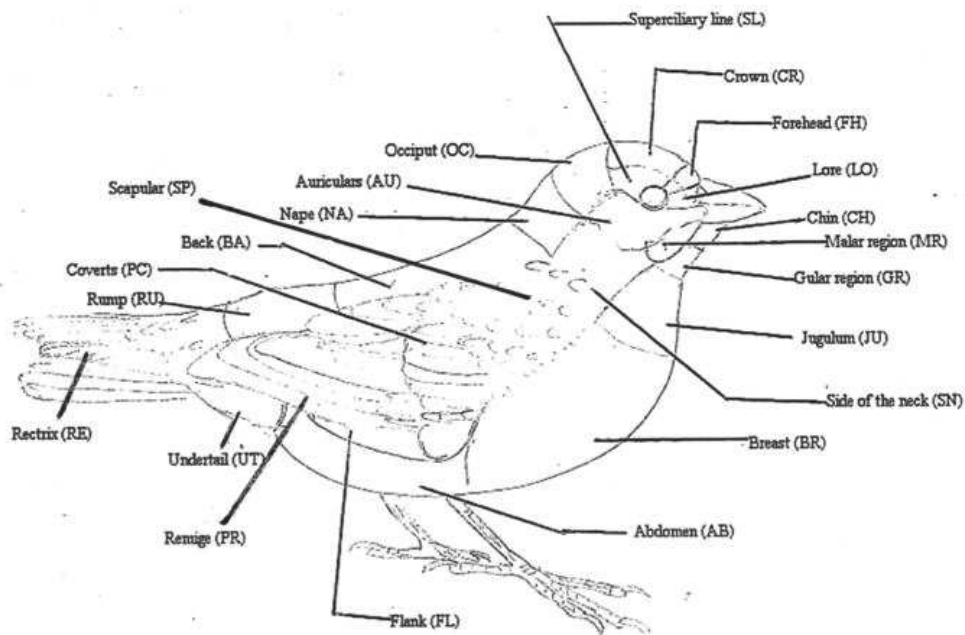


Figure S2. Topography of a house sparrow (*Passer domesticus*) representing the 22 zones defined to measure bird whole body colouration (abbreviation in brackets). The lore zone (LO) was first defined but removed from analyses due to bad conservation in several specimens.

**INSERT 2: THE BLUE TIT (*CYANISTES CAERULEUS*) AS A SPECIES MODEL IN THE STUDY OF
PLUMAGE COLOURATION**

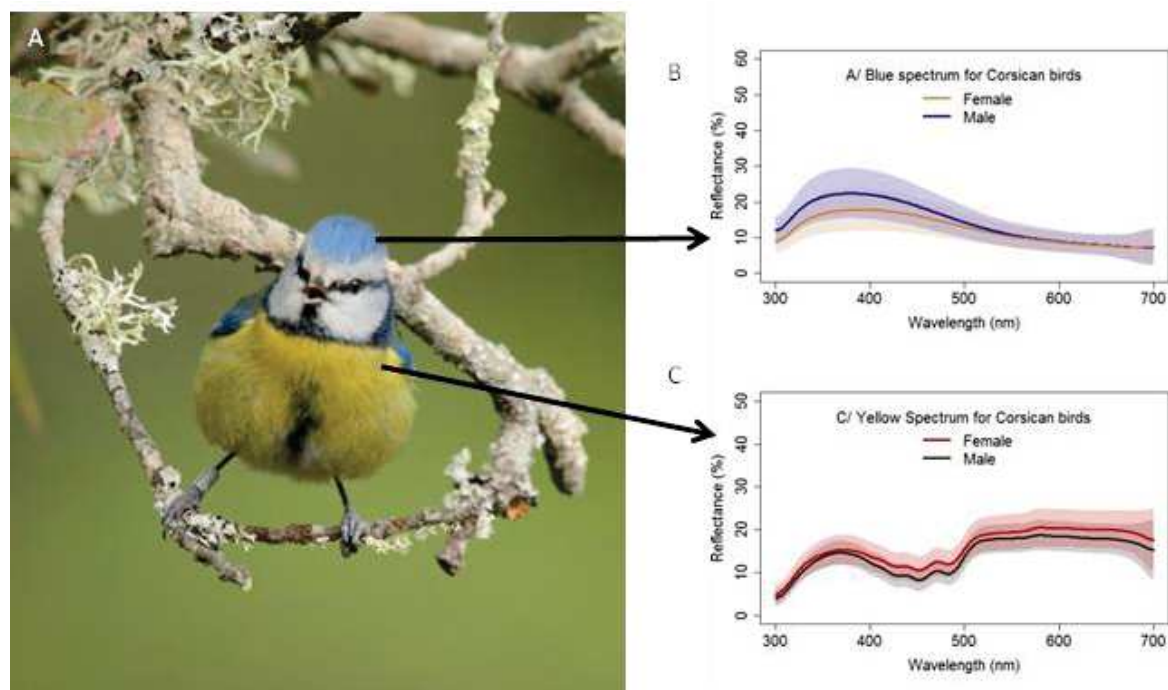


Figure 1 A/ Male blue tit photographed in Montestremo above Pirio field station (Corsica) at the beginning of the breeding season (April 2015). After identifying blue tits with a metal ring (here on its right leg), a sample of feathers from the blue crown and the yellow chest patches are plucked to enable future spectrophotometric measures in the lab. Picture courtesy of Stéphane Tillo. B/ Mean spectra extracted from measurements of blue feathers of the blue crown in males and females, showing sexual dichromatism. Samples from Corsican populations in 2011. C/ Mean spectra extracted from measurements of yellow feathers of the yellow chest patch in males and females showing a low sexual dichromatism. Samples from Corsican populations in 2011. Black arrows relate location of feather sampling to colour spectra. Mean spectra B and C have been obtained using package *pavo* (Maia et al. 2013) in R.

1. Information regarding species and populations

Blue tit (*Cyanistes caeruleus*) is a European species of songbird, socially monogamous and mostly resident although local migration may occur during winter. Males and females defend a territory during breeding season and nest is built by the female alone in an already excavated hole. Male feeds the female while she incubates eggs and broods hatchlings. Then both parents share the care of feeding nestlings and fledglings (Cramp and Simmons 2009). Social pair may attempt a new clutch after clutch failure, or after breeding success if it is still early in the season. In our southern populations, except in the mainland population (D-ROUVIERE), second clutches are rare. Most pairs remain together for the whole breeding season (Perrins 1979). Some pairs also remain together across breeding seasons but high mortality during winter lessens the

probability that both members survive winter. Divorce also occurs and has been related to first-year breeders (Culina et al. 2015) or habitat quality (Blondel et al. 2000). Blue tit readily breeds in nest box, and has been a species model for European ecologists working on wild populations (review on colouration see Parker 2013; review on clutch size see Møller et al. 2014).

In 1975, Jacques Blondel initiated the long-term project on Mediterranean blue tits in Corsica and near Mount Ventoux (Blondel et al. 2006). 40 years later, the project had widened with four different populations of blue tits intensively monitored each year (Figure 2). One population is located in the north-west of Montpellier and differs from the three populations situated in Corsica by morphological and life-history traits. The three Corsican populations also varied in their habitat, their phenology and show evidence of local adaptation (see MS A1: Charmantier, Doutrelant, Dubuc Messier, Fargevieille and Szulkin 2016).

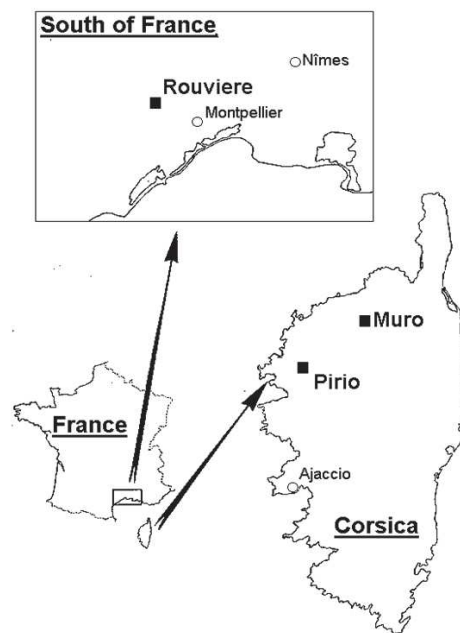


Figure 2 Map figuring the location of the four populations. D-MURO and E-MURO are represented as “Muro” since they are only 5 km away from each other. Map courtesy of Claire Doutrelant.

In each population, every breeding season, birds are captured while feeding at the nest. Birds are identified by a uniquely-numbered metal ring and morphological measures are taken. Blood is also sampled at first capture. Each year, all chicks are measured and ringed before fledging (around 15 days old). In addition, since 2005, feather collection is systematically

performed on each breeding parent. As I was using the historical and large data set collected by previous students and researchers, I contributed to the data collection during three to four months each year of my Ph.D. (for contribution to nest information see Lambrechts et al. 2014, 2016a,b). I managed field seasons 2014 and 2015 on the continental site from mid-March to mid-July and also actively contributed to the heart of season 2016.

As an ongoing scientific project started by Claire Doutrelant in 2005, samples of feathers have been plucked on each breeding bird (males and females) each year in each population. Feathers were plucked on two patches: the ultraviolet-blue crown and the yellow chest patch (Figure 1). These feathers were measured with a spectrometer in the lab and colour variables were extracted with Avicol software (Gomez 2006) from the spectra obtained. During my Ph.D., I compiled in a single database all these measurements gathering colouration information, identification of individuals associated with their nest site, life-history traits and morphological measures. The colour database consists of more than 6000 lines and 32 columns for 10 years and four populations.

2. Functions of blue tit colouration: what do we know?

Blue tit colouration has been the object of many researches since the discover that males reflected more towards ultraviolet compared to females (Andersson et al. 1998; Hunt et al. 1998) and that females biased offspring sex ratio towards males when they were associated with a male reflecting more towards ultraviolet (Sheldon et al. 1999). Many experimental and correlational studies were performed to determine their link with sexual and social selection. At the beginning, most studies focused on ultraviolet colouration. Studies conducted in the lab found that both sexes preferred a mate with a blue crown reflecting more towards ultraviolet (Hunt et al. 1999; but see Kurvers et al. 2010). Studies conducted in the field suggested assortative mating on ultraviolet colouration of the blue crown (Andersson et al. 1998; but see Garcia-Navas et al. 2009). Experimental studies conducted in aviaries or nature suggested a role of ultraviolet colouration in intra-sexual interactions during breeding season (see Alonso-Alvarez et al. 2004; Vedder et al. 2008; Remy et al. 2010 for males; see Midamegbe et al. 2011 for females). Ultraviolet colouration of the blue crown has been experimentally related to mate investment in parental care for both sexes (see Kingma et al. 2009 for males; see Limbourg et al. 2013 for both sexes) and bias in maternal investment in reproduction (Johnsen et al. 2005). Last, female

ultraviolet colouration on the blue crown was also related to proxies of reproductive success (Henderson et al. 2013).

The study of yellow colouration is scarcer. Yellow chest is not sexually dimorphic in northern Europe (Hunt et al. 1998; Doutrelant et al. 2008) but is sexually dichromatic in Corsica (see MS A1). Yellow colouration of the chest patch was related to parental investment in both sexes (Senar et al. 2002; Midamegbe et al. 2013), to female investment in reproduction for male colouration (Biard et al. 2009) and to reproductive success for female colouration (Doutrelant et al. 2008). The two colour patches, the yellow chest patch and the blue crown, were last found to be condition-dependent (see Doutrelant et al. 2012 but see Peters et al. 2011 for ultraviolet colouration).

All these studies suggested a role of the ultraviolet-blue and yellow ornaments in sexual and social selection. However, a recent meta-analysis reviewing all studies performed on male colouration in blue tits revealed a lack of consistency across studies (Parker 2013). Based on his study, Parker concluded that the only consistent result was the presence of sexual dichromatism for ultraviolet colouration of the blue crown and an age-related correlation with ultraviolet colouration. This conclusion was based on the fact that replicated studies gave different results and on the limited sample size in most of the studies. In regard of his results, Parker thus outlined the necessity to conduct replicated studies with larger sample sizes across a long period of time. The colour database of our project was created to test hypotheses related to both colouration in males and females blue tits in while considering spatiotemporal variation as well as recommendations from Parker's meta-analysis.

A lack of replication of results and spatiotemporal variation are commonly found in natural populations. Working on sex ratio in polygynous red deers, Clutton-Brock and collaborators showed that dominant females were producing significantly more sons than subordinate females (Clutton-Brock et al. 1984) confirming the Trivers-Willard hypothesis (Trivers and Willard 1973); years later, density had changed in the population and they failed to find a similar result. This story illustrates well the importance to consider spatiotemporal variation and to quote Cockburn (2014): "If the study had started later, the original exciting result would not have been obtained".

CHAPTER III

INTRASPECIFIC LEVEL: COLOURATION IN MALE AND FEMALE BLUE TITS, ASSORTATIVE MATING AND SPATIOTEMPORAL VARIATION



Pair of blue tits (female on the left, male on the right) photographed in the Regino valley (around Muro field stations) in Corsica in March 2016. Sexual dichromatism is very low in blue tits and is situated in the UV wavelengths, invisible to human vision. Similarity in colouration in males and females offers the opportunity to test a process related to mutual ornamentation: assortative mating. Picture courtesy of Stéphan Tillo

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MS2: Colour ornamentation in the blue tit: quantitative genetic (co)variances across sexes. Charmantier Anne, Matthew E. Wolak, Arnaud Grégoire, Amélie Fargevieille and Claire Doutrelant. <i>Heredity</i> Published online on August 31 st 2016	95
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A - Assessing conditions leading to the evolution of colouration

Part1: Extracts from MS A1

Only parts of the article available as MS A1 relate to phenotypic divergence in colouration in the four blue tit populations. Here, I only present the model system, methods related to measurements, tables and figures associated. References to bibliography available in Appendix MS A1.

The geographic configuration of Mediterranean landscapes, characterized by a heterogeneous, fine-grained mosaic of habitats, provides an exceptional study system for investigating plastic and adaptive responses in spatially structured populations (Blondel and Aronson 1999; Blondel et al. 2010). In this system, we have focused on the variability expressed by a passerine bird, the blue tit, whose ecological niche is preferentially linked to oak woodlands. The blue tit is present in a narrower range of habitats compared to the great tit *Parus major* (Snow 1954; Lack 1971), and yet is a common breeder throughout Europe and western Asia where it readily breeds in nest boxes, which makes it an ideal model to study adaptation to environmental heterogeneity. In our study area, the breeding activity of about 350 blue tit pairs is monitored each year in more than 1000 nest boxes erected across eight study sites, in a series of habitats that strongly differ in being dominated by patches of either deciduous (*Quercus pubescens*) or evergreen (*Quercus ilex*) oaks (Box 2). In deciduous forest patches, the leafing process occurs ca. one month (3-5 weeks depending on the scale of analysis) earlier compared to evergreen forests, and all leaves are renewed. This results in an earlier and more abundant caterpillar peak in deciduous patches (Zandt et al. 1990; Dias et al. 1994), which are considered as higher quality breeding habitats (Blondel et al. 2006). These differences between habitats are clearly visible to the human eye (Box 2) and can be mapped at a large scale using vegetation reflectance data from satellite sensors, the latter correlating

remarkably well with the number of either type of oak quantified on the ground within a 50m radius from each nest box (Szulkin and Marrot, unpub data). Apart from the notable heterogeneity of the Mediterranean landscape, these study populations are also situated at the species range-edge, a position known to be conducive to geographic differentiation (Mayr 1970; Endler 1977). Hence the study of these populations may be of particular conservation value because they represent significant components of intraspecific biodiversity (Hardie and Hutchings 2010) and potential sources of evolutionary innovation and persistence during rapid environmental change such as global warming (Sexton et al. 2009).

Over the last forty years, the Montpellier blue tit group has ringed 6653 blue tit parents and 33489 blue tit chicks in these study populations. This sustained hard work aimed at elucidating the phenotypic variation displayed by blue tits at three spatial scales (Blondel et al. 2006): mainland *versus* Corsica (min. distance of 85 km off mainland Italy, and 170 km off the French mainland), a scale that is not the main focus of the present paper (but more details about this inference level can be found in Dias and Blondel 1996; Lambrechts et al. 1997b; Blondel et al. 2001; Doutrelant et al. 2001); Muro *versus* Pirio valleys (24.1 km between D-Muro and E-Pirio, where “D” stands for Deciduous and “E” for Evergreen), and three deciduous plots (D-Muro) *versus* three evergreen plots (E-Muro) within the Muro valley (separated by 5.6 km). Although these distinct study plots within the Muro valley have been detailed in some previous publications (e.g. Lambrechts et al. 2004), we have pooled their blue tit populations here since they never display any significant differentiation, neither phenotypically nor genetically.

Because of the low rate of capture-recapture data using multi-sites, our knowledge on natal and breeding dispersal in the blue tit remains approximate, with very different results in different studies. For example, while a maximum dispersal distances of 14.5 km was

recorded in Belgium (Tufto et al 2005), an earlier study over 1200 km² in Germany revealed that natal dispersal could reach distances of 24 km in males, and 470 km in females (Winkel and Frantzen 1991). Note that in the same study, breeding dispersal (that is the distance between successive reproductive events) reached maximal distances of 0.75 km for males and 37 km for females. At the same time, the mode of the distribution is likely to be much smaller, especially in Corsican blue tits (Blondel et al. 1999), and most of our dispersal events are recorded within sites even when other populations are monitored close by. Over the last 17 years of ringing in Corsica, we observed 2 dispersal events between D-Muro and E-Muro (5.6 km), 2 reverse events between the same sites, and none between the Muro valley and E-Pirio (24.1 km).

Colouration measurement:

Feather colouration was measured in the lab using AVASPEC 2048 spectrometer (Avantes, Apeldoorn NL) with an AVALIGHT-DHS deuterium-halogen lamp (Avantes, Apeldoorn NL) and a 200 µm optic fibre allowing measurement between 300 and 700 nm, conducting light at a fixed angle of 90° and a fixed distance of 2 mm from the sample. Samples were disposed on a square of black cloth with a white standard reference estimated regularly. Each sample consisted of three feathers from the blue crown or four yellow feathers from the chest patch piled in a pack to recreate natural feather assemblage. We measured each sample three times, the fibre being removed between each measure. For each individual and each colouration, we made measurements on two different samples. Spectra were extracted using software Avicol (Gomez 2006). After controlling for repeatability (Fargevieuille, Grégoire, Charmantier, Del Rey Granado, & Doutrelant, Submitted), we calculated mean spectrum based on the six measurements for each individual and each colouration and extracted five variables. For the blue crown, we calculated an achromatic variable blue brightness (area below the reflectance curve divided by the width of the interval

300 – 700 nm) and two chromatic variables UV-blue hue (wavelength (in nm) where reflectance is maximal) and UV-blue chroma (proportion of the total reflectance falling in the range 300-400 nm). For the yellow patch of the chest, yellow brightness was calculated following the same function as for blue brightness; yellow chroma was calculated as $(R_{700}-R_{450})/R_{700}$ with R representing percentage of reflectance for a given wavelength. Correlation among colour variables were up to 0.42 except between UV-blue hue and UV-blue chroma where correlation was -0.74 (see ESM Fargevieille et al., Submitted).

For statistical analysis, see comments in Figure 1

Results and discussion

Table 1 illustrates in detail how each population of blue tits presented in box 2 displays a unique colour phenotype (for detailed statistical results see Fargevieille et al., in prep.) when combining measures on the blue crown patch and the yellow chest.

Compared to Corsican blue tits, birds from the mainland have brighter yellow and UV blue colorations and less dichromatic yellow chroma. Corsican populations also differ from one another. First, the chromatic values of the UV blue coloration separate D-Muro from E-Muro and E-Pirio: deciduous birds display more UV (lower hue and higher UV chroma) and are more dichromatic (Fig. 1). Second, the chromatic values of the yellow coloration separate E-Muro from D-Muro and E-Pirio: the yellow chroma of E-Muro birds is lower and less dichromatic. Finally, the population of E-Pirio shows different brightness patterns, with duller UV-blue coloration and brighter yellow coloration. More studies are needed to understand the origin of these population differences and whether or not they are adaptive but it is noteworthy that D-Muro and E-Muro birds differ so strongly in both UV blue and yellow coloration. These differences may contribute to the observed genetic differentiation if mate choice preference for color signal differs between these populations or if individuals do not settle randomly in respect to their coloration. In the face of a habitat-specific divergence at a

micro-geographic scale, the observed differences in the UV blue coloration between the two Muro populations are particularly interesting to illustrate this possible divergent intra-sexual selection. Indeed, the blue crown patch is involved in social and intrasexual interactions (Rémy et al. 2010; Midamegbe et al. 2011) and since the deciduous forest of D-Muro offers a richer breeding habitat than the evergreen forests of E-Muro and E-Pirio, birds in D-Muro might display more UV because they are socially dominant over the birds breeding in evergreen patches (Braillet et al. 2002). Another hypothesis, involving plasticity in colouration, is that the stronger UV in D-Muro results from a lower cost of breeding in this population. We indeed experimentally demonstrated that increasing the cost of reproduction affects the parents' coloration in the following year (Doutrelant et al. 2012).

Table 1 Phenotypic divergence between blue tit populations in deciduous (D-) and evergreen (E-) patches on the mainland (D-Rouvière), and in Corsica (D-Muro, E-Muro, E-Pirio). Mean, sample size (*n*, number of individuals), variance and coefficient of variance (CV) are provided for five colour traits measured on breeding birds during the whole reproductive period. Data was collected between 2005 and 2013.

Colour ornament traits					
Male Blue Brightness	Mean (<i>n</i>)	16.6 (886)	15.4 (472)	16.1 (297)	15.4 (498)
	Variance (CV)	26.5 (0.31)	20.2 (0.29)	23.2 (0.30)	20.0 (0.30)
Female Blue Brightness	Mean (<i>n</i>)	14.1 (949)	13.13 (515)	13.4 (305)	12.4 (519)
	Variance (CV)	28.9 (0.38)	15.0 (0.30)	15.2 (0.29)	16.6 (0.33)
Male UV-Blue Hue	Mean (<i>n</i>)	376.3 (886)	370.1 (472)	377.0 (297)	377.6 (498)
	Variance (CV)	129.0 (0.03)	93.1 (0.03)	179.5 (0.04)	149.4 (0.03)
Female UV-Blue Hue	Mean (<i>n</i>)	387.9 (949)	381.2 (515)	384.3 (305)	384.2 (519)
	Variance (CV)	131.9 (0.03)	203.4 (0.04)	266.4 (0.04)	152.0 (0.03)
Male Blue UV Chroma	Mean (<i>n</i>)	0.38 (886)	0.39 (472)	0.37 (297)	0.38 (498)
	Variance (CV)	0.0013 (0.09)	0.0014 (0.10)	0.0007 (0.073)	0.0013 (0.10)
Female Blue UV Chroma	Mean (<i>n</i>)	0.34 (949)	0.35 (515)	0.34 (305)	0.34 (519)
	Variance (CV)	0.0011 (0.10)	0.0013 (0.10)	0.0009 (0.086)	0.0011 (0.10)
Male Yellow Brightness	Mean (<i>n</i>)	17.0 (854)	15.7 (472)	16.1 (297)	16.4 (500)
	Variance (CV)	12.0 (0.20)	11.3 (0.21)	12.5 (0.22)	11.1 (0.20)
Female Yellow Brightness	Mean (<i>n</i>)	17.1 (912)	16.5 (523)	16.7 (310)	17.0 (528)
	Variance (CV)	15.1 (0.23)	12.6 (0.22)	9.9 (0.19)	12.7 (0.21)
Male Yellow Chroma	Mean (<i>n</i>)	0.62 (854)	0.82 (472)	0.69 (297)	0.81 (500)
	Variance (CV)	0.03 (0.27)	0.03 (0.20)	0.02 (0.23)	0.02 (0.18)
Female Yellow Chroma	Mean (<i>n</i>)	0.61 (912)	0.74 (523)	0.65 (310)	0.70 (528)
	Variance (CV)	0.03 (0.28)	0.02 (0.20)	0.03 (0.26)	0.02 (0.18)

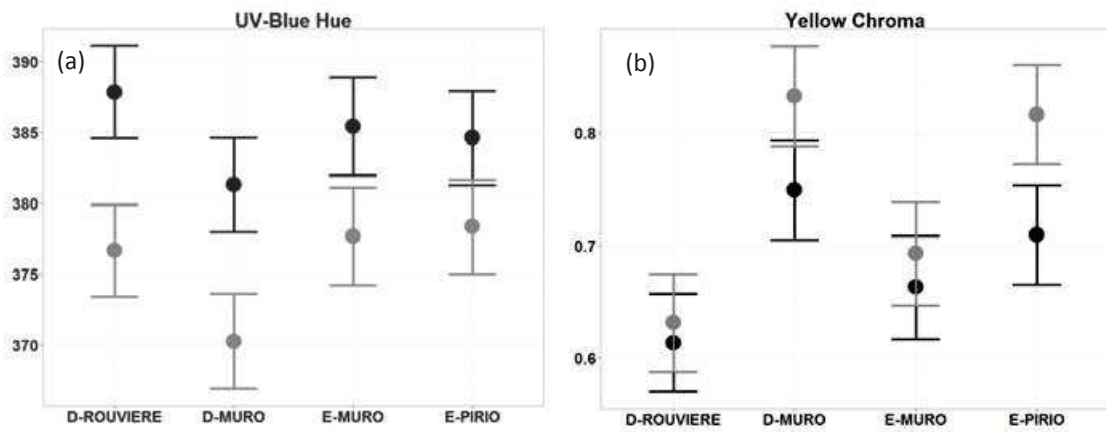


Figure 1 Differentiation in (a) UV/blue spectral hue of the crown patch and (b) purity (or chroma) of the yellow chest for males (in grey) and females (in black) across our blue tit populations. Data were collected on breeding birds between 2005 and 2013. Within Corsican populations, D-Muro birds display lower UV-blue hue compared to E-Muro and E-Pirio birds (values for males: D-Muro: 370, 95% CI=[367;374]; E-Muro:377, 95% CI=[374;381]; E-Pirio: 378, 95% CI=[375;382]) whereas E-Muro birds display lower yellow chroma compared to D-Muro and E-Pirio birds (values for males: E-Muro: 0.69, 95% CI=[0.65;0.74]; D-Muro: 0.83, 95% CI=[0.79;0.88]; E-Pirio: 0.82, 95% CI=[0.77;0.86]). Means and confidence intervals are derived from linear mixed-models (REML process) with age, sex and station as fixed effects and bird identity and year as random effects.

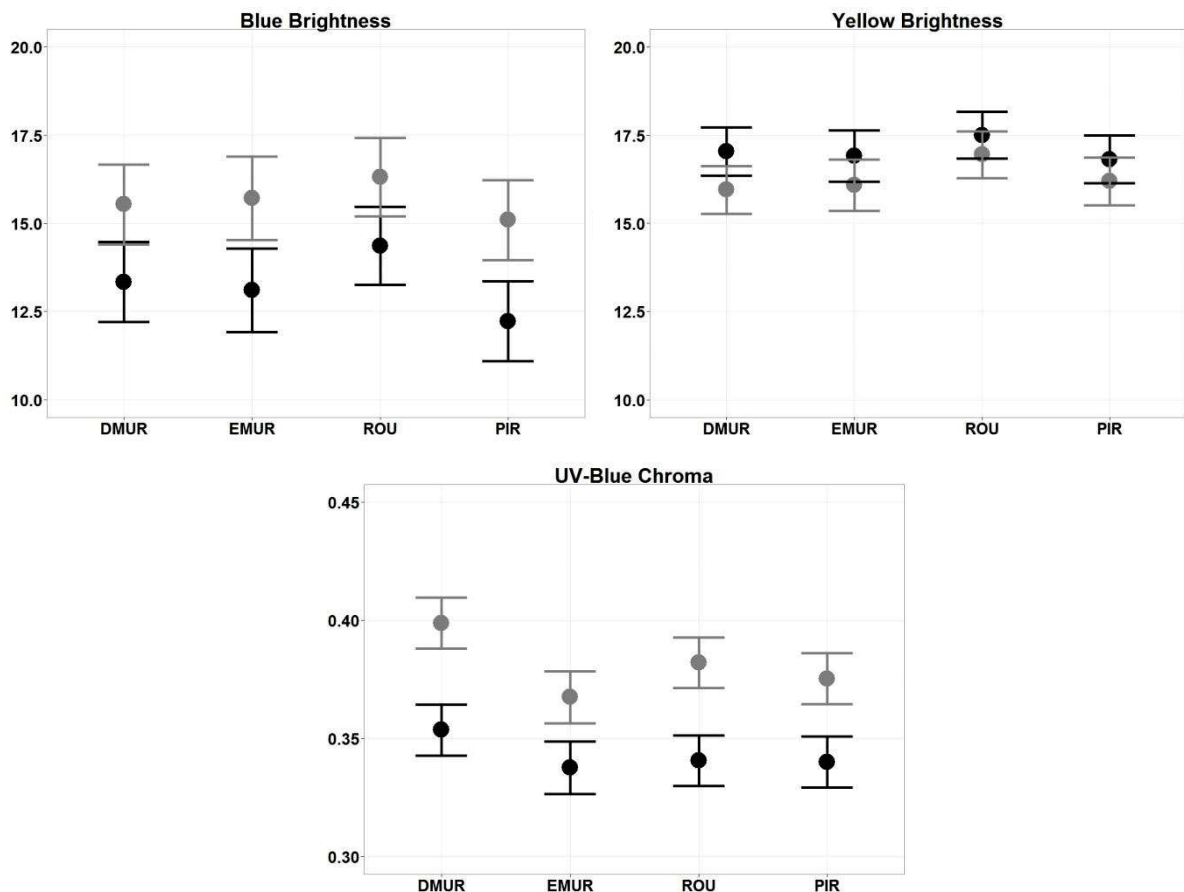


Figure 2 Additional figures (unpublished) with differentiation for brightness A/ of the blue crown, B/ of the yellow chest patch and C/ UV-blue chroma of the blue crown. For other details, see Figure 1

ORIGINAL ARTICLE

Colour ornamentation in the blue tit: quantitative genetic (co)variances across sexes

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Although secondary sexual traits are commonly more developed in males than females, in many animal species females also display elaborate ornaments or weaponry. Indirect selection on correlated traits in males and/or direct sexual or social selection in females are hypothesized to drive the evolution and maintenance of female ornaments. Yet, the relative roles of these evolutionary processes remain unidentified, because little is known about the genetic correlation that might exist between the ornaments of both sexes, and few estimates of sex-specific autosomal or sex-linked genetic variances are available. In this study, we used two wild blue tit populations with 9 years of measurements on two colour ornaments: one structurally based (blue crown) and one carotenoid based (yellow chest). We found significant autosomal heritability for the chromatic part of the structurally based colouration in both sexes, whereas carotenoid chroma was heritable only in males, and the achromatic part of both colour patches was mostly non heritable. Power limitations, which are probably common among most data sets collected so far in wild populations, prevented estimation of sex-linked genetic variance. Bivariate analyses revealed very strong cross-sex genetic correlations in all heritable traits, although the strength of these correlations was not related to the level of sexual dimorphism. In total, our results suggest that males and females share a majority of their genetic variation underlying colour ornamentation, and hence the evolution of these sex-specific traits may depend greatly on correlated responses to selection in the opposite sex.

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INTRODUCTION

Since Darwin's development of sexual selection theory (Darwin, 1871), theoretical and empirical work has greatly progressed towards explaining the mechanisms responsible for the evolution and maintenance of exaggerated male ornaments and weaponry (see, for example, Andersson, 1994; Savalli, 2001). Although secondary sexual characters are commonly more developed in males than females, in many animal species females also display elaborate ornaments (for example, conspicuous colours) or weaponry. After ignoring the issue for decades, evolutionary biologists have struggled to explain the evolution and maintenance of secondary sexual characteristics that are also exaggerated in females (Amundsen, 2000; Lebas, 2006; Clutton-Brock, 2007; Kraaijeveld *et al.*, 2007; Tobias *et al.*, 2012). Two nonexclusive hypotheses have been suggested as explanations for the evolution and maintenance of female ornaments: (1) indirect selection for elaborate female traits through direct selection on correlated male traits (*correlated response hypothesis*) (Lande, 1980; Price, 1996) and (2) direct selection on female traits occurring through either female–female competition for mates (Dijkstra *et al.*, 2008), male mate choice (Griggio *et al.*, 2005; Byrne and Rice, 2006) or social competition for ecological resources (Heinsohn *et al.*, 2005). Although each of these hypotheses has received some empirical support, there is currently no consensus about whether one plays a prevailing role, even within a given taxonomic group such as birds. A recent comparative analysis on 6000 species of passerines concluded that both female and male

plumage colourations are more extravagant in larger species and in tropical species (Dale *et al.*, 2015). Yet, the strength of sexual selection has antagonistic effects in the two sexes as it increases male colouration while decreasing female colouration (Dale *et al.*, 2015), supporting the possibility of independent evolution as suggested by previous studies (Amundsen, 2000). The work by Dale *et al.* (2015) also confirms that the general focus on male ornamentation has limited our understanding of the evolution of colour ornaments in both sexes.

Even though the presence of strong cross-sex genetic covariances is a crucial assumption underlying the correlated response hypothesis, sex-specific estimates of key quantitative genetic (co)variances underlying secondary sexual traits that are expressed in both sexes have been conspicuously scarce in the empirical literature (but see Price and Burley, 1993; Price, 1996; Chenoweth and Blows, 2003; Roulin and Jensen, 2015). In particular, studies on the role of ornamentation have largely focussed on sexually dimorphic species while neglecting species with low or no sexual dimorphism (see reviews in Kraaijeveld *et al.*, 2007; Poissant *et al.*, 2010). In the absence of further investigation into the heritability of sexual ornaments/weapons and their cross-sex genetic covariance, no generality can be drawn from the present empirical data regarding the importance of the hypotheses cited above. For example, in the review of Poissant *et al.* (2010), only 14 out of 549 estimations of cross-sex genetic correlations concerned ornaments or weaponry and the strength of these correlations varied substantially

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across taxa and across trait types (for example, between morphological traits and traits linked to communication).

In addition, theory for the evolution of sexual dimorphism also predicts that the degree of phenotypic difference between the sexes should be negatively associated with the cross-sex additive genetic covariance (and, to a certain extent, the cross-sex additive genetic correlation), and positively associated with the amount of sex-linked genetic variance (Fairbairn and Roff, 2006). Evidence supporting these predictions is presently very limited (Dean and Mank, 2014). Quantitative genetic analyses in natural populations based on long-term observations of individual phenotypes and relatedness (pedigrees) could offer a means to estimate sex-linked genetic variance. However, the large majority of studies in wild populations estimate additive genetic (co)variances while assuming only autosomal inheritance (Charmantier *et al.*, 2014). Recent investigations on colour variation have revealed Z-linked genetic variance in the collared flycatcher *Ficedulla albicollis* (explaining 40% of total phenotypic variance in wing patch size; Husby *et al.*, 2013), the barn owl *Tyto alba* (30% of variance in eumelanin spot diameter; Larsen *et al.*, 2014) and W-linked genetic variance in the zebra finch *Taeniopygia guttata* (2.6% of variance in beak colouration; Evans *et al.*, 2014). In many other cases however, investigations show no evidence for sex-linked genetic variance in colour ornamentation (for example, the Florida scrub-jay *Aphelocoma coerulescens*; Tringali *et al.*, 2015). Overall, contributions of sex-linked genetic variance to phenotypic variance in sexually selected and morphological traits measured in pedigreed populations is usually weak, yet it is commonly acknowledged that this could be because of low power to distinguish autosomal from sex-linked genetic variance (Husby *et al.*, 2013). It is indeed currently unclear whether wild population pedigrees used for quantitative genetic analyses confer sufficient power to disentangle autosomal additive genetic variance from other components of genetic variance (Wolak and Keller, 2014), as power analyses are not performed in these studies.

Colouration is often a sexually and/or socially selected trait that can signal individual quality and identity, as well as signal species identity, enhance crypsis, provide thermoregulatory benefits and protect against bacteria (Hill and McGraw, 2006), and is therefore central to an animal's fitness. However, to date, we know very little on the heritability of colouration (Svensson and Wong, 2011) or the genetic correlation that might exist between the sexes (Kraaijeveld *et al.*, 2007; Roulin and Ducrest, 2013). Comparative analyses have shown that colouration can evolve conjointly or separately in the two sexes (Amundsen, 2000; Dale *et al.*, 2015), but quantitative genetic studies of colouration are required to determine the main factors driving the observed sex-specific evolutionary patterns.

Although blue tits can appear sexually monomorphic to a human eye, spectrophotometry analyses have shown that blue tits from the subspecies *Cyanistes caeruleus caeruleus* are sexually dichromatic in the ultraviolet (UV) blue of the crown patch but monomorphic in their yellow carotenoid-based chest colouration (Andersson *et al.*, 1998; Hunt *et al.*, 1998; Doutrelant *et al.*, 2008). Both male and female UV blue colouration influences intrasexual interactions (see, for example, Alonso-Alvarez *et al.*, 2004; Rémy *et al.*, 2010; but see Vedder *et al.*, 2008), mutual mate choice (Hunt *et al.*, 1999) and mate reproductive investments (see, for example, Sheldon *et al.*, 1999; Limbourg *et al.*, 2004; Kingma *et al.*, 2009; but see Dreiss *et al.*, 2006 for males and Limbourg *et al.*, 2013 for females). In addition, male and female UV blue and yellow adult colouration is condition dependent (Doutrelant *et al.*, 2012; but see Peters *et al.*, 2011) and can be linked to parental investment or success (see, for example, Garcia-Navas *et al.*, 2012;

Midamegbe *et al.*, 2013) and to parasite levels (del Cerro *et al.*, 2010). Overall, all these studies suggest that both UV blue and yellow colouration can be sexually selected in both sexes, yet Parker and colleagues (Parker *et al.*, 2011; Parker, 2013) have recently challenged this view. Parker *et al.* (2011) found weak but contrasted evidence of fecundity selection on colouration for both sexes over 3 years. Following a meta-analysis that considered all previous studies with the same strength, regardless of the pertinence and robustness of their methodology, Parker (2013) further concluded that the sexual and/or social functions of blue and yellow colouration in blue tits remains to be demonstrated. This debate highlights the need for more studies on the colour patches in this species, and the examination of the cross-sex genetic correlation is an essential step to advance our understanding of the evolution in ornaments in both sexes. Despite many documented and proposed selective advantages to colour ornaments in blue tits, only three quantitative genetic studies have been conducted on colouration in this species (Johnsen *et al.*, 2003; Hadfield *et al.*, 2006a, 2007; Drobnik *et al.*, 2013), showing low autosomal heritability for both types of colourations. Furthermore, the indirect selection hypothesis remains untested as there are no estimates to date of cross-sex additive genetic covariance.

We used 9 years of colour measures in long-term monitored blue tits located in a Mediterranean mainland population (subspecies *C. c. caeruleus*) and on the island of Corsica (subspecies *C. c. ogliastrae*) to investigate the sex-specific and cross-sex additive genetic (co)variances underlying colour ornamentation traits that show a gradient of sexual dimorphism, and have been suggested to be involved in intra- or inter-sexual selection. Colour features were measured in one structurally based (blue crown) and one carotenoid-based (yellow chest) ornament.

In the context of improving our understanding of the evolution and maintenance of sexual ornaments and the importance of genetic correlations in the evolution of female ornaments, our aims were originally threefold:

- (1) Assessing whether there is autosomal and/or sex-linked genetic variation for colour ornamentation in the blue tit;
- (2) Measuring the strength of cross-sex genetic covariances, with the particular aim to evaluate whether female ornament evolution could be driven by such covariances;
- (3) Testing the theoretical predictions that the degree of sexual dimorphism is negatively associated with the cross-sex additive genetic covariance and positively associated with the amount of sex-linked genetic variance (Fairbairn and Roff, 2006; Poissant *et al.*, 2010).

MATERIALS AND METHODS

Sampling procedure and colour measurement

Blue tits have been monitored in the Rouvière forest (mainland France) since 1991 and at two localities in Corsica since 1976 (Pirio) and 1994 (Muro). Details on these study sites can be found in (Blondel *et al.* (2006) and Charmantier *et al.* (2016). Blue tits from Corsica belong to a different subspecies from blue tits found in the French Mediterranean mainland. The distance between Muro and Pirio in Corsica is 25 km. In order to improve our power for quantitative genetic models, individuals from these two valleys were pooled in one common Corsican data set. Supplementary Information A2 provides statistical justification for this choice based on a test for equality of additive genetic variances between the two populations.

Each year, breeding parents were captured in nest boxes between April and June. A small proportion of individuals were caught before the breeding period in January–March (in 2008–2009 and 2011–2013, $n=577$ or 15.9% of

measures). Each bird was equipped with a uniquely numbered metal ring provided by the Museum National d'Histoire Naturelle in Paris, six blue feathers were collected from the bird's blue crown and eight yellow feathers from the yellow chest to allow colour measurements in the lab. Bird sex and age were determined based on the capture–recapture database or on the colour of wing coverts for unringed birds. Chicks were ringed after 9 days of age that allowed building social pedigrees for each population. Genotyping of parents and offspring in 2000–2003 has shown that up to 29.3% (annual range: 18.2–29.3%, Charmantier *et al.*, 2004) of chicks were the result of extra-pair matings in Corsica, and 18.2% (annual range: 11.5–18.2%, Charmantier and Perret, 2004) on the mainland. The social pedigree used in this study was corrected for extra pair paternities only for chicks born in 2000–2003 in both populations. In these years, molecular genetic data allowed to identify 53% of extra-pair sires, whereas nonidentified genetic fathers were attributed a dummy identity. The Corsican pruned pedigree included 1507 individuals over 14 generations and the mainland pedigree 1233 individuals over 12 generations.

Feather colouration was measured in laboratory conditions, using a spectrometer (AVASPEC-2048, Avantes BV, Apeldoorn, Netherlands) and a deuterium-halogen light source (AVALIGHT-DH-S lamp, Avantes BV) covering the range 300–700 nm (Doutrelant *et al.*, 2008, 2012) and kept at a constant angle of 90° from the feathers. For each bird and colour patch (crown and chest), we computed the mean of six reflectance spectra taken on two sets of three blue and four yellow feathers (Doutrelant *et al.*, 2008, 2012). We used the software Avicol v2 (Gomez, 2006) to compute chromatic and achromatic colour variables based on the shape of the spectra (Andersson *et al.*, 1998; Doutrelant *et al.*, 2008), following previous studies on blue tits in our populations (see, for example, Doutrelant *et al.*, 2008, 2012) and others (see, for example, Alonso-Alvarez *et al.*, 2004; but see Parker *et al.*, 2011). For the UV blue crown colouration, we computed one achromatic variable: blue brightness (area under the reflectance curve divided by the width of the interval 300–700 nm); and two chromatic variables: blue hue (wavelength at maximal reflectance) and blue UV chroma (proportion of the total reflectance falling in the range 300–400 nm). Lower values of hue and higher values of UV chroma mean that the signal is stronger in the UV. For the yellow chest colouration, in addition to yellow brightness, we computed yellow chroma as

$(R_{700} - R_{450})/R_{700}$. Higher values of yellow chroma are linked to higher carotenoid contents in the plumage (Isaksson *et al.*, 2008). We have shown previously that our measures of these five colour traits using a spectrometer are highly repeatable (see, for example, Doutrelant *et al.*, 2008, 2012; Midamegbe *et al.*, 2013), suggesting acceptable measurement error. Figure 1 displays average spectra for blue crown and yellow chest measures in 2011.

The complete data set included 3629 observations with at least one colour parameter measured ($n = 1659$ in Rouvière (mainland), $n = 1035$ observations in Muro (Corsica) and $n = 935$ in Piriò (Corsica)) for a total of 2177 birds (see Table 1 for detailed sampling efforts on males and females). Supplementary Figure S1 presents the distribution of each colour parameter in Rouvière and in Corsica and Supplementary Table S1 shows the phenotypic correlation between each pair of traits in the mainland and the Corsican populations (Supplementary Information). As Supplementary Table S1 illustrates, among the five classically used and biologically relevant measures of colouration, some are phenotypically correlated yet Spearman rank's correlation did not exceed 0.672 in absolute value. The strongest phenotypic correlation was between blue UV chroma and blue hue (Spearman rank's correlation ranging from -0.476 to -0.672). All other trait combinations showed correlations of absolute value less than 0.4.

Sexual dimorphism

For each trait in both data sets, we measured the degree of sexual dimorphism in colour ornamentation by calculating a standardized effect size: Cohen's d , and its associated standard error (equations 10 and 16 in Nakagawa and Cuthill, 2007). Cohen's d effect size is a dimensionless statistic; a value of 0.2 would typically be suggestive of small sexual dimorphism, whereas a value of 0.8 would be interpreted as revealing strong sexual dimorphism.

Quantitative genetics

Exploring fixed effects. Previous to conducting quantitative genetic models, we conducted linear mixed models to explore the contribution of fixed effects (year of measure, year of birth, period of measurement and individual age) to the various colour parameters in both data sets. For all traits, only year of

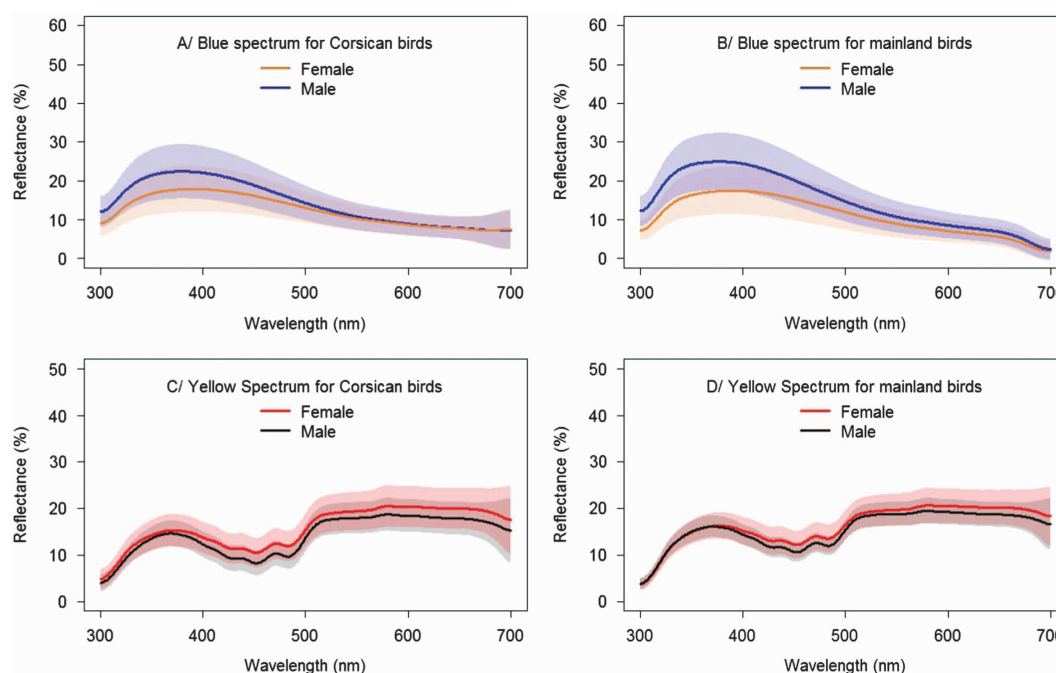


Figure 1 Average UV blue crown spectra for male (blue) and female (orange) blue tits sampled in 2011 (a) in Corsica and (b) on the mainland. Average yellow chest spectra for male (black) and female (red) blue tits sampled in 2011 (c) in Corsica and (d) on the mainland. Thick lines represent mean spectra and shaded areas associated s.d. values. Plots were realized using the R package 'pavo' (www.rafaelmaia.net/r-packages/pavo).

Table 1 Sampling effort and mean values (with associated s.d. values) for colour traits measured in blue tit males and females in Rouvière (mainland) and in Corsica between 2005 and 2013

	Blue brightness	Blue hue	Blue UV chroma	Yellow brightness	Yellow chroma
<i>Corsica, males</i>					
Nb measures	882	882	882	870	958
Mean (s.d.)	15.6 (4.8)	375.1 (11.7)	0.39 (0.04)	16.1 (3.7)	0.80 (0.18)
<i>Corsica, females</i>					
Nb measures	865	865	865	825	930
Mean (s.d.)	12.9 (4.3)	383.3 (12.2)	0.35 (0.04)	16.8 (3.6)	0.70 (0.16)
<i>Rouvière, males</i>					
Nb measures	810	810	810	769	769
Mean (s.d.)	16.6 (5.2)	376.5 (11.3)	0.38 (0.03)	17.0 (3.5)	0.63 (0.17)
<i>Rouvière, females</i>					
Nb measures	840	840	840	801	801
Mean (s.d.)	14.2 (5.4)	388.1 (11.3)	0.34 (0.03)	17.3 (3.9)	0.61 (0.17)

Abbreviation: UV, ultraviolet.
Hue is in nm.

measure was retained in all models as a categorical fixed effect (see details in Supplementary Information A1).

Univariate animal models. Genetic (co)variances, heritabilities and genetic correlations were estimated using restricted maximum-likelihood (REML) estimation procedures implemented in the software ASReml v3.0 (Gilmour *et al.*, 2009). For each data set and colour measure, we first implemented a sex-specific univariate ‘animal model’ that combined the phenotypic measures for a given sex with the pedigree information to partition the phenotypic variance into an additive genetic variance (V_A), a variance due to permanent environment effects (V_{PE} , based on repeated observations of individuals) and a residual variance (V_R), while controlling for annual fluctuations using year as a single fixed effect. In such a model the phenotypic value of an individual i is written as:

$$y_i = \mu + \text{YEAR} + a_i + PE_i + \varepsilon_i \quad (1)$$

The additive genetic effect on individual i (a_i), was assumed to be normally distributed with mean of zero and variance of V_A . The permanent environment effect (PE_i) and residual errors (ε_i) were also assumed to be normally distributed, with zero means and variances V_{PE} and V_R . Residual errors were assumed to be uncorrelated within individuals across measurements. In the Corsican model, a genetic group determined the Muro/Pirio origin for each bird.

The additive genetic variance estimates were tested against a null hypothesis of zero by carrying out likelihood ratio tests, where minus two times the difference in log likelihood between a model including the variance and a model without it was tested against the χ^2 distribution with one degree of freedom.

Bivariate animal models and cross-sex additive genetic variance. In order to estimate the cross-sex additive genetic covariance for each colour measurement, we expanded Equation 1 to a bivariate model where the phenotypic values of males (m_i) and of females (f_i) are explained by fixed (year of measure) and random effects (as previously, additive genetic, permanent environment and residual effects):

$$\begin{aligned} m_i &= \mu_m + \text{YEAR}_m + a_{mi} + PE_{mi} + \varepsilon_{mi} \\ f_i &= \mu_f + \text{YEAR}_f + a_{fi} + PE_{fi} + \varepsilon_{fi} \end{aligned} \quad (2)$$

This bivariate animal model provides sex-specific estimations of additive genetic variances (V_{A_m} , V_{A_f}), permanent environment variances (V_{PE_m} , V_{PE_f}) and residual variances (V_{R_m} , V_{R_f}). In this model, each character is sex specific and cannot be measured in males and females simultaneously, and hence this model cannot fit any between-individual (permanent environment) or within-

individual (residual) covariance. However, it can fit a cross-sex additive genetic covariance ($COV_{A_{mf}}$) from which we estimate the cross-sex additive genetic correlation:

$$r_{A_{mf}} = \frac{COV_{A_{mf}}}{\sqrt{V_{A_m} \times V_{A_f}}} \quad (3)$$

A bivariate animal model was fitted for each colour trait in each population, with a genetic group specified for Muro and Pirio individuals in the case of the Corsican data set. The additive genetic covariance estimates were tested against a null hypothesis of zero by carrying out a likelihood ratio test using the χ^2 distribution with one degree of freedom. In order to test for a genotype \times sex interaction, which occurs when a given genotype has different phenotypic expressions in males versus females, we compared the original model with a model where $V_{A_m} = V_{A_f} = COV_{A_{mf}}$ using likelihood ratio tests and the χ^2 distribution with two degrees of freedom. To allow comparisons between traits and populations, we also report sex-specific coefficients of additive genetic variance CV_{A_m} and CV_{A_f} (Houle, 1992) in which the square root of the additive genetic variance is scaled by the trait mean:

$$CV_A = 100 \times \sqrt{V_A / \bar{X}} \quad (4)$$

Including a Z-linked genetic variance. We conducted power analyses to determine the ability of the animal model to estimate sex-chromosomal and autosomal additive genetic variance given our blue tit pedigrees and data structures. Specifically, our goal was to determine whether we could detect Z-chromosome-linked additive genetic variance (V_Z) in the blue tit colouration data. Our general approach was to use Monte Carlo simulation to reassign individual phenotypes with known (that is, simulated) sources of trait covariation in the population and then use animal models with each simulated data set to test the null hypothesis that Z-chromosomal additive genetic (co) variances were equal to zero. Over many replicate simulations, the proportion of significant P -values ($P < 0.05$) obtained from our null hypothesis tests reflect the power (the probability of rejecting the null hypothesis when it is false) of the animal model to estimate V_Z . We note that this does not determine the power of the animal model to provide unbiased estimates of autosomal (V_A) and sex-linked (V_Z) additive genetic variances (Supplementary Information).

We simulated random effects underlying observed phenotypes similar to those modeled for the observed data (Equation 2): additive genetic (autosomal and Z-linked), permanent environment and residual effects (Supplementary Information A3). We used 27 unique combinations of autosomal additive genetic, permanent environment and residual variances along with cross-sex autosomal and sex-linked additive genetic correlations (Supplementary

Table S2). Within each of these unique combinations, the Z-linked additive genetic variance was set to one of seven values: $\sigma_{Z\text{-male}}^2 = 1, 10, 30, 50, 60, 70$ or 90 to assess the power at each level.

For each of the above parameter combinations, in each of the two data sets (Corsica and Rouvière), we simulated phenotypes for every individual (Supplementary Equation S1 in Supplementary Information) a total of 1000 different times. We used R (R Core Team, 2014) and the R package *nadiv* (Wolak, 2012) to simulate each of the above effects (Supplementary Information A3). We used the model of sex-chromosomal additive genetic variance of Fernando and Grossman (1990) that assumes no global sex chromosomal dosage compensation or recombination between the Z and W chromosomes.

Simulated phenotypes were analysed with an animal model implemented in the ASReml R package (v3.0, Butler *et al.*, 2009). Models were conducted with and without the Z-linked additive genetic (co)variance terms, and minus two times the difference in these model log likelihoods was used to calculate a likelihood ratio test statistic. Probabilities of obtaining a difference in log likelihoods were assigned assuming an asymptotically χ^2 distributed test statistic with three degrees of freedom. For a given set of parameters, we used the proportion of *P*-values < 0.05 as an estimate of power. Full details are available in the Supplementary Information A3.

RESULTS

Additive genetic (co)variances and heritabilities

As detailed in Table 2, the bivariate animal models revealed that the chromatic part of the crown colouration (blue UV chroma and hue) was overall heritable (except for 2 of the 8 estimated colour parameters: male hue and female UV chroma in Corsica) with heritability estimates ranging from 0.07 to 0.19 in Corsica (0.73–4.06 for CV_A) and from 0.18 to 0.23 in Rouvière (1.10–3.98 for CV_A). In contrast, the achromatic part of the crown colouration (brightness) was heritable for both sexes (with heritabilities of 0.18 and 0.10 for males and females) in Corsica but not heritable for both sexes in the mainland Rouvière population (although CV_A s were high), suggesting it is more sensitive to nongenetic variation than chromatic parameters. Similarly, the achromatic part of the yellow colouration was nonheritable in both sexes and populations, whereas the chromatic part was significantly heritable in males (heritabilities of 0.13 and 0.25 in Corsica and the mainland), but not in females.

Differences in model log likelihoods where sex-specific additive genetic variances— V_{A_m} and V_{A_f} —were unconstrained or constrained

to be equal indicated genotype by sex interactions only in two cases: in Corsica for blue hue ($P=0.003$) and blue UV chroma ($P<0.0001$). Estimated $COV_{A_{m,f}}$ (Table 2) for all blue measures and for Corsican yellow chroma were large and significantly greater than zero. $COV_{A_{m,f}}$ was not significantly different from zero for yellow chroma in Rouvière, yet this is most likely explained by the very small V_{A_f} that prevents a correct estimation of the covariance.

Power analysis for sex-linked genetic variance

Overall, the power simulations revealed low power to estimate Z-linked additive genetic variance in our two data sets (see partial results in Figure 2 and Supplementary Information A3). Using a common rule of thumb for power, the Corsican data only achieve a minimum level of desired power (80%) when the Z-linked between-sex additive genetic correlation is one (bottom row, Supplementary Figure S2a), Z-linked additive genetic variance is very high (>70) and autosomal additive genetic variance is two. The animal model combined with the Rouvière population structure (Supplementary Figure S2b) achieves 80% power under less restrictive conditions, although this still requires Z-linked additive genetic variance to comprise at least 50% of total phenotypic variance (that is, $h^2_{Z\text{-linked}} > 0.5$).

Sexual colour dimorphism

All colour traits displayed some sexual dimorphism, apart from yellow brightness in the Rouvière population (Figure 3, all paired one-sided Student's *t*-tests with $P<0.016$ except for yellow brightness in the Rouvière: $P=0.061$), with males being more colourful than females for both ornaments, with brighter blue and slightly brighter yellow.

DISCUSSION

Autosomal and sex-linked genetic variation for colour ornamentation in the blue tit

Autosomal genetic variation. Our quantitative genetic analyses reveal higher heritabilities for the crown blue UV colour than previously estimated (Hadfield *et al.*, 2006a), confirm that the chromatic part of yellow colouration can be heritable in males (Evans and Sheldon, 2012) and reveal a lower heritability for yellow chroma in females than males.

Table 2 Heritability of colour features in male and female blue tits (h_m^2 and h_f^2) and cross-sex additive genetic covariances ($COV_{A_{m,f}}$) and correlations ($r_{A_{m,f}}$) estimated using bivariate animal models (with s.e. values)

	<i>nb obs</i>	V_{A_m}	CV_{A_m}	h_m^2	V_{A_f}	CV_{A_f}	h_f^2	$COV_{A_{m,f}}$	$r_{A_{m,f}}$
<i>Corsica</i>									
Blue brightness	1795	3.73 (1.02)	12.34	0.18 (0.05)	1.59 (0.76)	9.81	0.10 (0.05)	2.37 (1.30)	0.97 (0.54)
Blue hue	1795	7.48 (4.98)	0.73	0.07 (0.04)	13.59 (6.18)	0.96	0.11 (0.05)	10.06 (7.98)	1.00 (0.87)
Blue UV chroma	1795	2.5E10⁻⁴ (5.3E10⁻⁵)	4.06	0.19 (0.06)	1.2E10 ⁻⁴ (1.2E10 ⁻⁴)	3.10	0.14 (0.14)	1.710⁻⁴ (7.9E10⁻⁵)	0.99 (0.68)
Yellow brightness	1772	0.95 (0.61)	6.05	0.07 (0.05)	0.73 (0.60)	5.07	0.06 (0.05)		
Yellow chroma	1957	3.6E10⁻³ (1.2E10⁻³)	7.56	0.13 (0.04)	3.6E10 ⁻³ (2.7E10 ⁻³)	8.51	0.15 (0.11)	3.6E10⁻³ (1.7E10⁻³)	1.00 (0.63)
<i>Rouvière</i>									
Blue brightness	1650	1.35 (1.05)	7.01	0.06 (0.05)	1.33 (1.27)	8.15	0.07 (0.07)		
Blue hue	1650	17.24 (7.48)	1.10	0.18 (0.07)	21.37 (5.58)	1.19	0.20 (0.05)	19.04 (5.65)	0.99 (0.28)
Blue UV chroma	1650	1.6E10⁻⁴ (5.7E10⁻⁵)	3.31	0.19 (0.07)	1.9E10⁻⁴ (4.3E10⁻⁵)	3.98	0.23 (0.05)	1.6E10⁻⁴ (4.5E10⁻⁵)	0.94 (0.25)
Yellow brightness	1570	0.74 (0.38)	5.07	0.09 (0.04)	0.75 (0.93)	5.00	0.07 (0.08)		
Yellow chroma	1570	4.9E10⁻³ (1.9E10⁻³)	11.16	0.25 (0.10)	3.6E10 ⁻³ (2.0E10 ⁻³)	9.91	0.16 (0.09)	9.3E10 ⁻⁴ (1.4E10 ⁻³)	0.22 (0.33)

Abbreviation: *nb obs*, number of measures for each trait; UV, ultraviolet.

The cross-sex additive genetic correlation is presented only for cases where at least one sex-specific additive genetic variance (V_{A_m} , V_{A_f}) was significant. All significant results ($P<0.05$) are in bold. Significance of $r_{A_{m,f}}$ was not assessed.

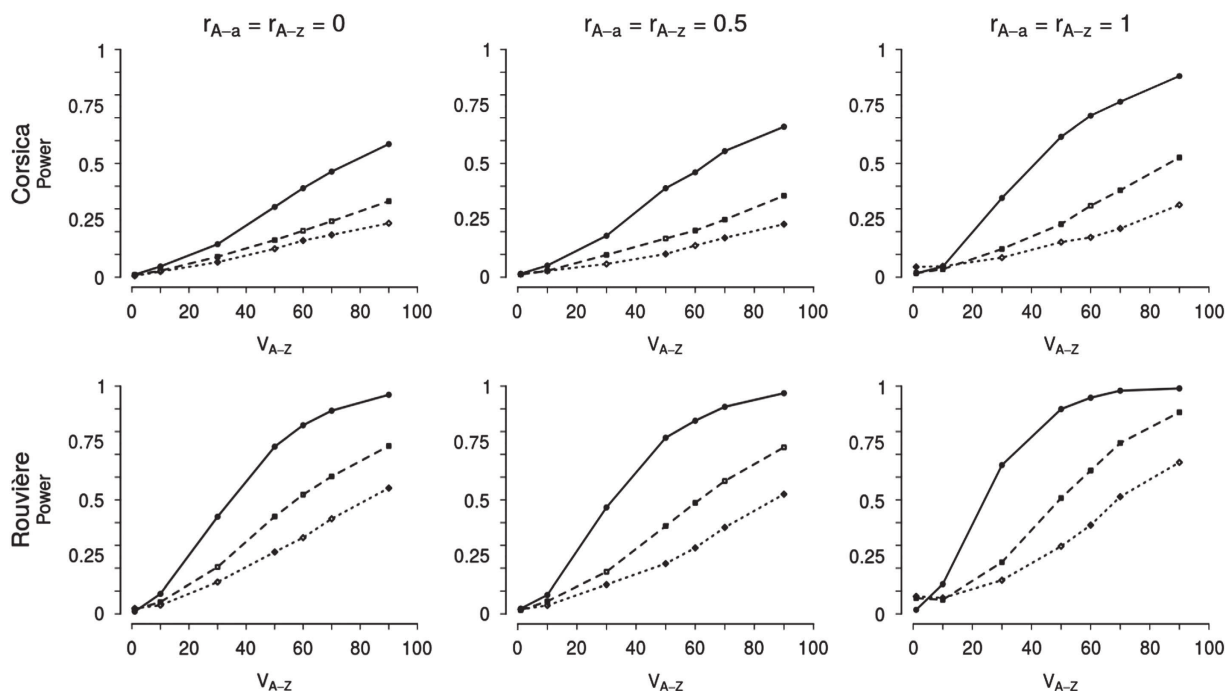


Figure 2 Power to estimate Z-chromosomal additive genetic variance in the Corsica (top row) and Rouvière (bottom row) populations. Between-sex additive genetic autosomal (r_{A-a}) and Z-chromosomal (r_{A-z}) correlations vary from zero to one. Power is calculated as the proportion of simulations for which the model with Z-chromosomal additive genetic (co)variances fitted significantly better than a model without. Power was assessed at seven values of Z-chromosomal additive genetic variance (values along the x axes) and three values of autosomal additive genetic variance (V_{A-a} : solid = 2, dashed = 50, and dotted = 100 lines).

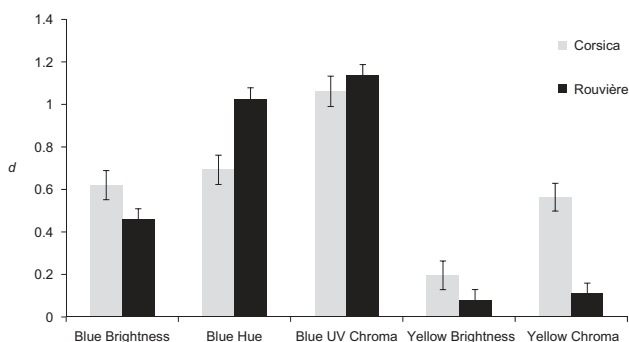


Figure 3 Sexual dimorphism (Cohen's d) on five colour traits in blue tits from Corsica (grey) and from the mainland (population of Rouvière, black). Bars represent s.e. values. See Table 1 for sample sizes and main text for statistics.

Blue UV colouration depends on the microstructure of the plumage (Prum, 2006), whereas yellow colouration is influenced by carotenoid contents (Partali *et al.*, 1987) and by microstructure (Shawkey and Hill, 2005). Food is the sole source of carotenoids for blue tits as animals cannot synthesize them. Hence, stronger environmental dependence is expected in carotenoid-based colouration compared with the structurally based colouration. This prediction is upheld by our results for females, but not so much for males where there is no strong contrast between heritabilities for carotenoid-based and structurally based colouration.

The fact that male yellow chroma is as heritable as the UV blue colouration in both populations, and displays high CV_{A_m} , is very interesting. As yellow chroma is related to carotenoid content in the

feathers, more chromatic individuals are often depicted as having higher foraging capacities and/or higher parasite resistance (see, for example, del Cerro *et al.*, 2010). Our results suggest that more chromatic males have male offspring that are more chromatic themselves. This could be interpreted as more chromatic males having higher abilities at finding food and/or at parasite resistance, and that their male offspring inherit these aptitudes, either genetically or nongenetically. Indeed, although the additive genetic variance is estimated here based on a variety of relatedness types, the animal model cannot always decipher accurately between genetic versus shared environmental or social resemblance between relatives when the large majority of individuals in the pedigree are siblings or parent-offspring (Wolak and Keller, 2014). A male-specific social rather than genetic inheritance of yellow chroma, for example, mediated by paternal care, could explain why this trait is less heritable in females (although note that CV_{A_s} are of similar magnitude). As females disperse longer distances than males in the Blue tit (Matthysen *et al.*, 2005), males sharing microhabitats could possibly lead to a male-specific nongenetic inheritance pattern. Such sex-specific environmental covariance between relatives needs to be investigated in future work, ideally using experimental approaches to isolate genetic and environmental effects. In any case, such father-son resemblance in yellow chroma makes it a good candidate for a sexually selected trait to optimize both direct and indirect benefits for females.

The moderate but significant heritabilities presented here are consistent with previous estimates in colour patches of blue tits (Hadfield and Owens, 2006) and great tits (Evans and Sheldon, 2012), yet they are much smaller than heritabilities associated with the sizes of melanin and white colour patches in other species (ranging from 0.28 to 0.90, see, for example, Saino *et al.*, 2013; Hubbard *et al.*, 2015;

Roulin and Jensen, 2015). Melanin and white patches have previously been suggested to be influenced by an individual's condition (Gustafsson *et al.*, 1995; Griffith, 2000), including long-lasting effects of early environments (Roulin, 2016). However, our recent understanding on the genetic determinism for melanism (Theron *et al.*, 2001; Ducrest *et al.*, 2008), as well as the comparison with our present results suggest that variation in black and white ornaments may be less susceptible to body condition than structural and carotenoid-based colourations. In particular, we found that additive genetic variance explains only a small proportion of total variation in the achromatic part of yellow chest colouration, consistent with findings in other blue and great tit populations (Hadfield and Owens, 2006; Hadfield *et al.*, 2006b; Drobniak *et al.*, 2013). These results suggest that most variation in this aspect of colouration is likely attributable to environmental sources, including individual condition. Differences in condition dependence across colouration signals have been demonstrated experimentally using drug or nutritional treatments (McGraw *et al.*, 2002; Hill *et al.*, 2009). Two comparative analyses have also revealed that sexual dichromatism (used as a proxy of sexual selection) is more intense for carotenoid-based or structurally based colouration than for melanin-based colouration (Badyaev and Hill, 2000; Taysom *et al.*, 2011).

Our variance partitioning in the blue UV crown colour reveals some striking differences between the two blue tit subspecies (for example, heritable blue brightness in Corsica only), although the absence of other comparable studies prevents any generalization. In addition, we found higher heritabilities overall than Hadfield *et al.* (2006a), thereby illustrating that the genetic determinism of colouration can vary across populations and requires further quantitative genetic investigations of colouration both within and across species (see review in Mundy, 2006).

Sex-linked genetic variation. Although it is now clear that many genes underlying sexual dimorphism are not sex linked (Badyaev, 2002) and that sex linkage is not a requirement for sexual dimorphism (Fairbairn and Roff, 2006; Dean and Mank, 2014; Roulin and Jensen, 2015), there is accumulating evidence for sex linkage of genes underlying sexually dimorphic traits, especially with the increasing accessibility of genetic mapping in nonmodel organisms (Charlesworth and Mank, 2010; Huang and Rabosky, 2015). Recent evidence suggests that Z-linked genetic variance can explain as much as 40% of the total phenotypic variation in colour ornaments of birds (see Introduction and Husby *et al.*, 2013). However, the statistical power to estimate Z-linked additive genetic variance in our two data sets was very low (see partial results in Figure 2 and Supplementary Information A3). Although we found more power in Rouvière than the Corsican populations (possibly because of a higher pedigree connectedness), it is unlikely, however, that an animal model using data collected from either population would have enough power to detect Z-linked additive genetic variance. Only when the simulated autosomal additive genetic variance was at its lowest value and the simulated Z-chromosomal additive genetic variance was among its highest values would conventional rules of thumb deem there to be sufficient power (that is, power > 80%) to calculate Z-chromosomal additive genetic variance. Although empirical estimates of sex-chromosomal additive genetic variance are few, it seems an unlikely condition to find such high sex-chromosomal heritability almost at the exclusion of autosomal heritability. Overall, these simulations revealed that we could not test the hypotheses involving sex-linked genetic variation, and that most if not all previously published results on sex-linked genetic variance suffered from similar lack of power. This is a worrying report that calls

for further simulations to determine the structure and size of pedigree and data required to estimate sex-linked genetic variance.

Cross-sex genetic covariances and female ornamentation

In the animal kingdom, dimorphic traits under sexual selection have been shown to be associated with a whole range of cross-sex genetic correlations: from low (for example, in *Drosophila serrata*; Chenoweth and Blows, 2003) to very strong correlations (for example, in the red deer *Cervus elaphus*; Pavitt *et al.*, 2014). The sparse and contrasted results prevent from drawing general conclusions on the link between genetic covariances across sexes and the evolution of sexual traits. In our study, estimated cross-sex additive genetic correlation— $r_{A_{m,f}}$ —were high (close to one), even in cases where the trait was not significantly heritable in one sex (for example, blue hue and yellow chroma in Corsica, see Table 2 for details). To our knowledge, only one other study explored $r_{A_{m,f}}$ for blue structural colours (in Florida scrub-jays, Tringali *et al.*, 2015), with similar results of very strong cross-sex genetic correlations. These results validate the fundamental assumption underlying the correlated response hypothesis (Lande, 1980). They suggest that evolution of female crown colouration could be drastically constrained by indirect selection acting on males.

Analogous conclusions can be drawn from estimates of $r_{A_{m,f}}$ in carotenoid-based ornaments: the evolution of colouration in one sex is likely to have a strong influence on the colouration in the other sex. However, we found large variability in our estimates, with $r_{A_{m,f}}$ of yellow chest chroma ranging from 0.22 in Rouvière to 1 in Corsica. The few estimates of cross-sex genetic correlations for carotenoid-based colour traits so far in the literature show a similarly large range of values for $r_{A_{m,f}}$. High additive genetic correlation was found for beak redness in the zebra finch ($r_{A_{m,f}} = 0.926$; Schielzeth *et al.*, 2012) but a study of yellow brightness, saturation and hue in blue tit nestlings showed $r_{A_{m,f}}$ ranging from -0.13 to 0.19 with very large confidence intervals (Drobniak *et al.*, 2013). These very divergent results call for further investigations on cross-sex genetic correlations in carotenoid-based ornaments in a wider range of species and populations. New genomic tools might also soon allow the identification of genomic regions involved in colour variation in both males and females, thereby revealing whether the same genes influence plumage colouration in both sexes (Roulin and Ducrest, 2013; Kraaijeveld, 2014; Huang and Rabosky, 2015).

Our quantitative genetic analyses used social pedigrees that were only partially corrected for extra-pair paternity. Hence, additive genetic variances and heritabilities in Table 2 could be underestimated, although as said above they were overall larger than reported in previous studies (Charmantier and Réale, 2005). Unfortunately, little is known on how errors in paternity assignment due to extra-pair reproduction can affect the estimation of genetic covariances and sex-linked genetic variance (Reid, 2014). A study combining data on extra-pair occurrence and parental colour is planned for our study populations so that we may quantify to what extent missassigned paternity will bias quantitative genetic (co)variance estimates.

Linking the degree of sexual dimorphism to cross-sex additive genetic covariance

In accordance with previous studies in this species (Andersson *et al.*, 1998; Hunt *et al.*, 1998; Delhey and Peters, 2008; Doutrelant *et al.*, 2008) blue characteristics were all highly dimorphic, with the strongest dimorphism expressed in the blue UV chroma, whereas yellow characteristics showed small or moderate dimorphism. Interestingly, Corsican subspecies of blue tits were significantly more dimorphic for yellow chroma than mainland birds (two-sided Student's

t -test, $P=4 \times 10^{-8}$), whereas the reverse was true for blue hue (two-sided t -test, $P=0.0001$). Although sexual dimorphism in yellow is usually considered very small for this species and possibly below the detectable level for birds (Delhey *et al.*, 2010), strong dichromatism has been reported once before, in central Spain (Garcia-Navas *et al.*, 2012). Our personal observations across the *ultramarinus* complex (C Doutrelant and G Sorci, unpublished data) suggest that the yellow sexual dimorphism might be a characteristic of blue tits in the southern part of the species distribution. These observations limited to the southern edge of the distribution could be explained by differences in selective forces acting on this ornament. Southern blue tit populations are subject to more drastic food limitation than northern ones. Comparative selection analyses would confirm whether these increased environmental constraints result in different selection pressures acting on male and/or female yellow colouration, in particular on yellow chroma, as it is directly linked to the carotenoid content of the feather, and is heritable.

Homologous characters in the two sexes, such as blue crown colour and yellow chest colour in blue tits, are presumably controlled, at least in the early evolution of these traits, by very similar sets of genes, leading to strong cross-sex genetic covariance. As any dimorphic character, these traits are likely to be under antagonistic selection in males and females (Rice, 1984) that, combined with a strong cross-sex genetic covariance, would create an intralocus sexual conflict (Lande, 1980; Bonduriansky and Rowe, 2005; Poissant *et al.*, 2010). This leads to the classic prediction that the degree of sexual dimorphism should be inversely correlated with the level of $COV_{A_{m,f}}$ (Fairbairn and Roff, 2006) and of $r_{A_{m,f}}$ (Lande, 1980; Bonduriansky and Rowe, 2005). The negative relationship between the cross-sex additive genetic covariance and the magnitude of sexual dimorphism is generally upheld over a range of trait types and across a variety of animal and plant species (Fairbairn and Roff, 2006; Bonduriansky, 2007; Poissant *et al.*, 2010). However, studies on the role of ornamentation in sexual selection have largely focussed on conspicuously sexually dimorphic species, neglecting species with low or no sexual dimorphism (see reviews in Kraaijeveld *et al.*, 2007; Poissant *et al.*, 2010). Estimating cross-sex genetic covariances for weakly dimorphic or nondimorphic species/traits is now a necessary stepping stone in our understanding of the evolution of sexual dimorphism (Lande, 1980). In our blue tit study, this prediction was not validated when comparing the five colour traits with varying degrees of dimorphism. Indeed, the most dimorphic traits (blue UV chroma and blue UV hue) displayed strong $COV_{A_{m,f}}$ in both data sets, with $r_{A_{m,f}}$ close to 1, and the only nonsignificant $COV_{A_{m,f}}$ was found in one of the least dimorphic traits (yellow chroma). These results imply that the evolution of sexual dimorphism in this species was not facilitated by low intersexual genetic covariance, suggesting other mechanisms should be considered.

First, the observed sexual dimorphism in colour could be driven by environmental differences rather than genetic ones, with a greater sensitivity of one sex to environmental variation. For instance, it has been shown in insects that sex-specific phenotypic plasticity can generate variation in sexual size dimorphism (Stillwell *et al.*, 2010). Differences in plasticity between males and females should lead to consistent differences in sex-specific heritabilities for similar levels of CV_A (Houle, 1992), but this is not a general result witnessed across the focal traits in Table 2. Second, genes linked to sex chromosomes could explain the sexual dimorphism over and above the autosomal genetic (co)variances estimated here, although we could not estimate such sex-linked genetic variance. Third, cross-sex genetic covariances may have changed over the course of the evolution of sexual dimorphism. Meagher (1992) has suggested that during the evolution of sexual

dimorphism, loci that show sex-specific expression should be strongly selected for and should become fixed, thereby no longer contributing to the additive genetic variance. This could explain how $COV_{A_{m,f}}$ could be temporarily low or negative during the evolution of dimorphism, but then large and positive once the sex-specific loci are fixed.

An important limitation of our study is that we could not adopt a truly multivariate approach where genetic covariances between suites of traits within and between the sexes might provide a different view on the genetic constraints for the evolution of sexual dimorphism. Indeed, the evolutionary trajectory of a given sex-specific character can be constrained or facilitated by selection acting on the variance displayed by the same trait expressed in the other sex, but also by positive or negative genetic correlations with other traits within and between both sexes (Blows and Hoffmann, 2005; Poissant *et al.*, 2010). For this reason, future studies will need to integrate cross-sex genetic covariances across traits with multivariate selection analyses (Lande, 1980; Chenoweth *et al.*, 2010) in order to fully uncover how sexual antagonistic selection and intralocus sexual conflicts can promote or constrain the evolution of divergent male and female traits (Wyman *et al.*, 2013). Such an approach has been adopted recently in a study of a laboratory population of *Drosophila melanogaster* (Ingleby *et al.*, 2014) and also in a natural population of barn owl (Roulin and Jensen, 2015). Yet, model complexity combined with data availability still largely prevent such multivariate analyses in many natural populations.

CONCLUSION

Overall, this study brought three major advancements in our understanding of the evolution of colour ornamentation and sexual dimorphism. First, the present analyses demonstrated heritability for UV colouration (in both sexes) and yellow colouration (in males), a major requirement for the evolution of colour through sexual or social selection. Second, our simulations revealed the low power of animal models to estimate sex-linked additive genetic variance in wild populations, thereby hampering our ability to test a major hypothesis for the evolution of sexual dimorphism. Third, in the current debate on the evolution of female ornaments, the present results suggest that cross-sex genetic correlations can be very high in colour traits across varying degrees of dimorphism. A fine-scale analysis of sex-specific forces of natural, social and sexual selection is now required to determine the role of indirect (selection acting on males) and direct selection for the evolution of female ornaments. Future genomic studies should be used to determine whether the same genes underlie colouration in males and females.

DATA ARCHIVING

Phenotypic and pedigree data sets available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.gp384>. The raw data will be embargoed for 5 years, but could be made available during this period upon request to the authors.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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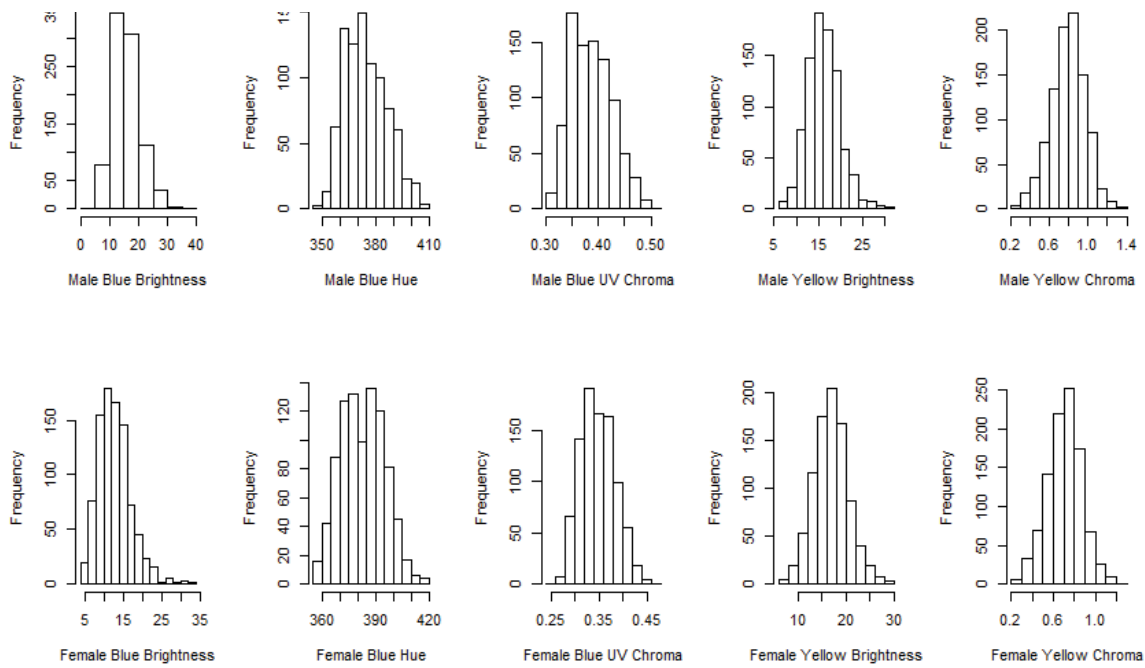
Supplementary Information

Supplemental material for ‘Colour ornamentation in the blue tit: quantitative genetic (co)variances across sexes’

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Doutrelant

Figure S1. Distributions of colour parameters measured in male and female blue tits a) in the combined Corsican populations of Muro and Piriò and b) in the Rouvière population (French mainland).

a) Corsica



b) Rouvière

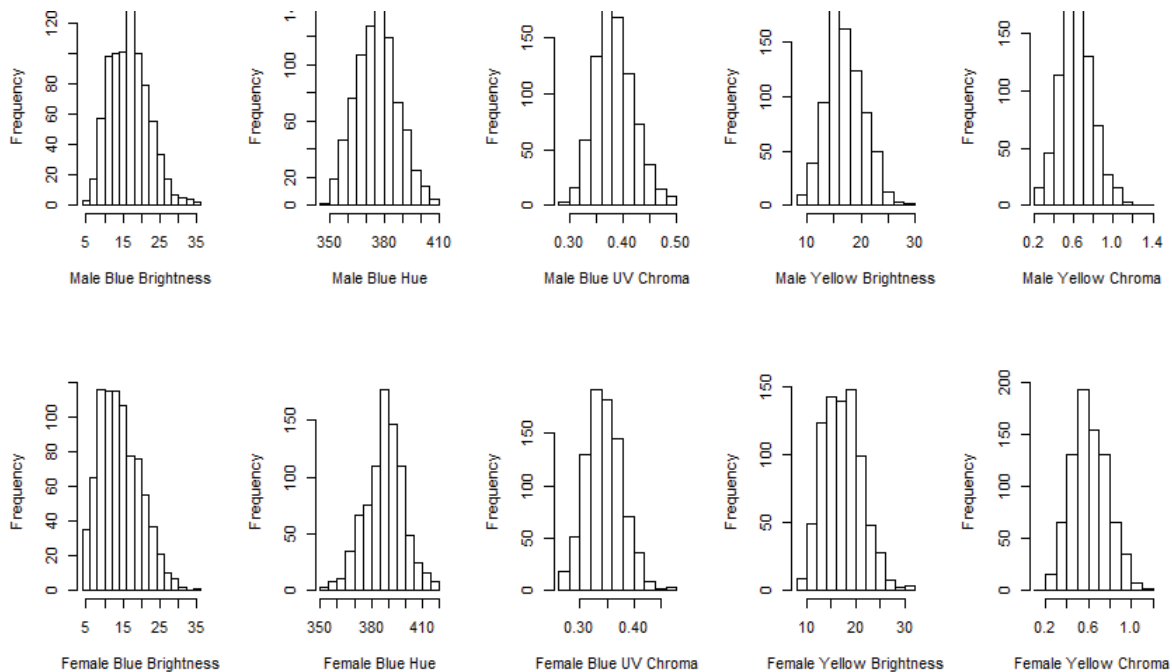


Table S1. Phenotypic Spearman's rank correlation rho and associated p -value for the five sex-specific colour measures, a) in the combined Corsican populations of Muro and Pirio; and b) in the Rouvière population (French mainland). In red (above diagonal) for females; in blue (below diagonal) for males. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

a) Corsica	Blue brightness	Blue hue	Blue UV chroma	Yellow brightness	Yellow chroma
Blue brightness		-0.181***	0.057	0.111**	0.028
Blue hue	0.036		-0.491***	-0.150***	-0.006
Blue UV chroma	-0.145***	-0.476***		0.041	0.142***
Yellow brightness	0.083*	-0.177***	0.012		-0.312***
Yellow chroma	-0.03	0.067*	0.246***	-0.372***	

b) Rouvière	Blue brightness	Blue hue	Blue UV chroma	Yellow brightness	Yellow chroma
Blue brightness		-0.063	-0.129***	0.180***	0.223***
Blue hue	0.191***		-0.672***	-0.043	0.021
Blue UV chroma	-0.360***	-0.663***		-0.101**	-0.071*
Yellow brightness	0.171***	-0.082*	0.048		-0.113**
Yellow chroma	0.143**	0.007	-0.05	-0.022	

APPENDIX A1. Exploring Fixed Effects in Quantitative Genetic Analyses

When running preliminary quantitative genetic models with/without year as a fixed effect, the comparison showed that annual influences on colour were such that, if omitted as a fixed effect, the heritability estimation could be twice as large as those presented in the results section and cross-sex genetic covariances were also often stronger and significant. This inflation of additive genetic variance, heritability, and cross-sex genetic covariance, is most probably due to year effects inducing resemblance between relatives, in particular sibs. For this reason, year of measure was included in all models as a fixed effect. The other fixed effects explored, *i.e.* year of birth, period of measurement (in three periods: January+February, March+April and May+June, roughly corresponding to winter, nest building and chick rearing), and age (coded as younger than 2 years old *versus* 2 years or older) had no such influence on the quantitative genetic parameters. In order to avoid upwardly biased heritability estimates classically resulting from overparameterized models (Wilson, 2008), these fixed effects were not included in the models.

APPENDIX A2. Combining the Two Corsican Populations

In order to test whether we could pool the data from the two Corsican populations in a single quantitative genetic model, we tested for an equality of additive genetic variances between the two populations (see similar between-population comparison of variance components in Husby *et al*, 2010). We first combined the datasets and pedigree information from both populations in a bivariate animal model where the trait expressed in each population was one dependent variable. Hence this bivariate model considered the colour trait expressed in the two populations as separate traits. Since our dataset did not include any individual measured in both populations, covariances between the population-specific traits were set to zero. For each sex-specific colour trait, we first used this bivariate model to estimate V_A , V_{PE} and V_R in the two populations similar to two univariate models as described in equation 1. Second, we constrained V_A to be equal between the two populations. Third, we used a likelihood ratio test (LRT) to compare the likelihood of both models, thereby testing for the equality of V_A between the two populations.

This procedure, comparing models where additive genetic variances for Muro and Pirio were constrained to be equal or unconstrained, was repeated 10 times across all five sex-specific traits. In four cases out of 10, the models were not significantly different ($p > 0.15$), indicating that the level of sex-specific additive genetic variance was similar in both populations. In two cases (female yellow brightness and female blue UV chroma), the bivariate sex-specific model showed convergence problems, but V_A was not significant in either the constrained or unconstrained model. In four cases (female blue brightness and blue hue, male and female yellow chroma) the model with distinct population-specific V_A showed a better fit than a model constraining both V_A to be equal ($p < 0.01$), but V_A was not significant in either

population for the constrained or the unconstrained model. These models highlighted the issue of low power when splitting the Corsican populations since out of five sex-specific traits significant in the final analysis presented in Table 2, only two remained significant when using separate datasets. Hence for all further results, the datasets from Muro and Pirio are pooled in a single Corsican dataset, while fitting two distinct genetic groups in the animal models.

APPENDIX A3. Power Calculations to Estimate Colour Ornamentation Sex-Chromosomal Additive Genetic Variance

The different inheritance pattern of autosomes versus sex chromosomes introduces two problems for quantitative genetic analyses: potential bias when assuming an autosomal only model of inheritance (Wolak, 2013) and problems disentangling autosomal from sex-linked sources of additive genetic variance in empirical data (Fairbairn and Roff, 2006; Meyer, 2008). The second point is particularly relevant in quantitative genetic studies of free-living populations, because the power of the animal model to statistically separate sex-chromosomal from autosomal additive genetic variation depends on the number and particular classes of relatives for which phenotypic information is available. Specifically, only certain groupings of pairwise relationships are informative to determine the sex-specific autosomal versus sex-chromosomal contributions to phenotypic resemblance among relatives (e.g., sets of double first cousins, where each parent of one of the double first cousins is either a same-sex or opposite-sex full sibling with one of the parents of the other double first cousin), which may or may not be prevalent in data collected from wild populations (Fairbairn and Roff, 2006; Wolak and Keller, 2014). Therefore, we conducted power simulations to directly quantify the ability of the blue tit data and pedigree to estimate sex-linked additive genetic (co)variances.

Phenotypes were simulated according to the number and pattern of observations on blue brightness. In both Corsica and Rouvière, blue brightness had the greatest number of records in the dataset, implying that our simulation uses the trait with the most powerful data structure (e.g., number of observations). For both Corsica and Rouvière rows of the data with missing values for blue brightness were discarded. We used a Monte Carlo simulation approach where the i^{th} individual's j^{th} phenotype was specified as:

$$y_{ij} = \mu + a_i + z_i + p_{ij} + r_{ij} \quad (\text{eq S1})$$

where μ was the overall population phenotypic mean (fixed at 0 for all simulations), a_i was the additive genetic effect of individual i 's autosomal genes (i.e., the autosomal breeding value and was the same for each of the j observations on i), z_i was the additive genetic effect of individual i 's Z-linked genes (i.e., the Z-chromosomal breeding value and was the same for each of the j observations on i), p_{ij} was the permanent individual effect of individual i on the j^{th} observed phenotype (assumed to reflect all non-additive genetic and repeatable environmental effects on the phenotype), and r_{ij} was a residual deviation for observation j of individual i . All effects were assumed independent of one another. Individual values of a_i , z_i , p_{ij} , and r_{ij} were sampled from normal distributions where the population values of these effects were assumed to be approximately distributed: $\mathbf{a} \sim \text{BVN}([0,0]^T, \mathbf{G}_A \otimes \mathbf{A})$, $\mathbf{z} \sim \text{BVN}([0,0]^T, \mathbf{G}_Z \otimes \mathbf{S})$, $\mathbf{p} \sim \text{N}(0, \mathbf{Z}_P \mathbf{Z}_P^T \sigma_P^2)$, $\mathbf{r} \sim \text{N}(0, \mathbf{I} \sigma_R^2)$, where lower and upper case bold letters denote vectors and matrices, respectively, \otimes denotes a Kronecker (direct) product, T a matrix transpose, BVN a bivariate normal distribution, N a univariate normal distribution, \mathbf{A} the numerator relationship matrix (expected autosomal relatedness among individuals), \mathbf{S} the Z-chromosomal relatedness matrix (expected sex-chromosomal relatedness among individuals), \mathbf{Z}_P is an n row (number of observations) by i column (number of individuals) matrix that assigns the n^{th} observation to the i^{th} individual, and \mathbf{I} an n by n identity matrix (1s along the diagonal and 0s as all the off-diagonal elements). Further, \mathbf{G}_A and \mathbf{G}_Z are the autosomal and sex-chromosomal additive genetic covariance matrices:

$$\mathbf{G}_A = \begin{bmatrix} \sigma_{A-male}^2 & \sigma_{A-male,female} \\ \sigma_{A-female,male} & \sigma_{A-female}^2 \end{bmatrix} \quad (\text{eq S2a})$$

$$\mathbf{G}_Z = \begin{bmatrix} \sigma_{Z-male}^2 & \sigma_{Z-male,female} \\ \sigma_{Z-female,male} & \sigma_{Z-female}^2 \end{bmatrix} \quad (\text{eq S2b}),$$

where σ_A^2 and σ_Z^2 are autosomal and Z-chromosomal additive genetic variances respectively, σ specifies a between-sex genetic covariance, and finally σ_P^2 and σ_R^2 are the permanent individual and residual variances, respectively. Female and male permanent individual and residual variances are each assumed to be uncorrelated and equal between the sexes

From the `nadiv` (Wolak, 2012) R package, we used the `'grfx'` function to simulate random effects in conjunction with the functions `'makeA'` and `'makeS'` (to create the autosomal and sex-chromosomal additive genetic relationship matrices, respectively).

The values of the permanent individual and residual variances were set equal to $\sigma_P^2=10$ and $\sigma_R^2=50$ for all simulations (we use a Greek sigma for the expected value of a variance, but V for estimated values of a variance). For simplicity, we modelled equal variances between the sexes for both autosomal and Z-chromosomal (when expressed in terms of variance in the homogametic sex) additive genetic variances. Although the sexes were given equal variances, autosomal and Z-chromosomal between-sex additive genetic correlations (r_{A-a} and r_{A-z} , respectively) were each set to equal either 0, 0.5, or 0.99 (effectively one). This yielded nine unique combinations of autosomal and Z-linked between-sex correlations (Table S2). For a given combination of between-sex additive genetic correlations, we set the autosomal additive genetic variance at three levels: $\sigma_{A-female}^2=\sigma_{A-male}^2=2, 50, \text{ or } 100$, thereby yielding 27 unique combinations of autosomal additive genetic, permanent environment, and residual variances along with cross-sex autosomal and sex-linked correlations (Table S2). Within each of these unique combinations, the Z-linked additive genetic variance was set to one of seven values: $\sigma_{Z-male}^2=1, 10, 30, 50, 60, 70, \text{ or } 90$ to assess the power at each level.

Models used to analyze the simulated data were:

$$\mathbf{y} = \mathbf{1}\boldsymbol{\beta} + \mathbf{Z}_A\mathbf{a} + \mathbf{Z}_{ZZ} + \mathbf{Z}_P\mathbf{p} + \mathbf{r} \quad (\text{eq S3a})$$

$$\mathbf{y} = \mathbf{1}\boldsymbol{\beta} + \mathbf{Z}_A\mathbf{a} + \mathbf{Z}_P\mathbf{p} + \mathbf{r} \quad (\text{eq S3b})$$

where $\mathbf{1}$ is a vector of ones (relating each observation to the model intercept, or population mean, $\boldsymbol{\beta}$), the \mathbf{Z} s denote design matrices relating each observation in \mathbf{y} to the appropriate random effect, and \mathbf{a} , \mathbf{z} , \mathbf{p} , and \mathbf{r} are vectors of random autosomal additive genetic, Z-chromosomal additive genetic, permanent individual, and residual effects, respectively. In ASReml-R we used the 'corgh' function to specify a correlation structure with heterogeneous variances for males and females of \mathbf{a} (linked to the inverse of the \mathbf{A} matrix created with the 'makeAinv' function in nadiv), the 'us' function to specify an unstructured covariance matrix for males and females of \mathbf{z} (linked to the inverse of the \mathbf{S} matrix created with the 'makeS' function in nadiv), and the 'idh' function to specify a diagonal covariance matrix with heterogeneous variances for males and females (and no between-sex covariance) for \mathbf{p} and \mathbf{r} . If the two models converged, we compared them using a likelihood ratio test statistic (Self and Liang, 1987).

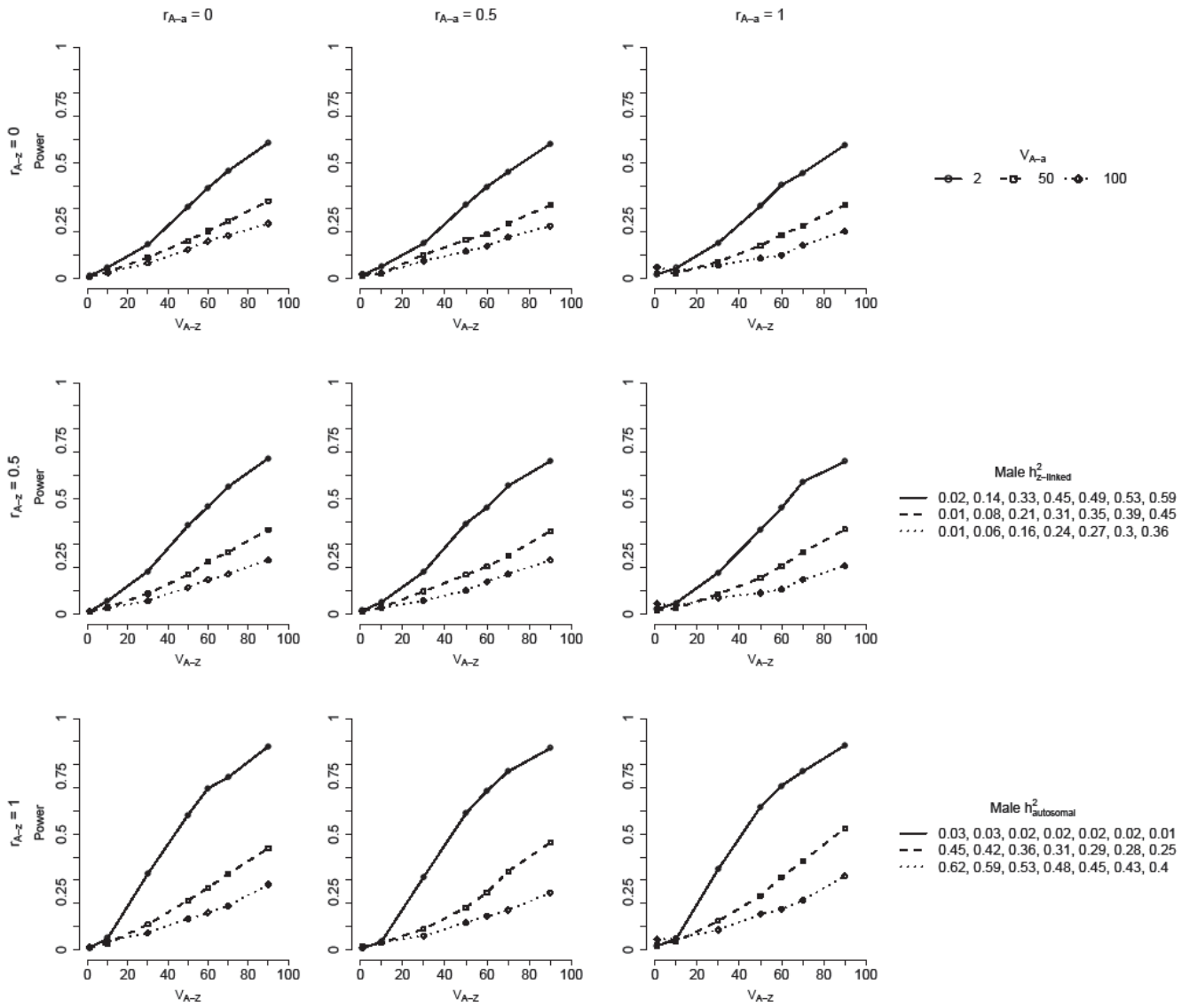
Because some of the variance in sex-chromosomal effects will contribute to the V_A estimate when the model does not include a term to estimate V_Z (Wolak, 2013), we interpret our null hypothesis test as a test of whether the amount of V_Z that is not explained by autosomal relatedness is greater than zero. Thus, if most phenotypic variance is sex-linked, but the model attributes this variance to V_A such that no additional variance can be attributed to the sex-chromosomal term, then our models cannot reject the null hypothesis.

Table S2. Parameter combinations of expected values used for Monte Carlo simulation.

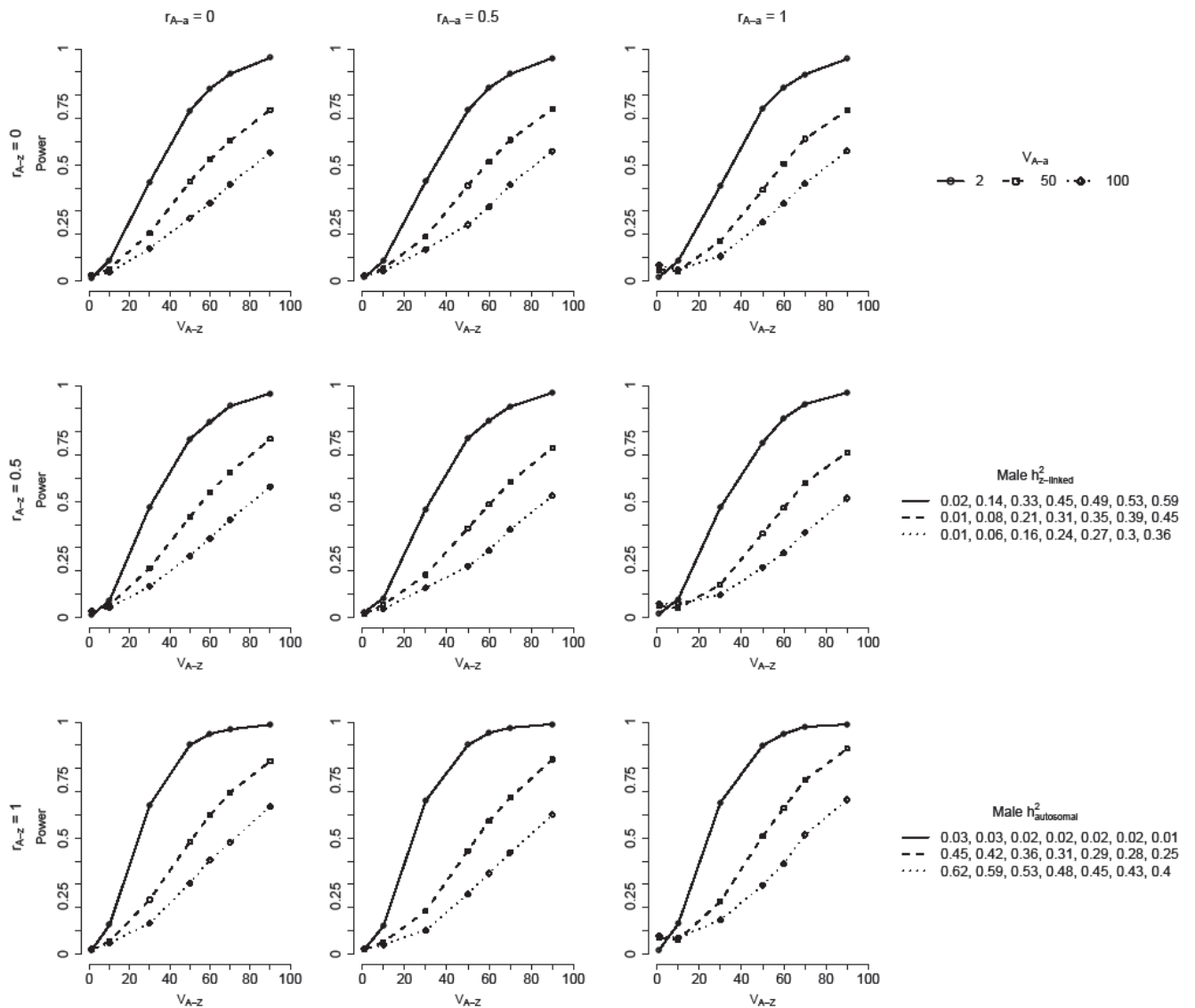
r_{A-a}	r_{A-z}	σ_A^2	σ_P^2	σ_R^2	σ_{Z-male}^2
	0				
	0	2	10	50	1, 10, 30, 50, 60, 70, 90
		50	10	50	1, 10, 30, 50, 60, 70, 90
		100	10	50	1, 10, 30, 50, 60, 70, 90
	0.5	2	10	50	1, 10, 30, 50, 60, 70, 90
		50	10	50	1, 10, 30, 50, 60, 70, 90
		100	10	50	1, 10, 30, 50, 60, 70, 90
	1	2	10	50	1, 10, 30, 50, 60, 70, 90
		50	10	50	1, 10, 30, 50, 60, 70, 90
		100	10	50	1, 10, 30, 50, 60, 70, 90
0.5	0	2	10	50	1, 10, 30, 50, 60, 70, 90
		50	10	50	1, 10, 30, 50, 60, 70, 90
		100	10	50	1, 10, 30, 50, 60, 70, 90
	0.5	2	10	50	1, 10, 30, 50, 60, 70, 90
		50	10	50	1, 10, 30, 50, 60, 70, 90
		100	10	50	1, 10, 30, 50, 60, 70, 90
	1	2	10	50	1, 10, 30, 50, 60, 70, 90
		50	10	50	1, 10, 30, 50, 60, 70, 90
		100	10	50	1, 10, 30, 50, 60, 70, 90
1	0	2	10	50	1, 10, 30, 50, 60, 70, 90
		50	10	50	1, 10, 30, 50, 60, 70, 90
		100	10	50	1, 10, 30, 50, 60, 70, 90
	0.5	2	10	50	1, 10, 30, 50, 60, 70, 90
		50	10	50	1, 10, 30, 50, 60, 70, 90
		100	10	50	1, 10, 30, 50, 60, 70, 90
	1	2	10	50	1, 10, 30, 50, 60, 70, 90
		50	10	50	1, 10, 30, 50, 60, 70, 90
		100	10	50	1, 10, 30, 50, 60, 70, 90

Figure S2. Power to estimate Z-chromosomal additive genetic variance in the a) Corsican and b) mainland Rouvière populations. Between-sex autosomal additive genetic correlations (r_{A-a}) vary from zero to one among columns moving left to right and Z-chromosomal additive genetic correlations (r_{A-z}) vary from zero to one among rows moving from top to bottom. Power is calculated as the proportion of simulations for which the model with Z-chromosomal additive genetic (co)variances fitted significantly better than a model without. Power was assessed at seven values of Z-chromosomal additive genetic variance (values along the x-axes) and three values of autosomal additive genetic variance (V_{A-a} ; solid, dashed, and dotted lines). This yielded different Z-chromosomal and autosomal heritabilities ($h^2_{Z-linked}$ and $h^2_{autosomal}$, respectively). The $h^2_{Z-linked}$ and $h^2_{autosomal}$ heritabilities are shown for each combination of V_{A-a} and Z-chromosomal additive genetic variance.

a)



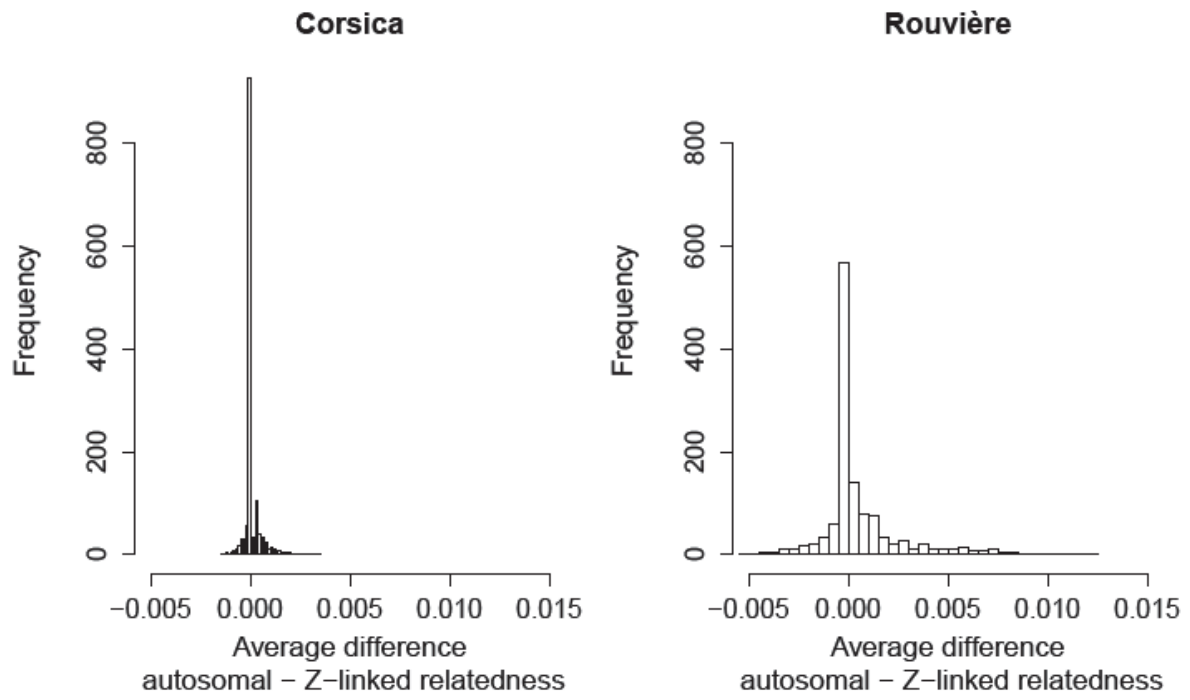
b)



Neither pedigree contains individuals that are double first cousins with one another, which partly explains the low power to estimate Z-linked additive genetic variance. The Rouvière pedigree is more connected than the combined Corsican pedigrees. This also explains why we found overall greater power to detect Z-linked additive genetic variance in the Rouvière data. In Rouvière ($n=1,233$), individuals have non-zero autosomal relatedness with a mean of 136.0 individuals (median=21, range=0-531) and non-zero Z-linked

relatedness with a mean of 56.9 individuals (median=7, range=0-388). Whereas in the Corsican pedigree (n=1,507) individuals have non-zero autosomal relatedness with a mean of 6.4 individuals (median=1, range=0-81) and non-zero Z-linked relatedness with a mean of 4.3 individuals (median=1, range=0-51). Consequently, the number of relatives useful for partitioning phenotypic resemblance into additive genetic sources (i.e., autosomal and Z-linked) is low in the Corsican populations. Separating Z-linked from autosomal additive genetic contributions to the resemblance among relatives requires a contrast between related individuals in the expected proportion of allele copies shared identical by descent on the autosomes versus the Z chromosome. This contrast is quantified by the difference between elements of pair-wise relatedness in the autosomal relatedness matrix compared to the Z chromosomal relatedness matrix (figure S3). Most elements of the autosomal and Z chromosomal relatedness matrices are very similar, more so in Corsica (figure S3 left panel) than Rouvière (figure S3 right panel).

Figure S3. Mean differences between elements in the autosomal and Z chromosomal additive genetic relatedness matrices in Corsica (left panel) and Rouvière (right panel).



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MS3 Quantification of selection on coloured traits in both sexes: selection for female colouration despite large spatiotemporal variation

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Preliminary note: this manuscript is in its early stage of preparation. It will be completed by more analyses that will also test the relationships between colouration and later proxies of reproductive success, namely number of fledglings produced. It is thus the first draft of a work in progress.

Introduction

For an evolutionary change in traits to be carried on, variation in the focal trait, relation with fitness and heritability are requested. In that context, certain forms (variation) of a trait are expected to confer a higher survival and/or reproductive success (fitness) to bearers; if heritable this trait is then transmitted to the offspring (Darwin, 1871). Here to determine how ornamentation is affected by reproductive selection in blue tits, we quantified relations between two traits of colouration (ultraviolet-blue colouration and yellow carotenoid-based colouration) and proxies of reproductive success. We quantified selection on ornaments in both sexes allowing interaction between male and female traits. Following Lande & Arnold method (1983), we quantified directional selection by regression-based approaches.

Given that selection may be spatially and temporally variable (Kuijper *et al.*, 2012; Parker, 2013) and that plumage colouration is expected to be affected by environmental conditions leading to variation among years and populations (McGraw, 2006), we used a large sample covering ten years of studies in four Mediterranean blue tit populations. After quantifying estimates of directional selection for males and females in each population, each year and each variable of colouration, we used a within-study meta-analysis approach (Nakagawa & Santos, 2012) to detect whether selection occurs in some colour traits.

Given that environment is often variable (Cockburn, 2014) we expected some spatiotemporal variation in selection. Given that we had only worked on early stages of breeding (laying date, clutch size) we expected our proxy of reproductive success to be more related to female estimates than male estimates if female ornaments are functional. Former papers on colourations in these populations found that both ultraviolet-blue and yellow colourations were condition-dependent in females (Doutrelant *et al.*, 2008, 2012; Midamegbe *et al.*, 2013). We thus expected to find selection in both colourations.

Methods

Feather collection

Four Mediterranean populations of blue tits have been monitored during breeding season for more than twenty years. One population D-Rouvière is located on mainland France, in a forest of deciduous oaks 18 km northwest from the city centre of Montpellier (43°39'N, 3°40'E). The three other populations are situated in the north of Corsican Island. Two populations D-Muro and E-Muro are situated respectively in a deciduous-oak habitat and in an evergreen-oak habitat are located in the Regino Valley and are 5km distance from each other (42°32'N, 8°54'E). The last population E-Pirio is situated in an evergreen-oak forest in the Fango Valley, which is 25 km away from the Regino Valley (42°22'N, 8°44'E). Males and females were captured on nest during chick attendance when nestling were between nine to 15 days old. Each bird was equipped and identified with a metal ring from CRBPO (research centre on bird population biology, Paris) programme STOC. Morphometric measures were collected (wing length, tarsus length, beak length, weight). Sex was determined based on the presence of a brood patch. Since 2005, on each bird, ten feathers from the yellow chest patch and eight feathers from the blue crown have been collected to allow colouration measurements in the lab.

Colouration measurement

Feather colouration was measured in the lab using AVASPEC 2048 spectrometer (Avantes, Apeldoorn NL) with an AVALIGHT-DHS deuterium-halogen lamp (Avantes, Apeldoorn NL) and a 200 μm optic fibre allowing measurement between 300 and 700 nm, conducting light at a fixed angle of 90° and a fixed distance of 2 mm from the sample. Samples were disposed on a square of black cloth with a white standard reference estimated regularly. Each sample consisted of three feathers from the blue crown or four yellow feathers from the chest patch piled in a pack to recreate natural feather assemblage. We measured each sample three times, the fibre being removed between each measure. For each individual and each colouration, we made measurements on two different samples. Spectra were extracted using software Avicol (Gomez, 2006). After controlling for repeatability (Fargevieille, Grégoire, Charmantier, Del Rey Granado, & Doutrelant, Submitted), we calculated mean spectrum based on the six measurements for each individual and each colouration and extracted five variables. For the blue crown, we calculated an achromatic variable blue brightness (area below the reflectance curve divided by the width of the interval 300 – 700 nm) and two chromatic variables UV-blue hue (wavelength (in nm) where reflectance is maximal) and UV-blue chroma (proportion of the total reflectance falling in the range 300-400 nm). For the yellow patch of the chest, yellow brightness was calculated following the same function as for blue brightness; yellow chroma was calculated as $(R_{700}-R_{450})/R_{700}$ with R representing percentage of reflectance for a given wavelength.

Former studies on our populations showed the spatiotemporal variations of colouration in blue crown and yellow chest patch (Charmantier *et al.*, 2016a). To allow a direct comparison of estimates, we standardized the five colour variables by sex, population and year. We also calculated correlations among variables within a sex (Table 1). Correlations among colour variables were up to 0.33 in absolute value except between UV-blue hue and UV-blue chroma

where correlation was -0.67 for both male and female values.

Table 1 Pearson correlation coefficients between variables for males and females (based on 1618 pairs). * refers to the p-value associated with * for $0.01 < p\text{-value} < 0.05$, ** for $0.001 < p\text{-value} < 0.01$ and *** for $p\text{-value} < 0.001$

Male (n=1618)					Female (n=1618)				
	UV-blue hue	UV-blue chroma	Yellow brightness	Yellow chroma		UV-blue hue	UV-blue chroma	Yellow brightness	Yellow chroma
Blue brightness	0.26	-0.33 (***)	0.02	0.04	Blue brightness	0.02	-0.03	0	0.04
UV-blue hue		-0.67 (***)	-0.02	0.02	UV-blue hue		-0.67 (***)	-0.06 (*)	0.08 (**)
UV blue chroma			0.004	0.007	UV blue chroma			0.02	-0.03
Yellow brightness				-0.28 (***)	Yellow brightness				-0.32 (***)

Proxies of reproductive success

Laying date: laying date was determined as the date when the first egg of a clutch was laid and was then calculated as the number of days since the 1st of March. Due to spatiotemporal variation in phenology in our four populations (Blondel *et al.*, 2006; Charmantier *et al.*, 2016) we used first egg laid of the year in a given population as day 1 for laying date and recalculated laying date for each bird in each population and each year following this rule. We then standardized these new values for laying date. Second attempts of reproduction were excluded from the analyses.

Clutch size: clutch size corresponded to the number of eggs laid in the first breeding attempt. Again, spatiotemporal variation on clutch size have been detected in our four populations (Blondel *et al.*, 2006; Charmantier *et al.*, 2016), we standardized clutch size per year per population.

Estimates of selection gradients

Laying date: multiple linear regression were performed for each year in each population. Laying date was used as the variable to explain. Male and female values (also allowing their interaction) for one colour variable (e.g. blue brightness, ultraviolet-blue hue, ultraviolet-blue chroma, yellow brightness and yellow chroma) were used as predictors. Calculation permitted to extract slope estimates corresponding to directional selection gradients associated with

male, female and their interaction. We also extracted variance associated to each slope estimates. We thus obtained 570 slope estimates and their associated variance value.

Clutch size: the same procedure was repeated with clutch size as the variable to explain.

Within-study meta-analysis

Since we were working with as many as five colour traits for birds from four populations sampled across ten years, a within-study meta-analysis was best suited for detecting potential selection gradients (Nakagawa & Santos, 2012; Hegyi *et al.*, 2015). This method takes into account any non-independence in the data within populations and among variables. The slope estimates and their associated variance we had calculated were used directly as effect size in the meta-analysis (Rosenberg *et al.*, 2013). We included year as a random effect. We ran 2x3 sets of models: for laying date and for clutch size, we ran a set of models for male slope estimates, female slope estimates and the interaction between male and female slope estimates. Colour variable and population were fixed effects, allowing also their interaction. Models were run using the *MCMCglimm* package (Hadfield, 2014) in R; 100000 iterations were performed, with a burn-in of 25000 iterations. We used a DIC model selection approach. The results of the best-fit models gave average directional selection gradients with 95 % confidence intervals (Nakagawa & Santos, 2012).

Results

Temporal variation in selection gradients

For both proxies, we found a large temporal variation in our estimates of selection (Figures 1 and 2 for laying date, Figures 3 and 4 for clutch size).

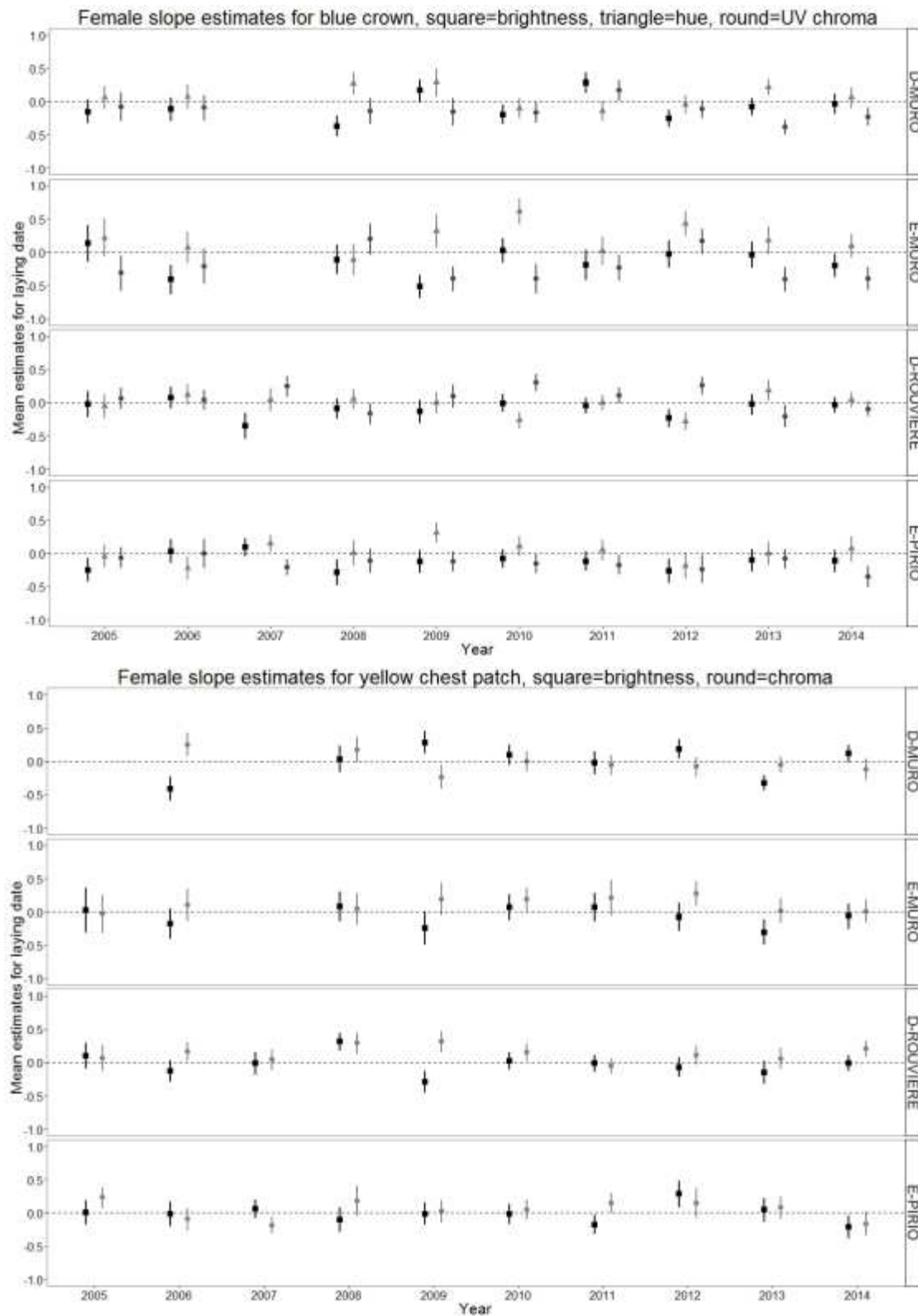


Figure 1 Values of slope estimates (selection gradients) associated with female variables by year and by population with laying date as the variable to explain. Bars correspond to mean slope estimate \pm slope standard-deviation

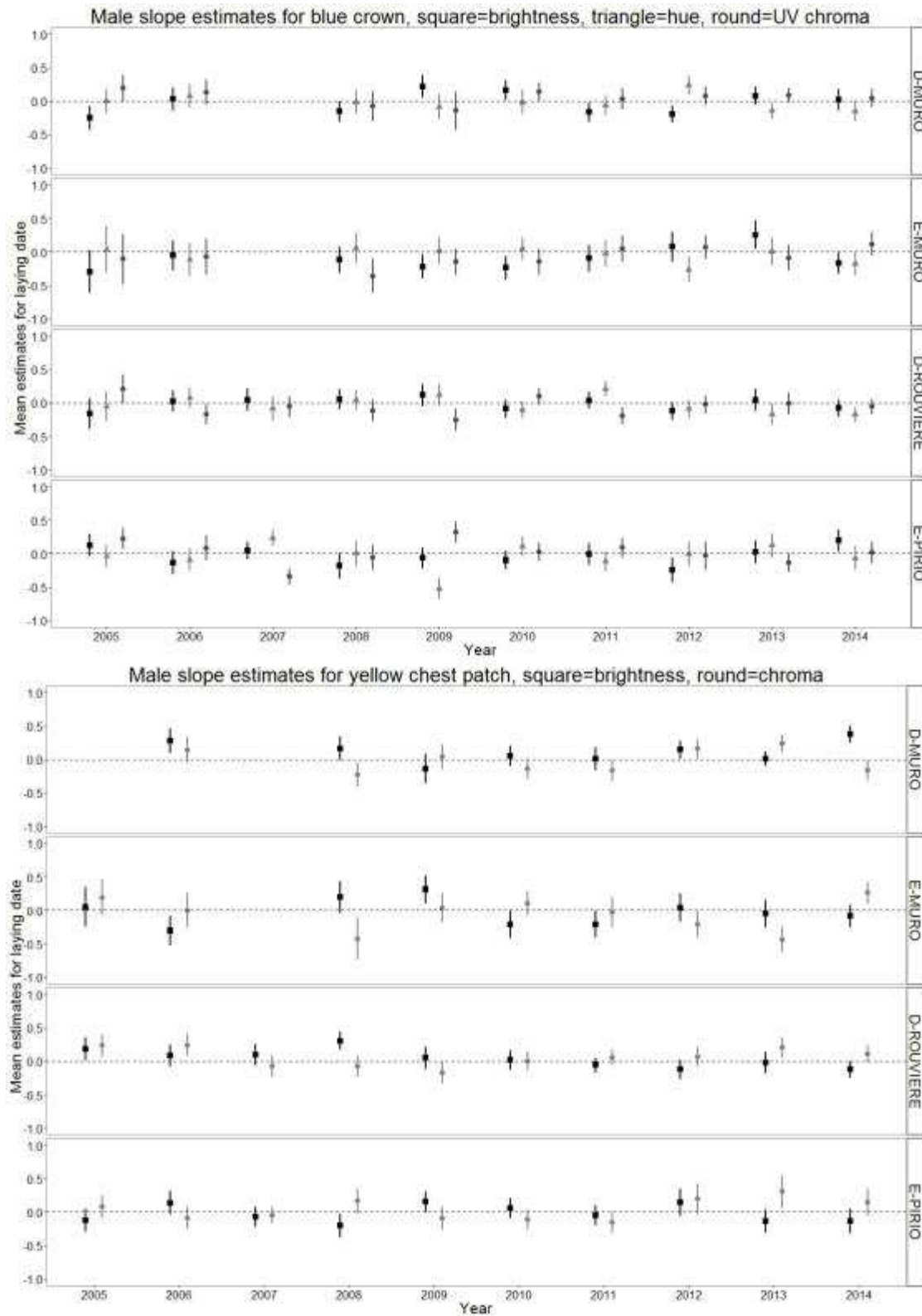


Figure 2 Values of slope estimates associated with male variables by year and by population with laying date as the variable to explain. Bars correspond to mean slope estimate \pm slope standard-deviation

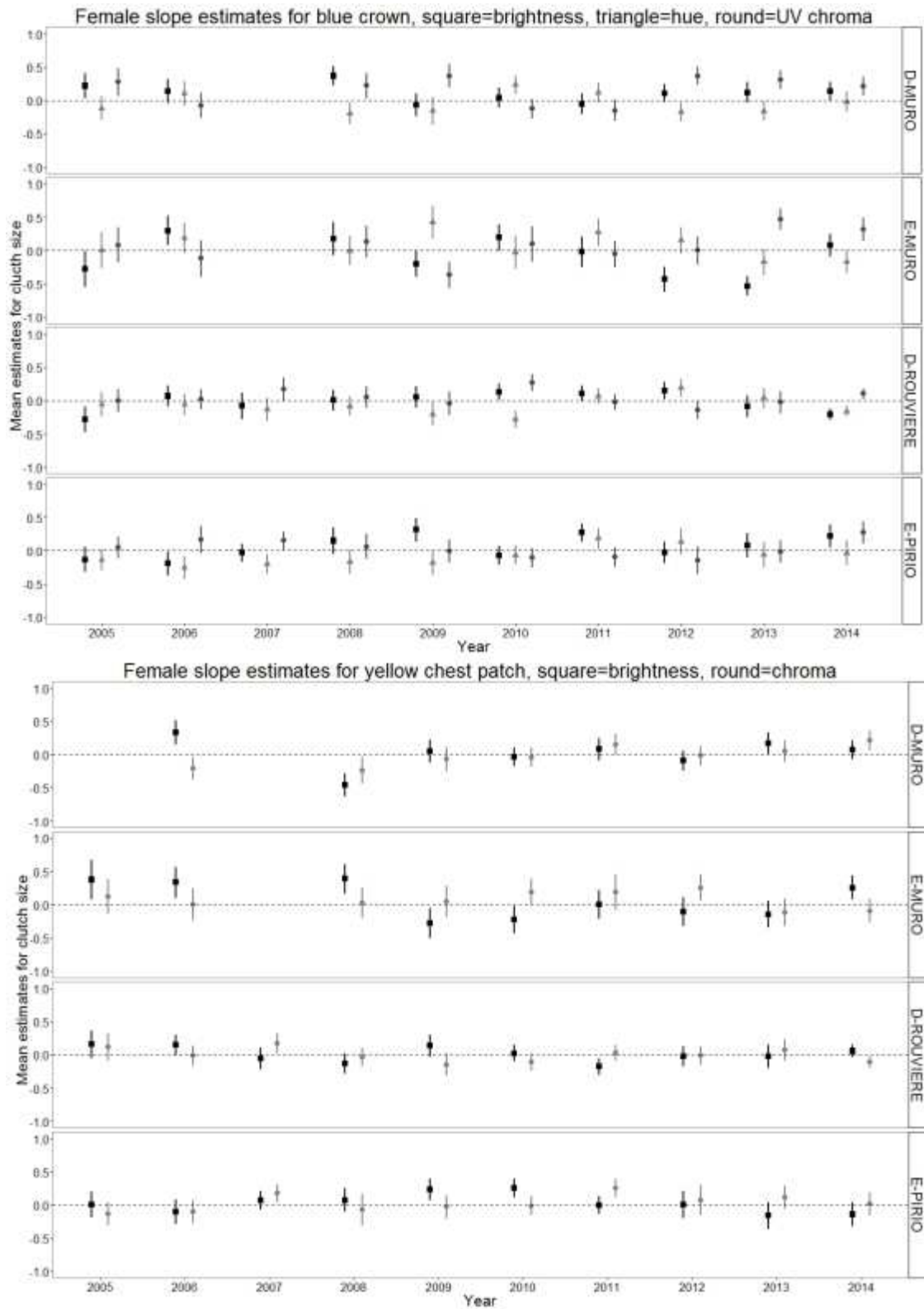


Figure 3. Values of slope estimates associated with female variables by year and by population with clutch size as the variable to explain. Bars correspond to mean slope estimate \pm slope standard-deviation

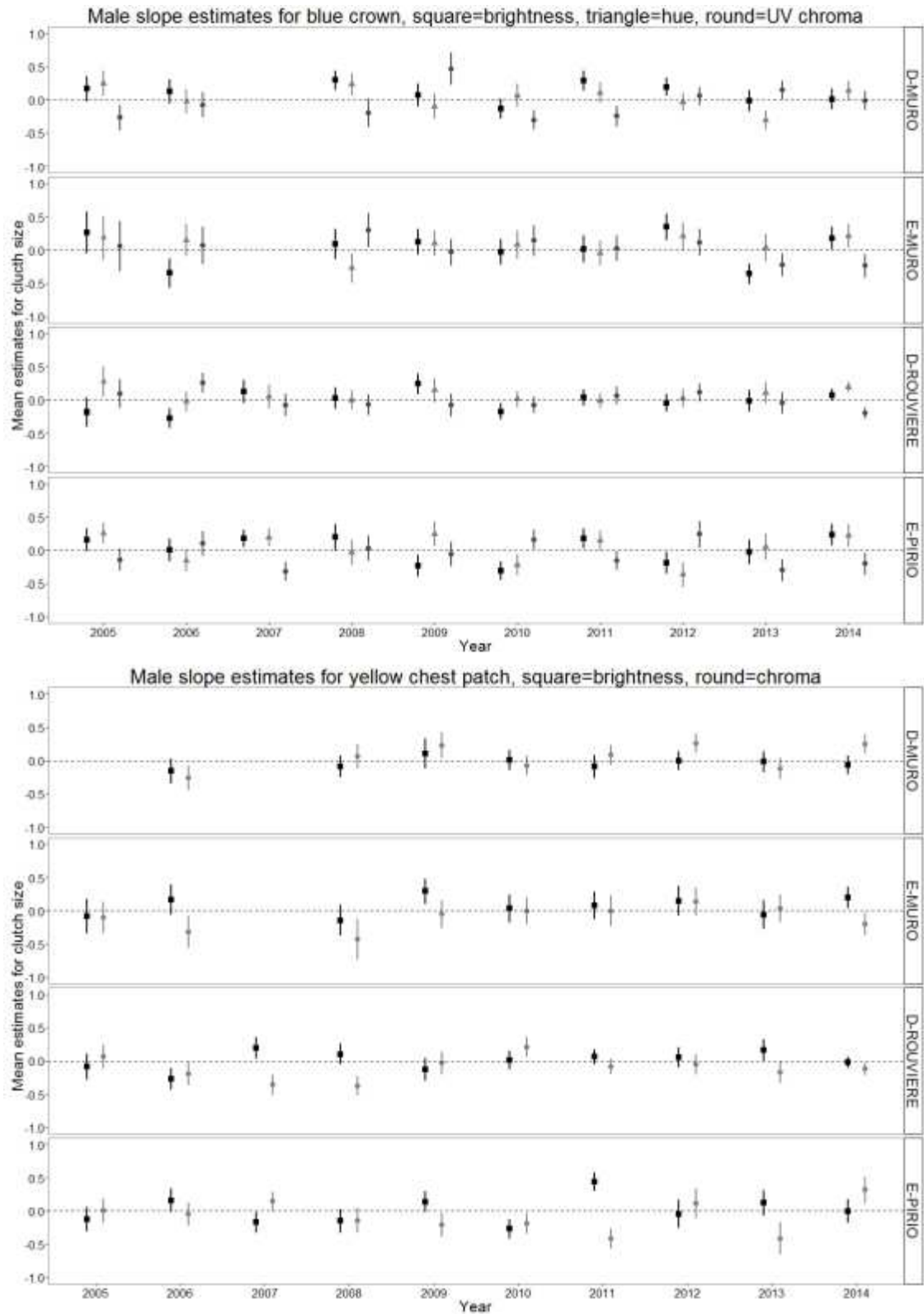


Figure 4. Values of slope estimates associated with male variables by year and by population with clutch size as the variable to explain. Bars correspond to mean slope estimate \pm slope standard-deviation

Model selection**Table 2.** Model selection on meta-analysis procedure for each type of slope estimate in each reproductive success proxy. Models with lowest DIC are in bold.

LAYING DATE						
Estimate type	Model	Variable	Population	Variable x Population	Year (random)	DIC
Male	1.1	x	x	x	x	-530.9
	1.2	x	x		x	-548.4
	1.3	x			x	-533.6
	1.4		x		x	-540.5
	1.5				x	-534.4
Female	2.1	x	x	x	x	-486.6
	2.2	x	x		x	-377.8
	2.3	x			x	-376.0
	2.4		x		x	-293.9
	2.5				x	-277.8
Male x Female	3.1	x	x	x	x	-366.9
	3.2	x	x		x	-402.6
	3.3	x			x	-410.7
	3.4		x		x	-414.3
	3.5				x	-407.6
CLUTCH SIZE						
Estimate type	Model	Variable	Population	Variable x Population	Year (random)	DIC
Male	4.1	x	x	x	x	-393.7
	4.2	x	x		x	-392.4
	4.3	x			x	-395.6
	4.4		x		x	-322.6
	4.5				x	-335.2
Female	5.1	x	x	x	x	-445.6
	5.2	x	x		x	-423.3
	5.3	x			x	-414.1
	5.4		x		x	-382.4
	5.5				x	-362.9
Male x Female	6.1	x	x	x	x	-375.3
	6.2	x	x		x	-422.0
	6.3	x			x	-438.9
	6.4		x		x	-394.1
	6.5				x	-426.1

Laying date: For male estimates, the model including “variable” and “population” as predictors was retained as the best model. For female estimates, the most complex model, including “variable”, “population” and their interaction was retained as the best model. Finally, the model containing “population” as a predictor was retained for the estimates of the interaction between male and female coloration (Table 2).

Clutch size: For male estimates and the estimates of the interaction between male and female, the model only including “variable” as predictor was retained as the best model. For female estimates, the most complex model, including “variable”, “population” and their interaction was retained as the best model (Table 2).

Average selection gradients

Laying date

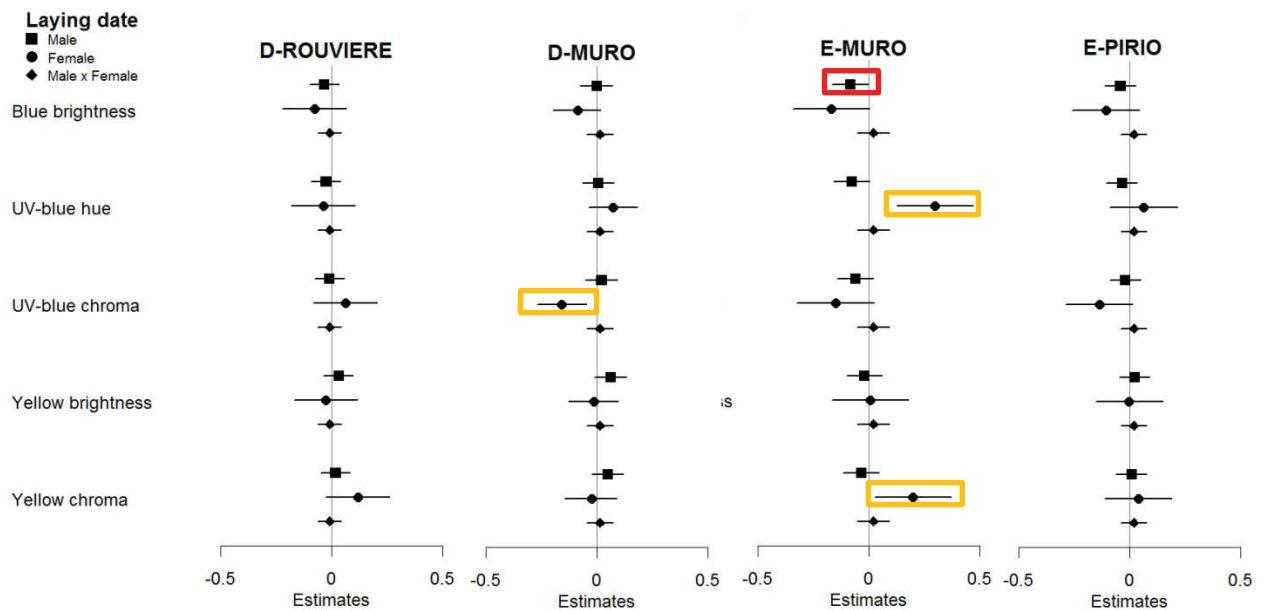


Figure 5. Forest plot with mean slope estimates from within-study meta-analysis procedure for laying date (by population and by type of variable) with associated 95% confidence intervals. Dashed line represents a slope estimate of 0. Orange rectangles correspond to significant female estimates, red one to significant male estimates. Earlier laying dates are generally associated with better reproductive success in most populations of passerines from Northern Europe.

- Male estimates: the only significant result (Figure 5, red rectangle) was a negative selection gradient for blue brightness in E-Muro. Males with brighter blue crown were associated with females laying eggs earlier.

- Female estimates: there were significant estimates (Figure 5, orange rectangles) in two populations. There was a negative selection gradient for UV-blue chroma in D-Muro. Females with a more intense blue crown colouration laid eggs earlier in the season. There were two positive selection gradients for UV-blue hue and yellow chroma in E-Muro. Females with the hue from the blue crown reflecting more towards ultraviolet laid eggs earlier. Also, females with less intense yellow colouration on the yellow chest laid eggs earlier.

- Male x Female estimates: there was no significant result associated with the average estimates of male and female interaction (Figure 5).

Clutch size

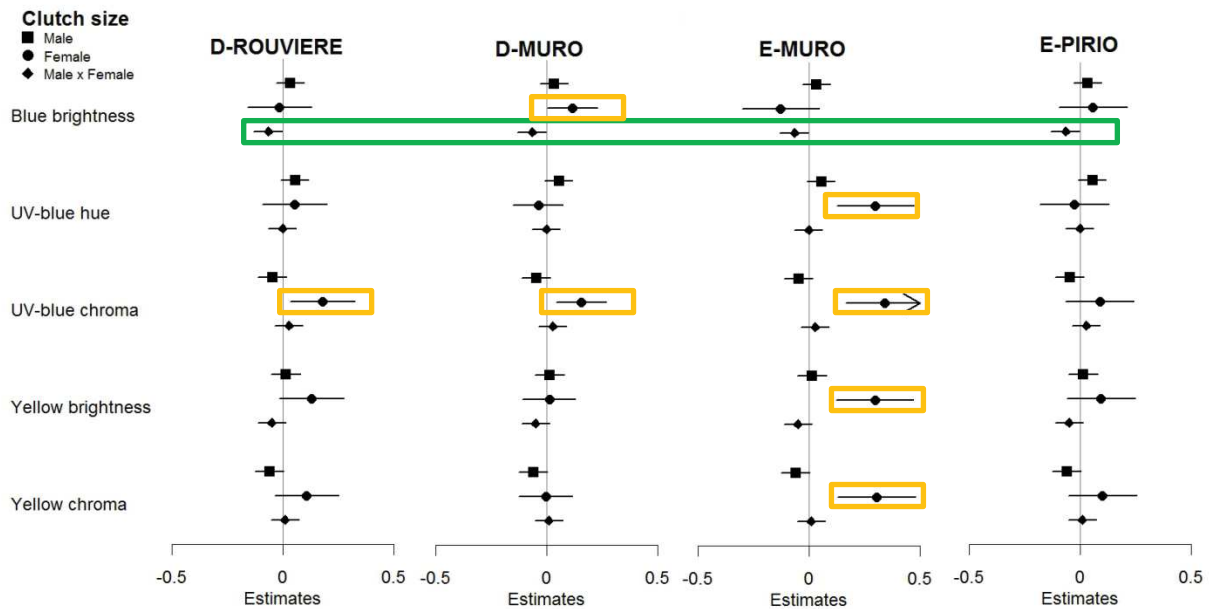


Figure 6. Forest plot with mean slope estimates from within-study meta-analysis procedure for clutch size (by population and by type of variable) with associated 95% confidence intervals. Dashed line represents a slope estimate of 0. Orange rectangles correspond to significant female estimates, green one to significant male x females estimates.

- Male estimates: there was no significant estimate associated with the average estimates of males (Figure 6).

- Female estimates: there were up to seven significant estimates (Figure 6, orange rectangles) in three populations. Four out of the five average selection gradients calculated in E-Muro were positive. For the blue crown, it meant females with a hue reflecting less towards ultraviolet but being more chromatic in ultraviolet (UV-blue chroma) produced a larger clutch. For the yellow chest patch, females with a brighter and more intense yellow colouration also produced a larger clutch. In D-Muro, average selection gradients for two variables of the blue crown were positive, brightness and ultraviolet-blue chroma. It meant females with a brighter and more intense ultraviolet-blue colouration were associated with a larger clutch. Finally, in E-Muro, there was a positive selection gradient for the UV-blue chroma of the blue crown. In this population, females with a more intense ultraviolet-blue colouration had a larger clutch. There was also a positive selection gradient on UV-blue chroma of the blue crown in three out of the four populations. There was no significant result for E-Pirio population.

- Male x female estimates: considering the fittest model only included “variable” as a predictor, there was no difference among populations. There was a significant negative selection gradient for the blue brightness of the blue crown (Figure 6, green rectangle). It can mean that pairs with mismatched blue brightness (either a bright male with a dull female or a bright female with a dull male) were associated to a lower clutch size.

Discussion

We found large temporal variation in selection gradients for both yellow and ultraviolet-blue colouration. Within-study meta-analyses results revealed selection was more frequent on female colouration than on male colouration. Selection was also more frequent on clutch size

than laying date and for the ultraviolet-blue chroma of the blue crown compared to other colour variables. The majority of the significant results concerned the E-Muro population, whereas no significant estimates were obtained in the E-Pirio population.

As expected (Cockburn *et al.*, 2008; Cornwallis & Uller, 2010; Cockburn, 2014), we found a large spatiotemporal variation associated with directional selection gradients for each variable. Interestingly, the temporal variation in the relationship between ornamentation and colouration seems larger in some populations. For instance, in relation to laying date, E-Pirio selection gradients were showing little variation and small values of estimates whereas E-Muro selection gradients were showing larger variation and more important values of estimates (*Statistical tests are needed to confirm the pattern observed*). The spatiotemporal variation we found can be explained by several factors. First, both colourations assessed were found to be condition-dependent and affected by investment in reproduction the year before (Doutrelant *et al.*, 2012). Also, each population has unique characteristics in term of vegetation, breeding success and food availability during moult (Blondel *et al.*, 2006). Some populations may thus provide better signals of quality than others. This large spatiotemporal variation between colouration and proxies of reproduction success may have consequences if colourations are used as a visual signal to assess mate; they can lessen the reliability of the signal. When both colourations are not affected by the same ecological factors, multiplying traits could offer a better reliability of a mate quality.

From within-study meta-analyses, selection models showed a simpler pattern of selection gradients in male colouration and the interaction between male and female colouration (Table 1, Figure 5 and Figure 6). On the contrary, patterns of selection gradients were complex in females with an interaction between “colour variable” and “population” predictors. This suggests a higher

local and temporal variability in selection estimates for females than males. Given that female is the sex producing eggs and that local conditions have a strong impact on egg production, this higher sensibility can be expected.

Selection gradients were more often significant in females than males. This could be explained by the fact that early proxies of reproductive success such as laying date and clutch size are more dependent on female abilities than males. However, if males compete for territories, the difficulty of acquiring a territory early in the season could have led males of lower quality to be associated with females less coloured females, laying eggs later. Such a scenario could have been possible given that blue crown colouration was found to be a badge of status used during male-male interactions (Remy *et al.*, 2010; Vedder *et al.*, 2010). The fact we found very few results related to male colouration or the interaction between male and female colouration suggest female quality has a predominant effect.

The frequency of significant selection gradients associated with female colouration estimates confirmed the potential for female evolution on colouration in blue tits. Because it is a conclusion based on 10 years of data across four populations - and not on two to three years in a single population (Parker, 2013) - we have confidence in this conclusion. However the next important step is to look at later proxies of reproductive success to evaluate the strength of these results.

In three out of the four populations, selection gradients on blue colouration were significant and so blue colouration was more frequently associated with significant values of selection gradients. In our populations, blue colouration is more consistently heritable than yellow colouration in females (Charmantier *et al.* 2016). It may signal a better genetic capacity to reproduce early and to invest a lot in reproduction. Also blue colouration has been associated to

female-female competition in our blue tit populations (Midamegbe *et al.*, 2011). It is also possible that females with more intense ultraviolet-blue colouration are more competitive females, which are able to secure a better territory or be in better conditions to lay earlier. These two explanations could explain why ultraviolet-blue colouration of the crown may be more associated with early stages of reproduction. Yellow colouration is a carotenoid-based colouration and carotenoids are pigments coming from diet, which can also be used as antioxidants (Burton & Ingold, 1984). This colouration can be more related to later stages of reproduction, especially with nestling survival or fledging survival (*this will be tested by the analyses I plan to conduct soon*).

Interestingly E-Muro was the population with the highest frequency of significant selection gradients. E-Muro is the population that faces the higher selective pressure to be in synchrony with its habitat of reproduction (Charmantier *et al.*, 2016). E-Pirio and D-Muro present laying date adapted to the local predominant vegetation. E-Muro is by contrast a patch of evergreen forest situated in a deciduous valley and presents a maladapted laying date (Porlier *et al.*, 2012). This population could be under stronger selection which may explain our results. Furthermore, D-Muro and E-Muro are close populations with different habitats and some insights of local adaptation (Charmantier *et al.*, 2016; Szulkin *et al.*, 2016). We also found a stronger assortative mating in those populations (Fargevieille *et al.*, Submitted). By contrast, there was no result associated with the E-Pirio population. Working on late stages of reproduction is needed to see whether selection on colouration is more related to these stages in this population where finding food for nestlings is a real constraint (Blondel *et al.*, 2006).

To conclude, the first results we present here brought two important conclusions: first female ornaments are associated with significant reproductive selection gradient and can thus evolve through selection, second our study confirms that more than a few years and populations are needed to conclude about selection in natural populations.

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B - Assortative mating on two coloured patches**1 Assortative mating by colored ornaments in blue tits: space and time matter**

2

3

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8

9 Summary

10 Assortative mating is a potential outcome of sexual selection and estimating its level
11 is important to better understand local adaptation and underlying trait evolution.
12 However, assortative mating studies frequently base their conclusions on small
13 numbers of individuals sampled over short periods of time and limited spatial scales
14 even though spatiotemporal variation is common. Here, we characterized assortative
15 mating patterns over 10 years in four populations of the blue tit (*Cyanistes caeruleus*),
16 a passerine bird. We focused on two plumage ornaments—the blue crown and the
17 yellow breast patch. Based on data for 1,657 pairs of birds, we found large interannual
18 variation: assortative mating varied from positive to negative. To determine whether
19 there was nonetheless a general trend in the data, we ran a within-study meta-analysis.
20 It revealed that assortative mating was moderately positive for both ornaments. It also

21 showed that mating patterns differed among populations, and especially between two
22 neighboring populations that displayed phenotypic divergence. Our results therefore
23 underscore that long-term studies are needed to draw broad conclusions about mating
24 patterns in natural populations. They also call for studying the potential role of
25 assortative mating in local adaptation and evolution of ornaments in both sexes in blue
26 tits.

27 **Keywords:** Color traits; *Cyanistes caeruleus*; mate choice; pairing association;
28 secondary sexual characters

29

30 **Introduction**

31 Studying sexual selection in the wild poses several methodological challenges. In
32 particular, in animal species with separate sexes, measuring the direction and force of
33 intra- and intersexual selection requires collecting information on difficult-to-quantify
34 behaviors from large numbers of individuals. An alternative approach is to study the
35 outcome of selection via mating patterns and more specifically positive or negative
36 assortative mating, which is when an organism pairs with a similar or dissimilar mate,
37 respectively (Jiang et al. 2013).

38 Assortative mating may be the consequence of many factors such as habitat choice or
39 timing of breeding but is often a consequence of mate choice (Andersson 1994; Price
40 2008). It is relatively straightforward to study assortative mating, and it has been the
41 subject of much short-term research conducted at small spatial scales (Jiang et al.
42 2013). However, the conclusions of such work may be unreliable because key studies
43 conducted on traits subject to sexual selection have clearly shown that mate choice

44 can vary in space and time (Svensson and Gosden 2007). For instance, female
45 preferences for male body size vary greatly among neighboring populations of the
46 damselfly *Ischnura elegans* (Svensson and Gosden 2007). Likewise, severe drought
47 can cause atypical dispersal events that fragment group structure and thus affect the
48 direction of sexual and kin selection in the white-winged chough, *Corcorax*
49 *melanorhampus*, a cooperative breeder (Heinsohn et al. 2000). Because most studies
50 examining secondary sexual traits in general, and assortative mating in particular, are
51 carried out at a small number of sites and over very few breeding seasons (Cornwallis
52 and Uller 2010 but see Svensson et al. 2005; Cockburn et al. 2008) longer-term
53 research that spans broader spatial scales is needed if we wish to more accurately
54 estimate the strength and consequences of mating patterns.

55 Assortative mating has broad-ranging consequences on trait evolution, local
56 adaptation, and speciation. Theoretical models have shown that, when it is associated
57 with disruptive selection, assortative mating by single traits or multiple ecologically
58 related traits facilitates sympatric speciation (Dieckmann 1999; Gavrilets 2003;
59 Bolnick and Fitzpatrick 2007) and local adaptation (Jiang et al. 2013). In a *Tilapia*
60 species complex in the early stages of speciation, there was strong assortative mating
61 by diet and color, which may promote species differentiation (Martin 2013).
62 However, in general, there is limited empirical evidence for assortative mating
63 playing such a role (Nosil et al. 2008; Branch et al. 2015). One reason may be the
64 existence of large temporal variation in assortative mating patterns: studies conducted
65 over one or two years are unlikely to be able to provide a representative view of long-
66 term patterns (Cockburn 2014). To date, temporal variation in assortative mating
67 patterns has remained unaddressed.

68 Assortative mating also influences the evolution of secondary sexual characters in
69 both sexes and therefore must be characterized if we wish to understand the basis for
70 female ornamentation. Indeed, quantifying mating patterns is the first step in
71 disentangling the still-debated factors underlying the evolution of female ornaments.
72 For instance, female ornaments may be an evolutionary by-product resulting from
73 selection for male ornaments and/or emerge from direct sexual or social selection on
74 female traits (Clutton-Brock 2007; Tobias et al. 2012).

75 In animals, both sexes often convey multiple signals to conspecifics; these signals
76 may provide redundant or complementary information about an individual's quality.
77 Therefore, males and females may assess each other using different signals and/or
78 different aspects of the same signal. However, few studies have investigated whether
79 the two sexes differ in the signals/signal aspects they use (Laczi et al. 2011; Hegyi et
80 al. 2015). When studying coloration, the influence of different signals on mate choice
81 is difficult to unravel because of multicollinearity among traits. In this study, we used
82 a commonality analysis to identify potential multicollinearity among explanatory
83 variables (Ray-Mukherjee et al. 2014). Exploring trait associations can help reveal
84 any complexity hidden in mating patterns.

85 Evidence for assortative mating is usually based on the value of the Pearson
86 correlation coefficient for pairs of traits. Although it is straightforward to calculate, its
87 use may be problematic when dealing with longitudinal data because of the risk of
88 pseudoreplication. The most common solution is to use the trait value for a given
89 individual for one year only; frequently, researchers choose the value associated with
90 the individual's first appearance or with a randomly selected time period (Row and
91 Weatherhead 2011; Van Rooij and Griffith 2012). Although this approach prevents
92 pseudoreplication, it dramatically decreases sample size. Furthermore, if an individual

93 changes mates every breeding season, the strategy leads to a major loss of
94 information, making it harder to accurately estimate the degree of interannual
95 variability. To assess the strength of assortative mating, we employed a powerful but
96 rarely applied method that can be used with multiple populations of a single species:
97 the within-study meta-analysis. This approach allowed us to draw generalized
98 conclusions while avoiding pseudoreplication (Nakagawa and Santos 2012).

99 We studied assortative mating in the blue tit (*Cyanistes caeruleus*), a passerine. Mate
100 choice is likely important in this socially monogamous species, in which birds pair
101 before the breeding season and both sexes provide parental care (see Fig. 1). We
102 focused on two ornaments—the blue/UV crown, which is produced by structural
103 coloration, and the yellow breast patch, which results from carotenoid-based
104 coloration. Both features are thought to be targets of social or sexual selection in both
105 males and females (Alonso-Alvarez et al. 2004; Limbourg et al. 2004; Kingma et al.
106 2009; Remy et al. 2010 but see Parker 2013 for males; Doutrelant et al. 2008, 2012;
107 Midamegbe et al. 2011, 2013; Parker et al. 2011; Henderson et al. 2013 for females).
108 If ornaments influence mate choice differently for males and females,
109 multicollinearity can occur among traits. Consequently, we anticipated that the final
110 pattern might be more complex than that normally expected from “simple” assortative
111 mating.

112 Two previous studies on assortative mating in blue tits (Andersson et al. 1998;
113 Garcia-Navas et al. 2009) focused on a single ornament, the blue crown, and obtained
114 data from a single population (using 18 and 26 breeding pairs, respectively) over a
115 single breeding season. They found opposite results: Andersson and colleagues (1998)
116 found evidence for positive assortative mating whereas Garcia-Navas and colleagues
117 did not. Here, not only did we use two ornaments, but we also collected data from

118 four different populations over 10 successive breeding seasons; we sampled a mean of
119 44 breeding pairs per year per population. Long-term studies that compile large data
120 sets are essential if we wish to better understand the potential strength of social and
121 sexual selection on ornaments (Parker 2013). Also, as assortative mating is a force
122 that contributes to local adaptation (Jiang et al. 2013), it is crucial to assess the
123 former's strength in closed populations that display phenotypic divergence. Of our
124 four study populations, two were separated by less than 5 km and experienced gene
125 flow (Szulkin et al. 2016); however, they nonetheless diverged in several fitness-
126 related life-history traits, including coloration (Charmantier et al. 2016a). They thus
127 present an ideal opportunity for investigating the link between assortative mating and
128 local adaptation.

129

130 **Materials and methods**

131 Study populations and sampling methodology

132 From 2005 to 2014, we collected feathers from birds in four blue tit populations in
133 France. One was located in mainland France, in a deciduous oak forest (La Rouvière)
134 found approximately 18 km northwest of Montpellier. The other three were located in
135 northwestern Corsica. Of the Corsican populations, two were found in the Regino
136 Valley (the Muro populations) and were separated by less than 5 km. Despite the
137 populations' spatial proximity, they occupied different habitat types: a deciduous oak
138 forest versus an evergreen oak forest (Lambrechts et al. 2004; Blondel et al. 2006;
139 Charmantier et al. 2016a). The third Corsican population, the Pirio population, was
140 found in the Fango Valley, which is located 25 km from the Regino Valley, in a forest
141 dominated by evergreen oaks. Hereafter, the four populations are referred to as D-

142 Rouvière, D-Muro, E-Muro, and E-Pirio, where D stands for deciduous oaks and E
143 stands for evergreen oaks.

144 We sampled feathers from breeding pairs that were trapped in their nestboxes as they
145 fed their chicks, approximately nine days after the chicks hatched. Sampling took
146 place in late April and May for the D-Rouvière, D-Muro, and E-Muro populations and
147 in June for the E-Pirio population. Because feather coloration changes over time as
148 feathers become soiled (Ornberg et al. 2002; Delhey et al. 2006), different mating
149 patterns could be present at the beginning versus the end of the breeding season.
150 Feathers were collected from a small number of breeding pairs both during the nest
151 construction period and the chick feeding period of the same season to determine
152 whether there was evidence for intraseasonal differences in assortative mating
153 patterns and strength among the study populations. Although assortative mating
154 increased in strength within breeding seasons, the population-level patterns were the
155 same as in the more extensive analysis, suggesting sampling period was not a concern
156 (see ESM Appendix 1 for more details on the methods and results).

157 Upon capture, each bird was equipped with a uniquely numbered metal band provided
158 by the French Natural History Museum in Paris; this effort was part of the CRBPO
159 banding program (#369). We collected 8 and 10 feathers from each bird's blue crown
160 and yellow breast patch, respectively (Doutrelant et al. 2008, 2012).

161

162 Measurements of color traits

163 We characterized five color traits that have been examined in previous studies of blue
164 tit populations (Sheldon et al. 1999; Delhey et al. 2003; Alonso-Alvarez et al. 2004;

165 Limbourg et al. 2004; Doutrelant et al. 2008, 2012; Remy et al. 2010; Midamegbe et
166 al. 2011, 2013). Measurements were performed in the laboratory using a
167 spectrophotometer (AVASPEC-2048, Avantes, Apeldoorn NL) and a deuterium-
168 halogen light source (AVALIGHT-DH-S lamp; range of 300–700 nm, Avantes,
169 Apeldoorn NL). A 200- μ m fiber optic probe was placed perpendicular to the feather's
170 surface at a fixed distance of 2 mm. A probe mount consisting of a black rubber cap
171 excluded all ambient light. Our reflectance data (R) were relative to a white standard
172 (WS-1; Ocean Optics, Dunedin (FL), USA) and a dark current (a black felt
173 background). We measured each sample three times, the fibre being removed between
174 each measure. For each ornament on each bird, we made measurements on two
175 different samples and found the mean of six reflectance spectra obtained from two
176 sets of three blue feathers and four yellow feathers (Doutrelant et al. 2008, 2012).
177 Samples within a year and a population were measured randomly, controlling a
178 potential effect of random drift due to measuring male and female samples
179 consecutively. We used Avicol v6 software (Gomez 2006) to estimate the color trait
180 values based on the shape of the spectra (Andersson et al. 1998; Doutrelant et al.
181 2008).

182 In the case of the blue crown, we characterized one achromatic trait, blue brightness
183 (i.e., the area under the reflectance curve divided by the width of the interval from
184 300–700 nm), and two chromatic traits, blue hue (wavelength at maximal reflectance)
185 and blue UV-chroma (proportion of the total reflectance falling in range from 300–
186 400 nm). Lower hue values and higher UV-chroma values correspond to a stronger
187 UV signal. In the case of the yellow breast patch, we calculated yellow brightness and
188 yellow chroma ($[(R_{700}-R_{450})/R_{700}]$). Higher yellow chroma values are associated with
189 higher levels of carotenoids in the plumage (Isaksson et al. 2008). Our measurements

190 were all highly repeatable, suggesting acceptable measurement error (ESM Appendix
191 2, Table S2.1).

192

193 Assortative mating database characteristics

194 In all our analyses, we only used pairs for which coloration data was available for
195 both the male and the female. Most of the time (97.4%), feathers were collected from
196 both members of a mated pair within three days. Within each population and breeding
197 season, pairs that reproduced 30 days after their first attempt were excluded from the
198 analysis because such clutches were potentially second or replacement clutches
199 (Nager and Vannoordwijk 1995).

200 Between 2005 and 2014, we analyzed color trait values for 1,657 mated pairs (Table
201 1), including 1,117 males and 1,110 females. The correlation (Pearson correlation
202 coefficient) between trait values never exceeded 0.42 (ESM Appendix 2, Table S2.2),
203 except in the case of blue hue and blue UV-chroma, which ranged from -0.33 to 0.74
204 (ESM Appendix 2, Table S2.2).

205

206 Statistics

207 Commonality analysis

208 We used commonality analysis to explore trait associations (i.e., which predictors
209 explain most of the variance in a given dependent variable) and to identify potential
210 multicollinearity (Ray-Mukherjee et al. 2014). We used the five standardized color
211 traits for one sex as predictors in additive multiple regression models in which the

212 trait values for the other sex were dependent variables (Table 2). The commonality
213 analysis was then performed on each of the ten multiple regression models using the
214 *yhat* package (Nimon et al. 2013) in R (R Core Team 2016) to determine which
215 variables should be retained in the analysis described below.

216

217 Characterizing assortative mating patterns

218 To look for evidence of assortative mating, for each population and each year, we
219 calculated the Pearson correlation coefficients between the trait values for mated
220 pairs; only the traits retained following the commonality analysis were used. Since we
221 were working with as many as five color traits for birds from four populations
222 sampled across ten years, a within-study meta-analysis was best suited for detecting
223 potential patterns of assortative mating (Nakagawa and Santos 2012; Hegyi et al.
224 2015). This method takes into account any non-independence in the data within
225 populations and among variables. The Pearson correlation coefficients and the
226 confidence intervals we had calculated were transformed to fit the meta-analysis and
227 were then used as effect sizes (Rosenberg et al. 2013). We included year as a random
228 effect.

229 In the first set of models, color trait and population were fixed effects; their
230 interaction was also included. Then, ornament variation was examined by grouping
231 the blue crown traits and yellow breast patch traits to create two levels of a new
232 variable: ornament. Ornament and population were then included as fixed effects, as
233 was their interaction. Models were run using the *MCMCglmm* package (Hadfield
234 2014) in R; 100,000 iterations were performed, with a burn-in of 25,000 iterations.
235 We used a DIC model selection approach. The results of the best-fit models were back

236 transformed to obtain new Pearson correlation coefficients (Nakagawa and Santos
237 2012; Rosenberg et al. 2013).

238 We examined temporal variation by two means. We first tested for temporal
239 autocorrelation among Pearson correlation coefficients on each color trait in each
240 population. For the D-MURO and E-MURO populations, due to the lack of data in
241 2007, we estimated temporal autocorrelation from 2008 to 2014. We also used a
242 within-study meta-analysis model that included year as a fixed effect rather than as a
243 random effect, which permitted the inclusion of year-by-population and year-by-
244 ornament interactions.

245

246 **Results**

247 Commonality analysis

248 For each of the ten multiple regression models tested in the commonality analysis, a
249 given color trait in one sex always explained most of the variance in the concordant
250 trait in the other sex. Explanatory ability ranged from 67% for yellow brightness to
251 92% for blue UV-chroma and yellow chroma (Table 2; see ESM Appendix 2, Table
252 S2.3 for details). This result means that corresponding traits (e.g., male blue
253 brightness and female blue brightness) were more likely to be associated than were
254 non-corresponding traits (e.g., male blue brightness and female blue UV- chroma). As
255 a result, we only retained concordant traits in our analyses of assortative mating
256 patterns.

257

258 Pearson correlation coefficients

259 For each population and each trait, there was notable variation among years in the
260 strength of assortative mating (see Fig. 2 for blue UV-chroma and yellow chroma; see
261 ESM Appendix 2, Fig. S2.1 for the other three traits). The most positive values were
262 around 0.60 (i.e., 0.60 for blue UV-chroma in D-Muro in 2009 and 0.57 for yellow
263 chroma in E-Muro in 2011) and the most negative values were around -0.40 (-0.39 for
264 blue brightness in E-Pirio in 2008).

265

266 Temporal autocorrelation

267 Overall, there was no effect of temporal autocorrelation among years for every
268 population and every color trait. Only a marginal negative effect of temporal
269 autocorrelation for yellow brightness was found in E-Pirio with a lag of two years (see
270 Fig. S2.2).

271

272 Within-study meta-analysis

273 The within-study meta-analysis showed that, on the whole, assortative mating was
274 positive in all four populations, even though there was a large degree of temporal
275 variation. In the analysis in which the color traits were included separately, the best-fit
276 model included only population (Table 3a), which means that there was variation in
277 assortative mating among populations but not among color traits (Fig. 2 and Appendix
278 2, S2.1). More specifically, assortative mating was strongest in the D-Muro
279 population and weakest in the D-Rouvière and E-Pirio populations. All the
280 populations had assortative mating values of 0.2 or less (Fig. 3), but the values were
281 all significantly different from zero, indicating that the two ornaments were serving as
282 the basis for assortative mating.

283 In the analysis in which the color traits were grouped, the best-fit model included
284 ornament and population as main effects (Table 3b). Looking more closely at the
285 results, there appeared to be somewhat stronger assortative mating by the yellow
286 breast patch than by the blue crown (Fig. 3).

287 In the analysis in which year was treated as a fixed effect, the best-fit model included
288 ornament and population as main effects but did not retain their interaction, which
289 suggests temporal variation was similar across all the four populations (ESM
290 Appendix 2, Table S2.4 and Fig S2.3).

291

292 **Discussion**

293 This study investigated spatiotemporal variability in assortative mating in four blue tit
294 populations in southern France; it focused on two ornaments, the blue crown and the
295 yellow breast patch. We found four major results. First, concordant color traits were
296 highly associated in breeding pairs: for instance, males with bright blue crowns paired
297 with females with bright blue crowns. Second, on the whole, assortative mating was
298 positive in all four populations, suggesting it influences ornament evolution in both
299 sexes in this species. Assortative mating was slightly more pronounced for the yellow
300 breast patch than for the blue crown. Third, we observed large temporal variation in
301 assortative mating patterns in the four populations, a result that underscores that long-
302 term studies are needed to draw conclusions about mate pairing in natural populations.
303 Fourth, there was spatial variation in assortative mating strength. The two closest
304 populations displayed the highest levels of assortative mating, which may have
305 interesting evolutionary implication on local adaptation and population divergence in
306 our study system.

307

308 Assortative mating is based on concordant color traits

309 First, our results show that both sexes use the same color traits to choose mates. In the
310 commonality analysis, concordant color traits in males and females were always much
311 more strongly associated than were non-concordant color traits. For instance, when all
312 five female color traits were used as predictors of male yellow chroma, female yellow
313 chroma alone explained 86% of the variation (Table 2 and ESM Appendix 2, Table
314 S2.3). This lack of multicollinearity also suggests that different traits do not form a
315 general, integrated signal. This finding is in line with recent discoveries in great tits
316 and collared flycatchers (Laczi et al. 2011; Hegyi et al. 2015).

317 Second, in all populations, there was a slightly greater degree of assortative mating by
318 the yellow breast patch than by the blue crown. This result could suggest that both
319 ornaments convey different information and that the yellow breast patch might be
320 more important in blue tit social and/or sexual interactions. Alternatively, if
321 assortative mating is related to sharing the same habitat (Kraaijeveld et al. 2007), a
322 stronger assortative mating by the yellow breast patch could mean that the yellow
323 breast patch is more condition-dependent than the blue crown (due to its sensitivity to
324 food availability – carotenoid - and parasites). Yet even if the best-fit meta-analysis
325 model retained a population effect (Table 3b), the ornament-specific differences in
326 assortative mating values were small and their confidence intervals overlapped (Fig.
327 3). Moreover, when the five color traits were treated as individual factors, the best-fit
328 meta-analysis model did not retain any of them (Table 3a). More studies are thus
329 needed to determine the role of sharing the same habitat on assortative mating value
330 and to determine whether the two ornaments convey different or similar information.

331 More specifically, it would be useful to carry experimental studies in which the
332 ornaments are manipulated (Jawor and Breitwisch 2004) to answer this question.

333

334 Assortative mating is positive overall

335 We found that, on the whole, assortative mating by the blue crown and by the yellow
336 breast patch was positive in the four study populations. Although its strength never
337 exceeded 0.2 across the 10 years of the study, it was nonetheless significantly
338 different from zero in each population. The large temporal variation observed may
339 help explain why its mean values were relatively low, even though it reached a
340 maximum of 0.6 (Fig. 2 and ESM Appendix 2, Fig. S2.1).

341 This overall level of assortative mating is slightly lower than what has been found for
342 birds in general, and for bird visual signals in particular, in a recent meta-analysis of
343 results from natural populations ($r=0.25$ with $[0.20;0.29]$ 95% confidence intervals
344 and $n=132$ for all traits in birds; $r=0.37$ with $[0.26;0.48]$ 95% confidence intervals and
345 $n=14$ for visual signals in birds; see ESM B Tables B1 and B4 in Jiang et al. 2013).
346 However, most studies of color signals in birds last no more than three years (but see
347 Roulin et al. 2003; Brommer et al. 2005), and because there is a strong publication
348 bias for significant results in science (Palmer 1999), these effects are probably
349 overestimates.

350 In the context of female ornament evolution, positive assortative mating by ornaments
351 could be strongly related to sexual selection (e.g., the mate choice hypothesis) or
352 social selection (e.g., the competition hypothesis) (Amundsen 2000; Clutton-Brock
353 2007, 2009; Tobias et al. 2012; Dale et al. 2015). If assortative mating is a

354 consequence of mate choice (either directional choice or mutual mate choice), then
355 choosing a mate based on the similarity of his/her ornaments ultimately favors the
356 presence of the ornament in the mate. Likewise, if assortative mating is a consequence
357 of competition (either for territory or resources), trait similarity could result because
358 the same signal conveys information about competitive status in both males and
359 females. Previous results obtained in the blue tit have suggested that female coloration
360 on the blue crown plays a role in male mate choice and competition (Hunt et al. 1999;
361 Alonso-Alvarez et al. 2004; Remy et al. 2010; Midamegbe et al. 2011). It has also
362 been shown experimentally that female coloration on the yellow breast patch is
363 condition-dependent and related to reproductive success and maternal reproductive
364 investment (Doutrelant et al. 2008; Midamegbe et al. 2013). Our results thus join with
365 others to suggest that female ornaments in this species may be directly selected. Of
366 course, this does not preclude genetic correlations from playing a role in the evolution
367 of female ornaments, especially since strong additive genetic correlations in chromatic
368 traits have been found between the sexes in our study populations (Charmantier et al.
369 2016b).

370

371 Large temporal variation exists in assortative mating

372 A longitudinal study examining multiple ornaments in a population of lark buntings
373 (*Calamospiza melanocorys*) found that sexual selection on male traits shifted
374 dramatically over time and even demonstrated reversals in directionality for given
375 traits (Chaine and Lyon 2008). Similarly in our study, the raw Pearson correlation
376 coefficients for the individual populations revealed large temporal variation in the
377 strength of assortative mating by color traits. For instance, in the D-Muro population

378 in 2011, there was significant positive assortative mating by blue UV-chroma
379 ($r_{\text{BUVC}}=0.29$; Fig. 2) but not by yellow chroma ($r_{\text{YC}}=-0.06$; Fig. 2). In the same
380 population in 2013, the opposite was observed ($r_{\text{BUVC}}=0.006$; $r_{\text{YC}}=0.33$; Fig. 2). This
381 finding illustrates that we should not draw general conclusions about assortative
382 mating based on a single breeding season because results may vary substantially from
383 year to year. In the review written by Jiang and colleagues (2013) on the topic of
384 assortative mating, most of the studies cited lasted no longer than a year.

385 Although ten years' worth of variation is insufficient if we wish to draw conclusions
386 about the potential ecological factors driving variability, the results of the analysis in
387 which year was treated as a fixed effect suggest that fluctuations in assortative mating
388 follow similar patterns across the four study populations (ESM Appendix 2, Fig S2.3)
389 and could therefore be explained by common ecological factors. This similar year
390 effect suggests that the large temporal variation observed is more than just random
391 fluctuation around the mean; furthermore the lack of temporal autocorrelation (Fig.
392 S2.2) indicates that patterns of assortative mating were independent across years.

393 To explain such interannual variability we speculate that the dramatic variation in the
394 strength of assortative mating by the two ornaments and their color traits could be
395 influenced by interannual plasticity in mate choice (Chaine and Lyon 2008; Kopp and
396 Hermisson 2008). This plasticity could stem from the fact that although the two
397 ornaments are condition dependent (in adults: Doutrelant et al. 2012 but see Peters et
398 al. 2011; in nestlings: Jacot and Kempenaers 2007), they display differential abilities
399 to respond to environmental conditions and are thus more or less informative
400 depending on the main factors affecting signaling in any given year (Bro-Jørgensen
401 2010). For instance, variable carotenoid availability or parasite prevalence during the
402 molt may mainly affect the reliability of the yellow breast patch as a signal while

403 leaving less mark on the blue crown, whose coloration is structural (McGraw et al.
404 2002). Variation in mortality and density may also affect mate choice or competitive
405 intensity, leading to a lesser degree of assortative mating during years when
406 conditions are harsher (Crowley et al. 1991).

407

408 Large spatial variation exists in assortative mating

409 Theory predicts that local adaptation will be reinforced when assortative mating
410 occurs (Jiang et al. 2013). Interestingly, we found a greater degree of assortative
411 mating in the two populations closest to each other: the Corsican Muro populations.
412 They display hints of local adaptation and strong phenotypic divergence for several
413 morphological, behavioral, and life-history (i.e., laying date, clutch size, fledgling
414 success) traits (Porlier et al. 2012; Charmantier et al. 2016a; Szulkin et al. 2016).

415 The Muro populations are separated by no more than 5 km and, although dispersal
416 between the two has been observed, recent genomic studies have revealed that the
417 populations display fine-scale genetic structures (Szulkin et al. 2016), which suggests
418 the presence of forces limiting random dispersal. The positive assortative mating we
419 observed in these populations may be one of those forces. These results, taken in
420 tandem with those of a previous study that found population-level differences in
421 coloration (Charmantier et al. 2016a), indicate that future research should determine
422 whether assortative mating can help reinforce population-specific selection pressures
423 on coloration. However, to truly test these ideas in our study system, we would need
424 to expand our number of study sites and sample localities closer to the E-Pirio and D-
425 Rouvière populations. We would also need to conduct mate choice experiments.

426

427 On the processes leading to assortative mating

428 Assortative mating can be the direct outcome of directional or mutual mate choice
429 (Kraaijeveld et al. 2007). But other processes can also lead indirectly to assortative
430 mating. For instance, if pairs remain together across years or if they choose to live in
431 the same micro-habitat, they can experience conditions affecting similarly their
432 plumage. Positive assortative mating can also be created by the scale-of-choice effect
433 (Rolan-Alvarez et al. 2015) when several neighboring populations are sampled and
434 pooled altogether for the purposes of analysis. Despite considering four distinct
435 populations, variations in micro-habitat within each population may lead to a scale-of-
436 choice effect. Analyses of assortative mating at the individual scale are thus needed to
437 determine the processes driving assortative mating.

438 Determining these processes was beyond the scope of this paper, aimed at quantifying
439 assortative mating at the population scale. In a parallel study we have tested at the
440 individual scale several processes comprising mate choice, age, habitat choice or
441 timing of breeding in our four populations. We found that assortative mating seemed
442 directly due to mate choice, with no effect of indirect processes (Fargevieille 2016).
443 This result suggests that mate choice is indeed an important process leading to
444 assortative mating.

445

446 Conclusions

447 First, this long-term study of assortative mating by two ornaments across multiple
448 populations has revealed that spatiotemporal variation cannot be ignored. Doing so

449 carries the strong risk of arriving at erroneous conclusions. Second, the within-study
450 meta-analysis approach, which allowed us to account for the dramatic spatiotemporal
451 variation we observed, revealed that assortative mating was positive overall and thus
452 might influence ornament evolution in both sexes. Third, assortative mating
453 demonstrated fine-scale variation, suggesting it could affect local adaptation and
454 population divergence in our study system, topics that should be explored in future
455 research.

456

457 Data accessibility

458 This section will provide the Dryad DOI if the manuscript is accepted.

459

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Table 1 Sample sizes for each population and year; total sample sizes are in bold

	D-Muro	E-Muro	D-Rouvière	E-Pirio
2005	33	19	62	46
2006	39	24	47	37
2007	0	0	41	58
2008	39	23	51	33
2009	37	26	42	37
2010	54	30	65	50
2011	55	32	76	51
2012	58	31	56	29
2013	50	30	44	38
2014	54	39	84	37
Total	419	254	568	416

Table 2 Percentage of variance explained by the concordant color trait in the opposite sex

Dependent variable	Predictor variables in the additive model	Concordant color trait	Variance explained
Female blue brightness (fBB)		mBB	72%
Female blue hue (fbH)		mBH	75%
Female blue UV-chroma (fbUVC)	mBB+mBH+mBUVC+mYB+mYC	mBUVC	78%
Female yellow brightness (fyB)		mYB	89%
Female yellow chroma (fyC)		mYC	92%
Male blue brightness (mBB)		fBB	82%
Male blue hue (mbH)		fbH	75%
Male blue UV-chroma (mBUVC)	fBB+fbH+fbUVC+fyB+fyC	fbUVC	92%
Male yellow brightness (myB)		fyB	67%
Male yellow chroma (myC)		fyC	86%

Table 3 Meta-analysis models in which year was a random effect. The best-fit model (in bold) was the model with the lowest DIC value. Ornament had two levels: blue crown and yellow breast patch.

Fixed effects	Random effect	DIC
a) Traits included separately		
Population	Year	-478.8
Trait+Population	Year	-455.1
Intercept	Year	-454.2
Trait	Year	-441.2
Trait*Population	Year	-404.0
b) Traits grouped by ornament		
Ornament+Population	Year	-468.9
Population	Year	-460.5
Ornament	Year	-448.1
Ornament*Population	Year	-447.6
Intercept	Year	-445.7



Figure 1. Pair of blue tits (female on the left, male on the right) photographed in the Regino valley (around Muro field stations) in Corsica in March 2016. Picture courtesy of Stéphan Tillo

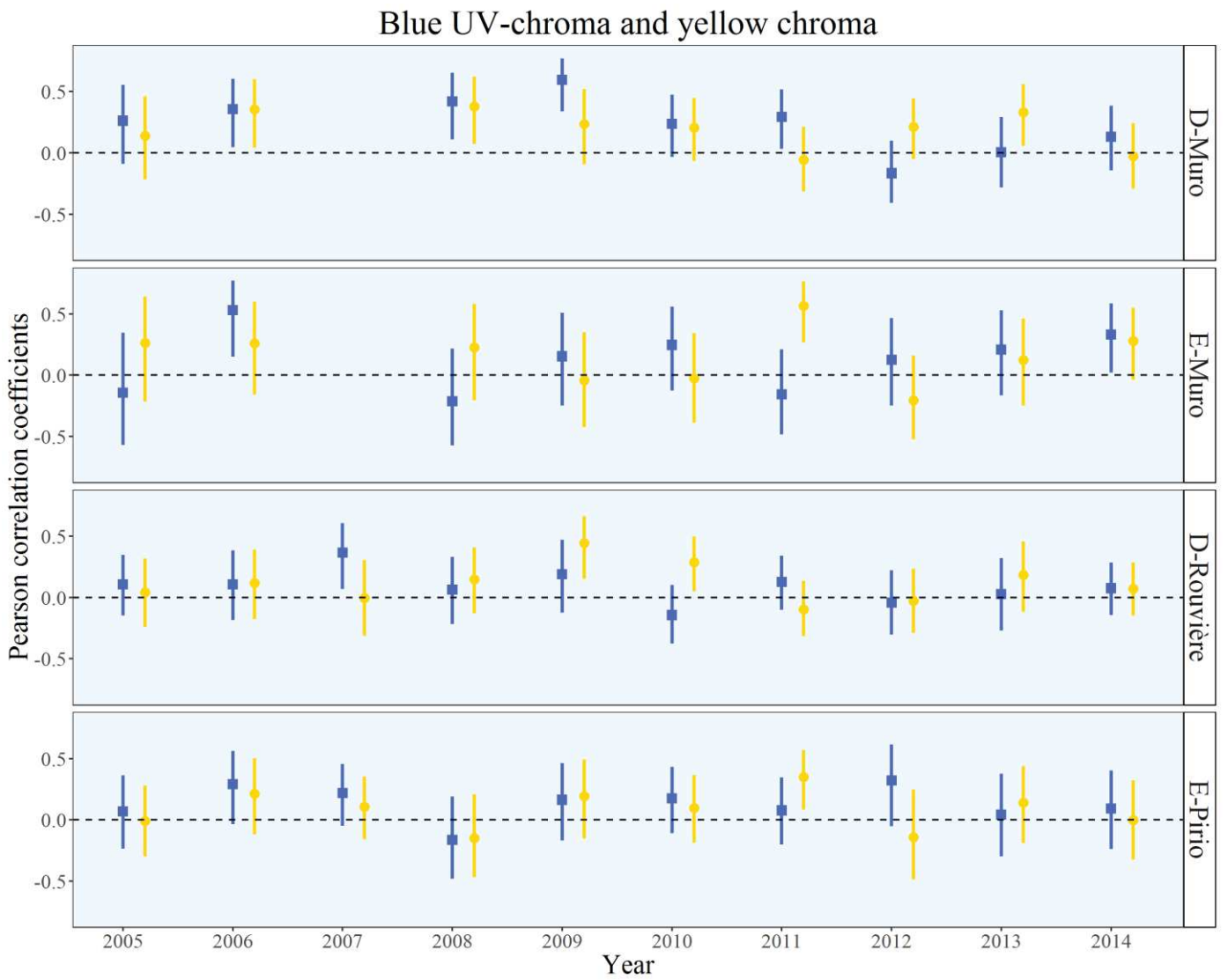


Figure 2. Pearson correlation coefficients for blue UV-chroma (blue squares) and yellow chroma (yellow circles) for each year for each population. The bars depict the 95% confidence intervals. The dashed lines indicate a coefficient value of zero, or the absence of assortative mating.

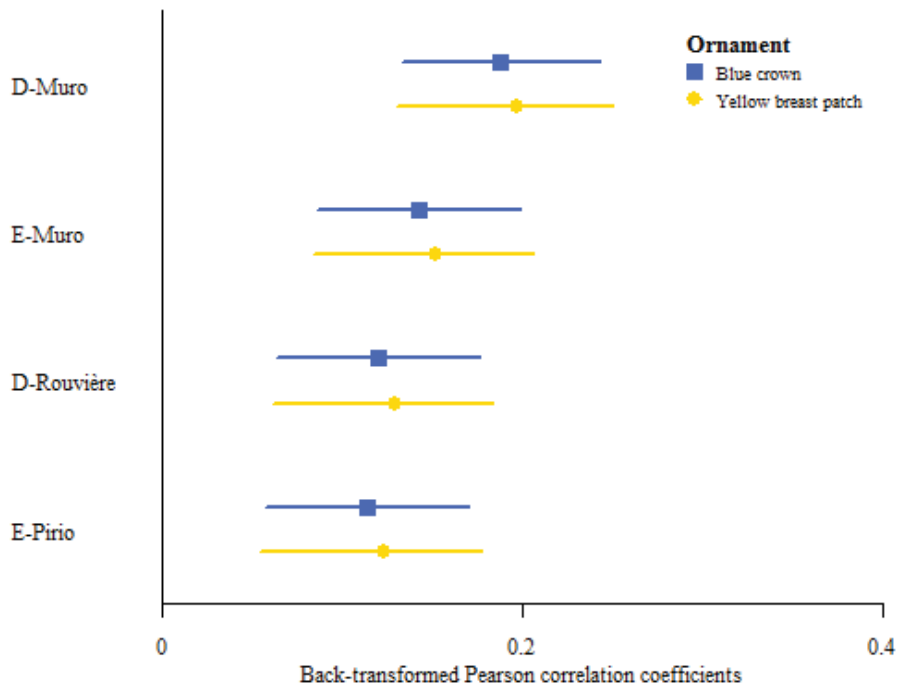


Figure 3. Back-transformed population-specific Pearson correlation coefficients for the two ornaments: the blue crown (blue squares) and the yellow breast patch (yellow circles). The bars depict the 95% confidence intervals.

Electronic Supplementary Material

Appendix 1 Assortative mating over the course of the breeding season

The aim of this additional analysis is to test whether assortative mating change over the course of the breeding season.

Methods

For some years - three/ four years of our ten years study - we collected feathers during the nest construction period (i.e. just before laying started, which was mid-March for the D-Rouvière, D-Muro, and E-Muro populations and mid-April for the E-Pirio population). At this point, the birds had already become territorial. Only mated pairs for which we had color trait information for both periods—nest construction and chick feeding—were included in the smaller-scale within-study meta-analysis. This restriction explains the relative small sample size in each of the study years (Table S1.1).

Table S1.1 Sample sizes (n) for the analysis of assortative mating patterns within breeding seasons

Population	Year	n
D-Muro	2011	10
	2012	13
	2013	16
	2014	16
E-Muro	2012	5
	2013	4
	2014	6
D-Rouvière	2008	11
	2009	15
	2011	7
E-Pirio	2011	10
	2012	4
	2013	4
	2014	8

After calculating the raw Pearson correlation coefficients, we carried out a within-study meta-analysis. Year was a random effect, and period (nest construction vs. chick feeding), population, and ornament were fixed effects. The interactions between period and ornament and between population and ornament were included.

Results

The best-fit model (based on DIC values) retained period and population (Table S1.2). Assortative mating increased in strength over the course of the breeding season (Fig. S1.1). However, the population-level patterns were the same as in the full analysis. We thus concluded that the assortative mating patterns and population differences observed in latter were reliable.

Table S1.2 Models from the meta-analysis examining assortative mating patterns within breeding seasons. The best-fit model (in bold) had the lowest DIC value. Ornament had two levels—blue crown and yellow chest patch—as did period—nest construction and chick feeding.

Fixed effects	Random effect	DIC value
Period+Population	Year	-332.8
Ornament*Population+Ornament*Period	Year	-315.6
Population+Ornament	Year	-312.2
Population+Ornament*Period	Year	-310.2
Ornament*Population+Period	Year	-307.8
Population	Year	-306.9
Ornament+Population+Period	Year	-302.3
Ornament*Population	Year	-296.5
Period	Year	-296.2
Ornament+Period	Year	-281.2
Ornament*Period	Year	-271.2
Ornament	Year	-265.5
Intercept	Year	-262.6

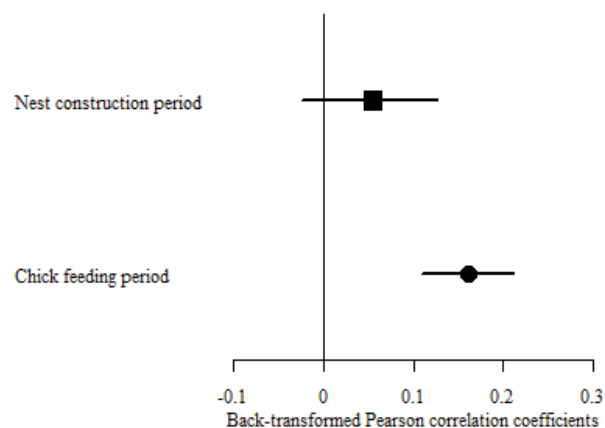


Figure S1.1 Back transformed Pearson correlation coefficients for the two periods of the breeding season. The values were taken from the model in which period was the sole predictor. The dashed line indicates a coefficient value of zero, or the absence of assortative mating.

Appendix 2

Table S2.1 Repeatability of color measurements estimated using an ANOVA. n_s is the sample size; n_m is the total number of measurements (three to six per individual); r_{BB} , r_{BH} , r_{BUVC} , r_{YB} , and r_{YC} are the repeatability estimates for blue brightness, blue hue, blue UV-chroma, yellow brightness, and yellow chroma, respectively. The D-Muro and E-Muro populations were pooled into a single category called “Muro.”

Population	Year	Blue crown					Yellow breast patch			
		n_s	n_m	r_{BB}	r_{BH}	r_{BUVC}	n_s	n_m	r_{YB}	r_{YC}
Muro	2005	129	754	0.843	0.704	0.848	130	777	0.748	0.674
	2006	137	815	0.801	0.785	0.845	139	837	0.714	0.611
	2007	0	0	NA	NA	NA	0	0	NA	NA
	2008	144	862	0.830	0.693	0.793	148	882	0.660	0.730
	2009	140	828	0.843	0.802	0.884	140	802	0.698	0.681
	2010	188	1108	0.924	0.845	0.905	188	1127	0.853	0.732
	2011	287	1620	0.796	0.782	0.863	280	1563	0.731	0.722
	2012	301	1692	0.811	0.718	0.829	308	1724	0.675	0.720
	2013	254	1382	0.803	0.702	0.756	269	1401	0.639	0.685
	2014	312	1859	0.927	0.699	0.878	312	1872	0.838	0.727
D-Rouvière	2005	224	1294	0.802	0.786	0.815	166	922	0.543	0.752
	2006	108	601	0.681	0.717	0.807	110	605	0.602	0.684
	2007	186	966	0.698	0.680	0.786	186	1059	0.540	0.821
	2008	322	1826	0.751	0.766	0.814	319	1931	0.437	0.594
	2009	348	1975	0.802	0.753	0.802	345	1945	0.590	0.719
	2010	161	952	0.918	0.742	0.871	160	930	0.819	0.720
	2011	209	1119	0.743	0.766	0.796	203	1146	0.486	0.680
	2012	154	883	0.749	0.684	0.781	155	852	0.504	0.612
	2013	114	541	0.751	0.688	0.770	104	510	0.605	0.580
	2014	189	1120	0.920	0.760	0.896	186	1116	0.805	0.821
E-Pirio	2005	92	288	0.789	0.715	0.842	99	282	0.562	0.644
	2006	80	434	0.729	0.681	0.777	81	435	0.646	0.772
	2007	128	743	0.695	0.651	0.798	132	739	0.509	0.687
	2008	83	482	0.773	0.645	0.686	81	435	0.671	0.577
	2009	90	523	0.854	0.683	0.735	88	522	0.633	0.719
	2010	110	650	0.818	0.684	0.835	111	666	0.772	0.611
	2011	166	915	0.688	0.672	0.720	163	924	0.637	0.725
	2012	140	803	0.797	0.699	0.805	141	761	0.580	0.600
	2013	120	643	0.808	0.677	0.860	118	558	0.791	0.707
	2014	157	926	0.907	0.723	0.862	157	942	0.725	0.651

Table S2.2 Pearson correlation coefficients for the five color traits in the four populations (the values for males and females are italicized and unitalicized, respectively; statistical significance: * $p < 0.05$; ** $p < 0.01$; $p < 0.001$).

Population	Color trait	Blue brightness	Blue hue	Blue UV-chroma	Yellow brightness	Yellow chroma
D-Muro	Blue brightness		-0.135**	0.026	0.042	0.096
	Blue hue	<i>0.075</i>		-0.555***	-0.230***	-0.037
	Blue UV-chroma	<i>-0.219***</i>	<i>-0.380***</i>		0.214***	0.212***
	Yellow brightness	<i>-0.020</i>	<i>-0.103*</i>	<i>0.138**</i>		-0.239***
	Yellow chroma	<i>0.116*</i>	<i>0.026</i>	<i>0.320***</i>	<i>-0.253***</i>	
E-Muro	Blue brightness		-0.057	0.170**	-0.059	0.154*
	Blue hue	<i>0.057</i>		-0.735***	0.061	-0.148*
	Blue UV-chroma	<i>-0.027</i>	<i>-0.668***</i>		-0.075	0.178
	Yellow brightness	<i>-0.0002</i>	<i>0.161*</i>	<i>-0.065</i>		-0.293***
	Yellow chroma	<i>0.138*</i>	<i>-0.077</i>	<i>0.105</i>	<i>-0.386***</i>	
D-Rouvière	Blue brightness		-0.005	-0.089*	0.102*	0.263***
	Blue hue	<i>0.218***</i>		-0.523***	-0.120**	0.084
	Blue UV-chroma	<i>-0.384***</i>	<i>-0.463***</i>		-0.107*	-0.083
	Yellow brightness	<i>0.0987*</i>	<i>-0.126**</i>	<i>-0.044</i>		-0.123**
	Yellow chroma	<i>0.217***</i>	<i>0.091*</i>	<i>-0.056</i>	<i>-0.018</i>	
E-Pirio	Blue brightness		-0.101*	0.064	0.183***	-0.096
	Blue hue	<i>0.082</i>		-0.329***	-0.191***	0.093
	Blue UV-chroma	<i>-0.131**</i>	<i>-0.418***</i>		-0.018	0.020
	Yellow brightness	<i>0.215***</i>	<i>-0.204***</i>	<i>-0.063</i>		-0.302***
	Yellow chroma	<i>0.006</i>	<i>0.156**</i>	<i>0.154**</i>	<i>-0.426***</i>	

Table S2.3. Coefficients from the commonality analysis performed for each color trait. Using additive multiple regression models, we tested the ability of each male color trait to predict the values of each female color trait. The process was then repeated using the female color traits as the predictor variables. “Unique” refers to the variance explained uniquely by the trait tested, while “common” refers to the variance jointly explained by the association of ornament-specific traits (e.g., the ability of male blue brightness, male blue hue, and male blue UV-chroma together to account for variance in female blue brightness); r^2 and r^2_{adj} are the two estimates of the proportion of the variance explained by the full model. The highest value for each model is in bold.

a/		Female blue brightness	Female blue hue	Female blue UV- chroma	Female yellow brightness	Female yellow chroma
r^2		0.123	0.166	0.274	0.113	0.236
r^2_{adj}		0.120	0.163	0.271	0.110	0.234
Male blue brightness	Unique	0.0924	0.0078	0.0207	0.0063	0.0008
	Common	0.0013	-0.0013	-0.0181	0.0048	0.0049
Male blue hue	Unique	0.0046	0.1398	0.0094	0.0059	0.0016
	Common	0.0019	-0.0046	0.0084	0.0028	-0.0005
Male blue UV- chroma	Unique	0.0093	0.0093	0.2098	0.0002	0.0005
	Common	-0.0017	-0.0018	0.0158	-0.0001	0.0045
Male yellow brightness	Unique	0.0129	0.0031	0	0.087	0.0021
	Common	0.009	0.0027	0.0007	0.0121	0.0032
Male yellow chroma	Unique	0.0002	0.0072	0.0101	0.0007	0.216
	Common	0.0003	-0.0035	0.0328	0.0014	0.015
b/		Male blue brightness	Male blue hue	Male blue UV- chroma	Male yellow brightness	Male yellow chroma
r^2		0.107	0.148	0.264	0.111	0.259
r^2_{adj}		0.104	0.145	0.262	0.108	0.257
Female blue brightness	Unique	0.0821	0.0022	0.0015	0.0112	0.0004
	Common	0.0121	0.0041	-0.0004	0.0077	0.0003
Female blue hue	Unique	0.001	0.1146	0.0377	0.0032	0.0033
	Common	0.0054	0.0228	-0.0302	0.0029	0.0003
Female blue UV- chroma	Unique	0.0003	0.0044	0.2522	0.0025	0.0261
	Common	0.0021	0.0135	-0.268	-0.0019	0.0168
Female yellow brightness	Unique	0.0068	0.0023	0.0016	0.076	0.0056
	Common	0.0047	0.0072	-0.0014	0.0193	-0.0042
Female yellow chroma	Unique	0.0034	0.0006	0.0003	0.0002	0.2113
	Common	0.0016	0.0002	0.0042	0.0049	0.0153

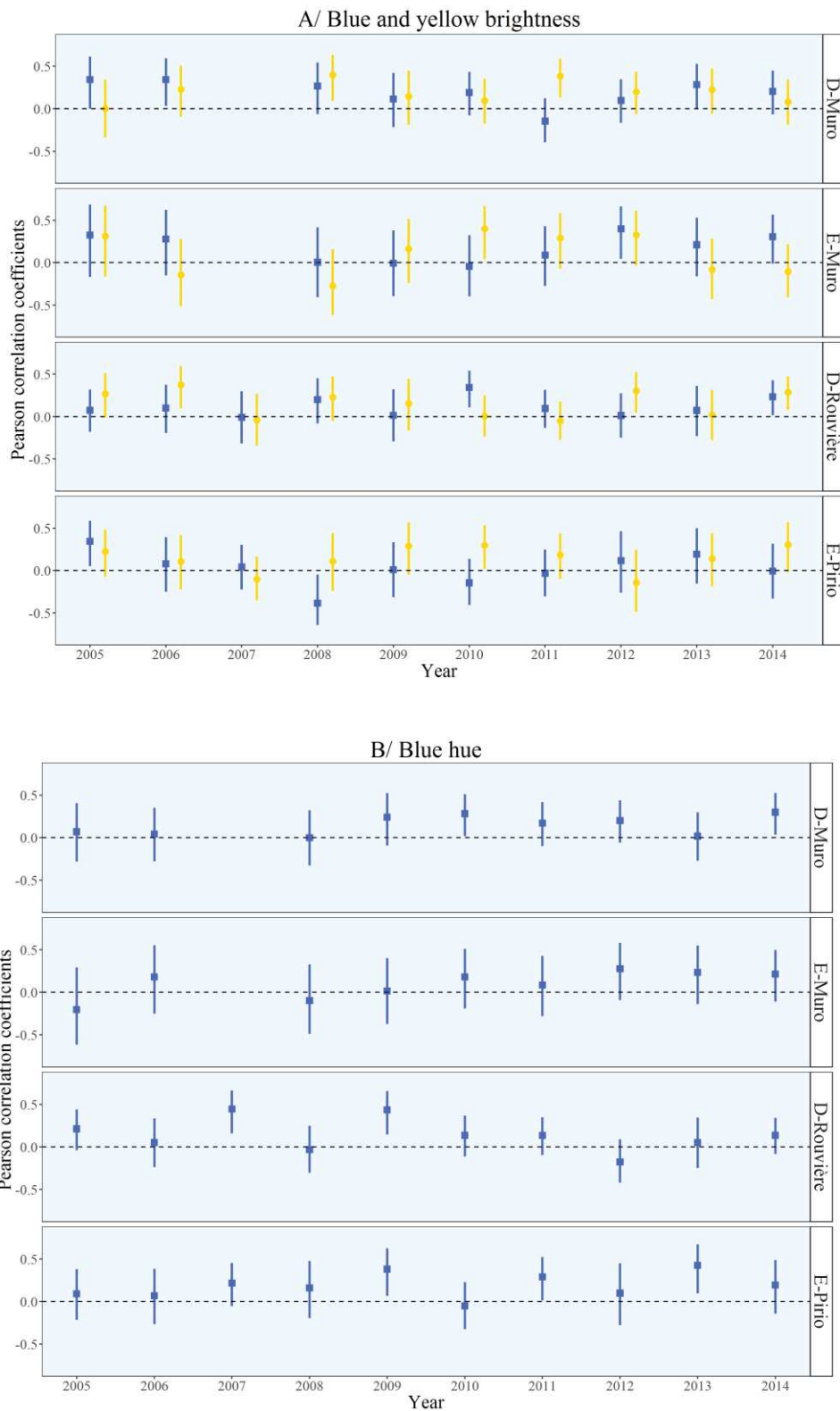


Figure S2.1 Pearson correlation coefficients for each population for each year for A/ blue brightness (blue squares) and yellow brightness (yellow circles) and B/ blue hue. The bars are the 95% confidence intervals. The dashed lines indicate a coefficient value of zero, or the absence of assortative mating.

Table S2.4 Meta-analysis models, in which year was a fixed effect. The best-fit model (in bold) was the model with the lowest DIC value. Ornament had two levels: blue crown and yellow chest patch.

Fixed effect	DIC value
Ornament+Year+Population	-456.8
Year+Population	-453.9
Population	-451.8
Population+Ornament	-449.0
Year*Population	-443.6
Ornament	-442.5
Ornament*Year+Population	-437.7
Ornament+Year	-437.4
Ornament*Year+Population*Year	-435.2
Ornament*Year	-434.5
Intercept	-433.8
Year	-431.5
Ornament+Population*Year	-426.7

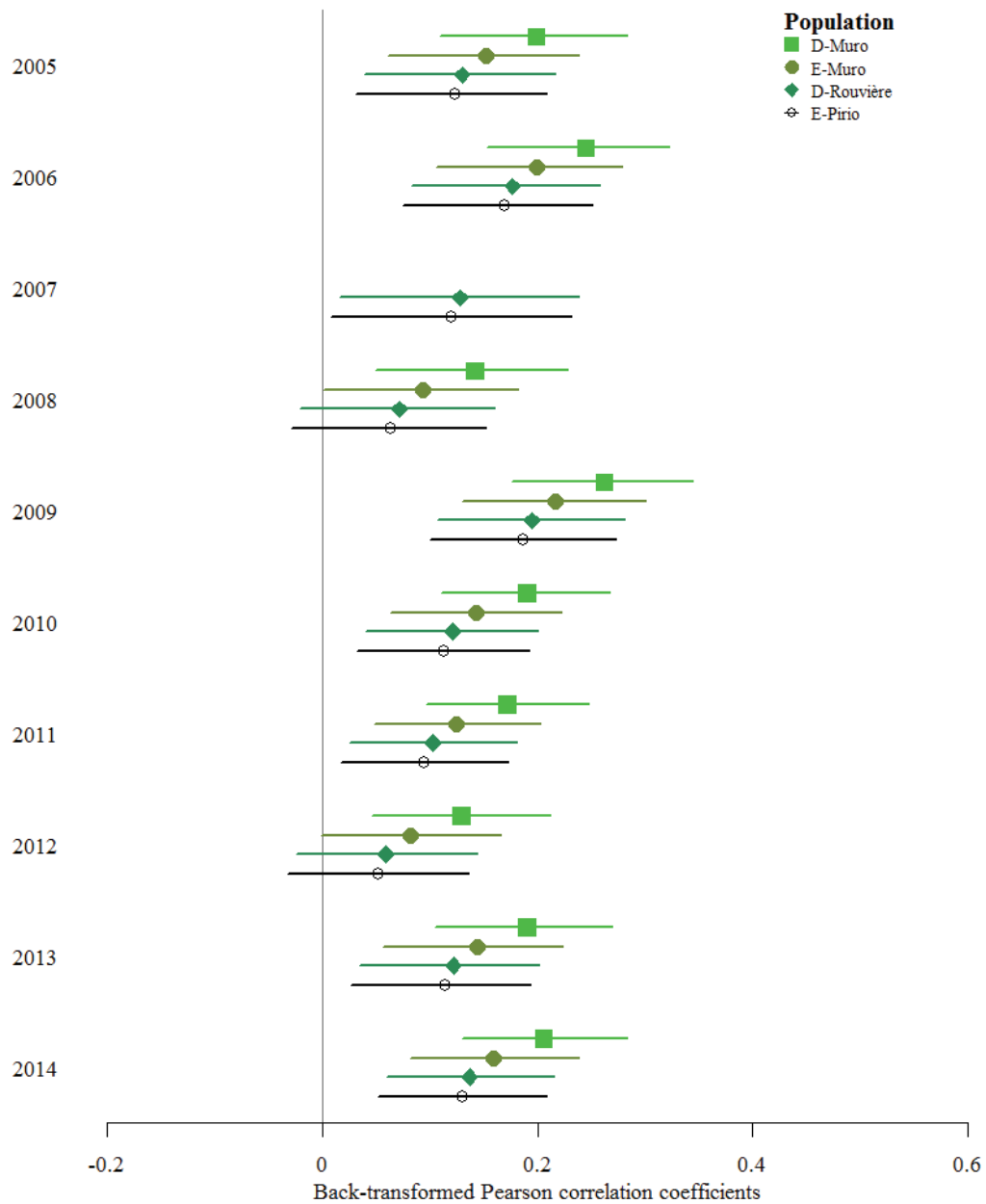


Figure S2.2 Back transformed Pearson correlation coefficients revealing the additive effects of population and year. The different study populations are represented with different symbols and different colors: D-Muro (squares, light green), E-Muro (solid circles, olive green), D-Rouvière (diamonds, dark green), and E-Pirio (open circles, black). The bars are the 95% confidence intervals. The dashed line indicates a coefficient value of zero, or the absence of assortative mating.

1 Assessing the main factors expected to drive assortative mating on colouration**2 Amélie Fargevaille, Arnaud Grégoire, Maria Del Rey Granado, Claire Doutrelant****3**
4 Abstract

5 Assortative mating refers to a non-random choice based on similarity between partners. On
6 evolutionary grounds, it may favour local adaptation and speciation. Assortative mating can
7 occur directly via mate choice thus reflecting sexual selection or can occur indirectly due to other
8 selective processes such as habitat choice or allochrony. Very few studies have assessed the
9 relative importance of these processes in wild populations and all the studies conducted so far
10 were on size-assortative mating. Here we worked on assortative mating for colour ornamentation
11 and assessed the effect of four proxies expected to affect the distance in the trait of interest
12 between partners: the level of expression of colouration in each partner, breeding time, habitat
13 quality and age of partners. We analysed assortative mating values within a pair - distance
14 between partners - for two patches of colouration in a bird, the Blue tit, with a data set
15 comprising up to 838 pairs. Our results revealed intermediate-coloured individuals were better
16 assorted than extreme individuals, a result that could be explained by a higher likelihood of
17 finding a similar mate for individuals with a more common phenotype. We did not find an effect
18 of breeding time, habitat quality or age. Assortative mating on colouration in blue tits seems thus
19 to be a direct consequence of mate choice but seems also to be strongly limited by the number of
20 mates which individuals can assess in natural populations.

21 **Introduction**

22 Assortative mating corresponds to a pattern of similarity in appearance between male and
23 female of a given pair (Jiang, Bolnick, & Kirkpatrick, 2013). The processes leading to assortative
24 mating have wide evolutionary consequences favouring local adaptation and speciation (Bolnick
25 & Kirkpatrick, 2012; Kondrashov & Shpak, 1998). They also reinforce the occurrence of a trait
26 in both sexes through sexual selection or stronger genetic correlation between trait expression and
27 preference (Kraaijeveld, Kraaijeveld-Smit, & Komdeur, 2007; Lande, 1981). Assortative mating
28 is thus a central pattern to understand in natural populations. To date most studies interested in
29 assortative mating focused on determining whether assortative mating occurs or not in natural
30 populations and to what extent. Rare are the studies trying to understand the mechanisms behind
31 it (but see Franceschi, Lemaitre, Cezilly, & Bollache, 2010; Montiglio, Wey, Chang, Fogarty, &
32 Sih, 2016; Taborsky, Guyer, & Demus, 2014).

33 Very different processes can lead to assortative mating and determining which factors
34 have more effects on assortative mating is essential to better predict its evolutionary
35 consequences at the species level. First, mate choice from one or both members of a pair can lead
36 to assortative mating if the individual is choosing its mate based on similarity to itself
37 (Kraaijeveld et al., 2007). Directional mate preference can also favour assortative mating; for
38 instance, if individuals with the highest value of a trait are better competitors and prefer highest
39 values of the same trait in their mates, it will form a pair of individuals of highest values.
40 Individuals with lower values will only have access to potential mates of lower values, creating
41 then assortative mating (McLain & Boromisa, 1987). If ornaments are correlated with age,
42 habitat choice and/or arrival on breeding site, individuals can also happen to pair together,
43 sharing incidentally the same ornaments (Gimelfarb, 1988). Finally, convergence of the degree of
44 ornaments within a pair can also lead to assortative mating if pairs remain together across

45 different breeding seasons, aged together and/or share the same habitat. This may reinforce
46 resemblance within a pair, favouring assortative mating. When assessing assortative mating as a
47 driver of the evolution of mutual ornamentation within a population, it is thus important to test
48 factors which may drive assortative mating before concluding it is due to mate choice. However,
49 few studies have attempted to do so, and most of them are related to size-assortative mating
50 (Montiglio et al., 2016).

51 *Level of expression of a trait*

52 The level of expression of a trait would be related to the first two of the four alternative
53 hypotheses developed above. If individuals are choosing their mate based on similarity to
54 themselves, we may expect the same level of assortative mating within a pair in all breeding
55 pairs. A strong directional mate preference could also lead to the same pattern. When the trait
56 preferred is positively correlated to intrinsic quality of its bearer, all individuals within a
57 population may show preferences for the same trait in their partner. In species with biparental
58 care, pair bond remains for the whole breeding season (Kokko & Monaghan, 2001). Thus only
59 high quality individuals, displaying a high level of expression of the trait of interest, would have
60 access to most-preferred mates. Individuals expressing a lower level of expression of a trait
61 would only have access to mates with a lower level of expression. Again, we may expect the
62 same level of assortative mating within a pair in all breeding pairs (Prediction 1).

63 Alternatively, individuals can show differences in their mating preferences. For instance
64 in zebra finches (*Taeniopygia guttata*), it has been shown experimentally that birds raised under a
65 given breeding condition (either low or high) preferentially choose to pair to birds having been
66 raised in similar breeding conditions and that better assorted pairs had a better reproductive
67 success than mismatched pairs (Holveck & Riebel, 2010). In Gouldian finches (*Erythrura*

68 *gouldiae*), high-quality females forced to socially paired with low-quality males experienced a
69 higher level of stress (Griffith, Pryke, & Buttemer, 2011). We may expect individuals with a high
70 level of expression of a trait to show a preference for mates with a high level of expression of a
71 trait, whereas individuals with a low level of expression of a trait would prefer mates with a low
72 level of expression of a trait. The pattern of assortative mating within a pair should then be higher
73 for individuals displaying either high or low levels of expression of a trait. Individuals displaying
74 an intermediate level of expression may be lesser assorted. When plotting assortative mating
75 within a pair against levels of expression of a trait, this should lead to a U-shape pattern of
76 assortative mating (Prediction 2).

77 *Timing in breeding*

78 In most species breeding seasonally, the earliest to seek for a mate may have more choice
79 and thus a better chance to choose a mate similar to itself than the latest ones (Dale, Rinden, &
80 Slagsvold, 1992). Thus, if mate choice leads to assortative mating, then earlier breeders would be
81 better assorted than later breeders, which may mate indiscriminately. We would thus expect a
82 positive relationship between the two variables (Prediction 3). However, timing in breeding may
83 affect assortative mating directly independently of any mate choice processes. Individuals may
84 breed earlier because they are more competitive or in better condition and may look like each
85 other because of these common characteristics (Crespi, 1989). Under this scenario both late and
86 early pairs would be highly assorted, leading to the same level of assortative mating regardless of
87 the timing of reproduction (Prediction 4).

88 *Habitat quality*

89 In species where both males and females defend a territory, higher quality individuals
90 have access to the best breeding habitat and thus higher quality individuals may have more

91 individuals interested to mate with them and be choosier than lower quality individuals that are
92 expected to pair afterwards. In consequence pairs installed in higher quality habitats may show a
93 higher assortative mating than pairs installed in lower quality habitats (Prediction 5). However,
94 having access to a better habitat may also affect diet and ecological factors related to phenotypic
95 traits. This may also lead to individuals breeding in similar habitat quality to look more alike
96 leading to reinforce positive assortative mating because of habitat similarity independently of any
97 mate choice processes (Prediction 6).

98 *Age*

99 Individuals with more experience may be better at defending a territory or acquiring a
100 mate and may be more assorted than younger individuals, which may mate indiscriminately
101 (Prediction 7). Alternatively, in the case of several breeding attempts, when a pair remains
102 together, partners may experience the same environment, affecting in the same way their own
103 phenotype (Jones, Moore, Kvarnemo, Walker, & Avise, 2003). This case can lead to a pattern of
104 assortative mating increasing or appearing after the first breeding attempt (Prediction 8).

105 Here we tested the effect of these four factors (levels of expression of a trait, timing in
106 breeding, habitat quality, age) on assortative mating on plumage coloration. Plumage colouration
107 has been related in many species of birds as the results of intra-sexual or intersexual selection
108 (see Hill, 1991 for an example of intersexual selection; Rohwer & Rohwer, 1978 for an example
109 of intrasexual selection). Also, being a secondary sexual character, plumage colouration is a
110 proxy of quality (Hill & McGraw 2006). Several studies have assessed the value of assortative
111 mating within a population on colouration in birds (e.g. Andersson et al. 1998 and Garcia-Navas
112 et al. 2009 for Blue tits; Jawor et al. 2003 for Northern cardinals; Silva et al. 2008 for European
113 rollers; Grunst and Grunst 2014 for Yellow warblers; Harris and Siefferman 2014 for Eastern

114 bluebirds) and a recent meta-analysis has determined a positive assortative mating on visual cues
115 including colouration (Jiang et al.). We used the Blue tit as our species model. We worked on the
116 variation in assortative mating within a pair presented by two colour patches in four populations
117 in a 10-year data set. To assess variation in assortative mating within a pair, we tested the effects
118 of 1) colouration quantification as the level of expression of a trait, 2) breeding time as a proxy of
119 timing of breeding, 3) habitat quality and 4) age.

120

121 **Material & Methods**

122 Study model

123 Blue tit (*Cyanistes caeruleus*) is a monogamous species of bird with low difference in colouration
124 between males and females. It presents two coloured patches that have been suggested to be
125 under sexual selection: the yellow patch on the chest and the ultraviolet blue patch on the crown
126 (see Kingma et al., 2009; Remy, Gregoire, Perret, & Doutrelant, 2010; Vedder, Schut, Magrath,
127 & Komdeur, 2010; Midamegbe, Grégoire, Perret, & Doutrelant, 2011; Limbourg, Mateman, &
128 Lessells, 2013 for ultraviolet blue crown; see C. Doutrelant et al., 2008; Peters, Kurvers, Roberts,
129 & Delhey, 2011; Claire Doutrelant, Grégoire, Midamegbe, Lambrechts, & Perret, 2012 for
130 yellow chest patch; but see Parker, 2013 for both patches). Assortative mating has been found in
131 this species on both colour traits (S. Andersson, Örnberg, & Andersson, 1998; Fargevieille,
132 Grégoire, Charmantier, Del Rey Granado, & Doutrelant, Submitted for blue crown but see
133 Garcia-Navas, Ortego, & Jose Sanz, 2009; Fargevieille et al., Submitted for yellow chest patch).

134 Feather collection

135 Since 2005, ten feathers from the yellow chest patch and eight feathers from the blue crown have
136 been collected on each breeding bird to allow colouration measurements in the lab. These

137 captured birds are located in four Mediterranean populations followed for reproduction (Blondel
138 et al. 2006, Charmantier et al. 2015). One population D-Rouvière is located on mainland France,
139 in a forest of deciduous oaks 18 km northwest from the city centre of Montpellier (43°39'N,
140 3°40'E). The three other populations are situated in the north of Corsican Island. Two
141 populations D-Muro and E-Muro are situated respectively in a deciduous-oak habitat and in an
142 evergreen-oak habitat and are located in the Regino Valley (42°32'N, 8°54'E). The last
143 population E-Pirio is situated in an evergreen-oak forest in the Fango Valley, which is 25 km
144 away from the Regino Valley (42°22'N, 8°44'E). Males and females were captured on nest
145 during chick attendance when nestling were between nine to 15 days old. Each bird was equipped
146 and identified with a metal ring from CRBPO (research centre on bird population biology, Paris)
147 programme STOC. Sex was determined based on the presence of a brood patch and age (first
148 year of reproduction or more) was determined based on wing covert colouration (Svensson,
149 1992). Laying date was determined by a weekly check of the nest-boxes. Laying date
150 corresponds to the date when the first egg of a clutch was laid and was then calculated as the
151 number of days since the 1st of March.

152 Colouration measurement

153 Feather colouration was measured in the lab using AVASPEC 2048 spectrometer (Avantes,
154 Apeldoorn NL) with an AVALIGHT-DHS deuterium-halogen lamp (Avantes, Apeldoorn NL)
155 and a 200 µm optic fibre allowing measurement between 300 and 700 nm, conducting light at a
156 fixed angle of 90° and a fixed distance of 2 mm from the sample. Samples were disposed on a
157 square of black cloth with a white standard reference estimated regularly. Each sample consisted
158 of three feathers from the blue crown or four yellow feathers from the chest patch piled in a pack
159 to recreate natural feather assemblage. We measured each sample three times, the fibre being

160 removed between each measure. For each individual and each colouration, we made
161 measurements on two different samples. Spectra were extracted using software Avicol (Gomez,
162 2006). After controlling for repeatability (Fargevieille, Grégoire, Charmantier, Del Rey Granado,
163 & Doutrelant, Submitted), we calculated mean spectrum based on the six measurements for each
164 individual and each colouration and extracted five variables. For the blue crown, we calculated an
165 achromatic variable - blue brightness (area below the reflectance curve divided by the width of
166 the interval 300 – 700 nm) and two chromatic variables - UV-blue hue (wavelength (in nm)
167 where reflectance is maximal) and UV-blue chroma (proportion of the total reflectance falling in
168 the range 300-400 nm). For the yellow patch of the chest, yellow brightness was calculated
169 following the same function as for blue brightness; yellow chroma was calculated as $(R_{700}-$
170 $R_{450})/R_{700}$ with R representing percentage of reflectance for a given wavelength. Correlation
171 among colour variables were up to 0.42 except between UV-blue hue and UV-blue chroma where
172 correlation was -0.74 (see ESM Fargevieille et al., Submitted)

173 Former studies on our populations showed the presence of sexual dichromatism and
174 spatiotemporal variations of colouration in blue crown and yellow chest patch (Charmantier,
175 Doutrelant, Dubuc-Messier, Fargevieille, & Szulkin, 2016). To avoid a potential confounding
176 effect for the comparison of mating pattern among pairs (i.e. distances as described below), we
177 standardized the five colour variables by sex, population and year.

178 Data set

179 A former study in our populations showed high spatiotemporal variation in assortative mating on
180 all colour variables with assortative mating varying between -0.385 and 0.597 (Fargevieille et al.,
181 Submitted). From this data set, we only included years with positive assortative mating as the aim
182 of the present work was to understand factors affecting variation around this traditional pattern.

183 We thus used the same database built as in Fargevieille et al. (1787 pairs) (Fargevieille et al.,
184 Submitted) but based on Pearson correlation coefficients calculated for each colour variable, each
185 year in each population (see Table S1), we restricted our sample size to pairs for which colour
186 variable, year and population presented a positive value of assortative mating (we selected values
187 with 95% confidence intervals not overlapping the -0.1 threshold). The sample size for our
188 analyses included between 506 and 838 pairs (Table 1). The annual assortative mating for this
189 data set varied between $r=0.134$ and $r=0.597$ (see Table 1 and S1). We also conducted the same
190 analyses on the complete dataset to compare and the results obtained were similar.

191 Distance between male and female colouration was used to assess assortative mating within a
192 pair and was calculated for each pair and each of the five colour variables. The distance was
193 calculated as the absolute value of the difference between male and female colour value within a
194 pair.

195 Explanatory variables explored in the analyses

196 *Level of expression of a trait* - We used the standardized value of coloration obtained in a given
197 year, for a given sex and population.

198 *Timing in breeding* - We used laying date as a proxy. Due to spatiotemporal variation in
199 phenology in our four populations (Blondel et al., 2006; Charmantier et al., 2016) we used first
200 egg laid of the year in a given population as day 1 for laying date and recalculated laying date for
201 each pair in each population and each year following this rule. We then standardized these new
202 values for laying date. Second attempts of reproduction were excluded from the analyses.

203 *Habitat quality* - We used a vegetation index based on the number of deciduous oaks and
204 evergreen oaks in a 50m radius around each nest-box. The number of evergreen oaks around nest
205 boxes has been related to laying date (Szulkin, Zelazowski, Marrot, & Charmantier, 2015). This

206 proxy of habitat quality offers the advantage of independency in time and among individuals
207 occupying the same nest-box among years. For D-Rouvière population, the forest is mainly
208 composed of deciduous oaks but a part of the forest is mixed with evergreen oaks. We summed
209 the values obtained for deciduous oaks and evergreen oaks for this population. The three other
210 populations had only one type of oaks and thus we did not have to sum oaks. Since we were
211 interested in the impact of within-site habitat on assortative mating and were not testing the
212 difference in habitat among populations, we standardized habitat quality value for each
213 population.

214 *Age* - The effect of age was estimated by three proxies: (i) the minimum age of the individual, (ii)
215 the age ratio of the population and (iii) the selection of pairs remaining together in two successive
216 breeding seasons. (i) Minimum age corresponds either to real age or to the age at the first capture
217 Birds ringed as chicks were estimated to be “zero” year old the year they were ringed. Birds
218 identified by their plumage as being ringed in their first year of breeding were estimated to be one
219 year old the year they were ringed. Birds identified by their plumage as being older than one year
220 old were estimated to be at least two years old the year they were ringed. Finally, the rare
221 breeding birds with indeterminate age when ringed were estimated to be at least one year old the
222 year they were ringed. Bird minimal age varied between one and nine years old in our final data
223 set. Due to a high mortality rate, most birds were less than five years old. Since bird minimal age
224 was used as a discrete factor, we pooled bird aged 5 or more as being “5+” years old. (ii)
225 Calculation was based on all birds captured during chick feeding period at the first attempt of
226 reproduction of the year between 2005 and 2014 in the four populations. Age ratio was calculated
227 for each sex, each year in each population as the number of birds in their first year of breeding
228 divided by the number of birds in their second or more year of breeding (Table 3). (iii) To
229 determine whether assortative mating was due to pairs remaining together in succeeding years,

230 we last restricted sample to pairs found breeding together in successive years from the initial data
231 set (all years and all populations). Since only a very restricted number of pairs were breeding
232 together three or four successive year, we only kept the two first years in our sample. Among the
233 populations, only 106 pairs (out of 1676 pairs) were retained in the sample and we assigned “1”
234 for the first year of reproduction and “2” for the successive year.

235 Statistical analyses

236 Models were analysed using linear mixed models and R package *lme4* (Pinheiro et al., 2016) with
237 an REML procedure.

238 To test the prediction that the colouration of the chooser, time of breeding, habitat quality or age
239 affects assortative mating, we ran five models that used the distance within a pair (i.e. pair
240 measurement of assortative mating) for each of the five colour variables as a dependent variable.
241 In these five models, we tested the following eight explanatory variables associated to each
242 distance value: male colouration value, male colouration squared value, female colouration value,
243 female colouration squared value, laying date, habitat quality and male and female minimal age.
244 Since a same pair could be associated in successive years, we used the identity of the pair as a
245 random effect. We used a backward stepwise model selection procedure to select our models. We
246 retained predictors for which p-values were lower than 0.1. If colour squared value was retained
247 in a model, we kept the corresponding linear value even with non-significant p-value. We then
248 calculated estimate values for each predictor retained in the final models with associated 95%
249 confidence intervals.

250 To test the prediction that levels of assortative mating within a pair were higher in years when
251 age ratio was skewed towards older birds (in their second or more year of reproduction), we used
252 linear mixed models with values of assortative mating for every year and every population.

253 Pearson correlation coefficient for assortative mating was used as the variable to explain, male
254 and female age ratios (allowing their interaction) were used as predictors, population and year
255 were added as random effect. Models were run for each of the five colour variables.

256 To test for the effect of remaining together as a pair in successive years, we used linear mixed
257 models on the restricted sample (see above). Distance of colouration within a pair was used as the
258 variable to explain, year of reproduction (either “1” or “2”) was used as a predictor, and identity
259 of the pair was used as a random effect. Models were run for each of the five colour variables.

260

261 **Results**

262 We found a positive quadratic effect of male and female colouration for each of the five colour
263 variables tested (Table 2 and Figure 2). Distance in colouration within a pair was smaller for
264 intermediate-coloured individuals (Figure 1). So, males and females displaying extreme
265 colouration values were associated to a mate more dissimilar to themselves than males and
266 females displaying intermediate colouration values. The effects were of similar magnitude
267 between males and females (Figure 2).

268 Laying date, habitat quality and male minimal age were dropped in all of our five final models.
269 Female minimal age was only retained in the final model testing yellow chest patch chroma. The
270 significant difference in distance was between female in her first year of reproduction (Female
271 age 1) and female in her second year of reproduction (Female age 2). The distance between male
272 and female yellow chroma was lower in females in the second year of reproduction than in
273 females of other ages. This means assortative mating within a pair on yellow chroma was
274 stronger when females in the pair were two year old than for any other age.

275 There was a positive effect of male age ratio on assortative mating only for the blue crown
276 brightness (Table 4). Assortative mating on blue crown brightness was stronger when the relative
277 number of males in their first year of breeding was higher.

278 Models testing the effect of remaining together in successive years show no effect on assortative
279 mating in any of the colour variables (Table 5).

280

281 **Discussion**

282 Our study aimed at understanding the main factors affecting variation in assortative
283 mating among pairs. We formulated eight predictions to start disentangling which main factors
284 drove assortative mating and thus to start revealing the main processes in action (mate choice,
285 timing in breeding, habitat quality or age). Working on assortative mating on colouration in four
286 populations of blue tits, we overall found very limited support for these predictions as only
287 female age had an effect in a single colour variable. Interestingly we found nonetheless that
288 individual colouration (either male or female) has a positive quadratic effect on assortative
289 mating. This means that individuals presenting extreme value of colouration were associated with
290 a partner with a more different colouration than individuals with a more common phenotype. This
291 suggests mate choice has to some extent a direct influence on variation in assortative mating in
292 our study species.

293 *Level of expression of colouration*

294 The positive quadratic effect of colouration on assortative mating we found (Figure 2)
295 suggests that mate choice processes lead to assortative mating but in an unexpected way. This
296 result however can be related to theoretical models showing a positive frequency-dependent
297 selection on assortative mating caused by a disadvantage of the phenotypes at the tail of

298 phenotypic distribution (Otto, Servedio, & Nuismer, 2008). The quadratic effect of colouration
299 on assortative mating could be due to an inability for extreme-coloured birds to find a mate of the
300 same level of colouration, due to the rarity of their own level of colouration. To evaluate this
301 explanation, we ran simple simulations based on the “best of n ” strategy (Uy, Patricelli, &
302 Borgia, 2001; Wittenberger, 1983) and offering the possibility to each male from the sample size
303 to choose the female presenting the closest level of colouration to their own colouration.
304 Simulations were run presenting two, four, ten of fifty potential mates to males. These
305 simulations (presented in Appendix 2) first revealed that the extreme individuals were always
306 worse assorted than others, regardless of the panel size of potential mates (Figure S1). Because of
307 the Gaussian distribution of most continuous traits, individuals with average phenotype would
308 have more chance to encounter potential mates similar with themselves than extreme phenotypes.

309 These simulations also showed a positive relationship between the panel size of potential
310 mates and the strength of assortative mating within a pair (Figure S1). This suggests that
311 individuals in our populations may only assess a limited number of potential mates and
312 individuals. An experiment testing female choice in Pied flycatchers (*Ficedula hypoleuca*)
313 already revealed females of this species were choosing among a very small panel of males (Dale,
314 Amundsen, Lifjeld, & Slagsvold, 1990; Dale et al., 1992). We need in addition to point that the
315 estimate values associated with colouration predictors were quite low (ranging from 0.10 to
316 0.18). This shows an overall identical degree of assortative mating within most values of
317 colouration, except for the extreme ones. This can mean that the degree of assortative mating
318 remains the same whatever the level of colouration, and may reveal that all individuals manage to
319 find a mate quite similar in colouration.

320 *Timing in breeding, habitat quality and age*

321 We did not find any effect of two of the three proxies expected to affect mating preference
322 or strength and/or to create assortative mating independently of mate choice processes. (i) Laying
323 dates had no effect on assortative mating. Based on this result, it is difficult to reject the
324 hypothesis that individuals initiating reproduction first might have more choice than later ones.
325 However, we cannot exclude the possibility that our proxy, laying date, might be not sufficiently
326 correlated to the actual date when blue tits form their bond. Many blue tits pair earlier in the
327 season (January to February) (Perrins, 1979). This actual pairing date is impossible to obtain in
328 natural population but a better estimation of the actual date of association within a pair is needed
329 to reject this effect. Winter social network data might help to have better estimates.

330 (ii) Our proxy of habitat quality had no effect on assortative mating. Territory quality
331 territories is variable in many species including tits (Cole, Long, Zelazowski, Szulkin, &
332 Sheldon, 2015; Hinks et al., 2015) and experimental tests have suggested the importance of the
333 blue crown patch for territoriality in both sexes (Alonso-Alvarez, Doutrelant, & Sorci, 2004;
334 Midamegbe et al., 2011; Remy et al., 2010; Vedder et al., 2010). Thus if most competitive birds
335 can choose first their territory and their mate, we would have expected to see a higher level of
336 assortative mating within a pair at least on variables related to the blue crown patch for birds
337 nesting in areas with a higher number of oaks. Because numbers of oaks are determinant for
338 laying date at least in evergreen forest (pairs breeding in a nest-box surrounded by a larger
339 number of evergreen oaks were laying earlier) (Szulkin et al., 2015), the fact that habitat quality
340 is not linked to variation in assortative mating suggests that assortative mating is not driven by
341 difference in habitat quality.

342 (iii) Our third factor, age, has an effect on assortative mating for yellow chroma but not
343 for the other variables. Our sixth prediction was that age might affect assortative mating if
344 individuals with more experience are better at defending a territory or acquiring a mate. Females

345 in their second year of reproduction were better assorted on yellow patch chroma than other
346 females. In our study species, moult occurs at the end of the breeding season except for fledglings
347 which only moult partially (acquisition of the blue crown for instance). The yellow colouration of
348 birds older than one year old may then have a plumage revealing a higher experience or quality
349 by their better ability to acquire resource before moult. The fact we found an effect for females
350 only may suggest better quality females are able to be choosier. The yellow chest patch is
351 carotenoid-based. Carotenoids are not naturally synthesised and are acquired in diet (McGraw,
352 2006). Used in colouration, they can signal the ability of the holder to acquire high quality
353 resource, and may be linked to its ability to invest in reproduction (C. Doutrelant et al., 2008;
354 Midamegbe et al., 2013 for female blue tits) or raise chicks (Biard, Surai, & Moller, 2007). This
355 could explain why females in their second year of reproduction are better assorted than females in
356 their first year of reproduction. However, this fails to explain the lack of difference we observe
357 for older females compare to first year breeders. Senescence might explain this absence of effect
358 for females older than two years. In short-live birds like tits senescence in laying processes
359 arrives around age four and can even appear earlier depending on the experience of the male she
360 is associated with (Auld & Charmantier, 2011). Senescence for colouration may follow the same
361 pattern. Thus, females designated as being more than two years may reveal cues of senescence,
362 which may explain why they were worse assorted than two-year-old females. For the other colour
363 variables and in particular for colouration of the blue crown, we found no effect of age, age ratio,
364 nor the fact of remaining as a pair in successive years on assortative mating. This rejects the
365 hypothesis that the pattern of assortative mating observed is explained by fidelity in pairs among
366 years for both colourations and by a better experience and quality for ultraviolet blue colouration.

367 As a general observation, our sampling protocol may also explain our results. We
368 captured birds and collected feathers during chick feeding, when chicks were at least nine days
369 old. This means we only collected feathers for pairs with a relative breeding success (ability to
370 raise chicks to nine days old) and this data set may only include good quality individuals. This
371 could affect two of the factors we tested: level of expression of the colouration and habitat
372 quality. Highly mismatched pairs or birds breeding in a very poor habitat may not access to the
373 stage of feeding nine-day-old chicks. Being able to collect feathers on pairs with hatching failure
374 or with precocious mortality in hatchling may have led to marked differences.

375 The evolution of ornamental traits in both sexes is still a pattern which needs to be
376 precisely understood in natural populations (Kraaijeveld et al., 2007). Understanding what drives
377 assortative mating is a key to determine the importance of mutual mate choice in natural
378 population but also to assess the role of mate choice in local adaptation (Jiang et al., 2013). Here
379 we tested several factors identified previously as driving assortative mating. Based on the largest
380 database ever used, we tested important factors which may explain assortative mating but did not
381 find an effect of most of these factors. However we did find a significant quadratic relation
382 between assortative mating and colouration in both sexes which suggested a direct assortative
383 mating based on choice in similarity in colouration but also suggested a difficulty for extreme
384 birds to be assorted. This effect needs to be confirmed by experimental approaches in natural
385 populations and mathematical modelling.

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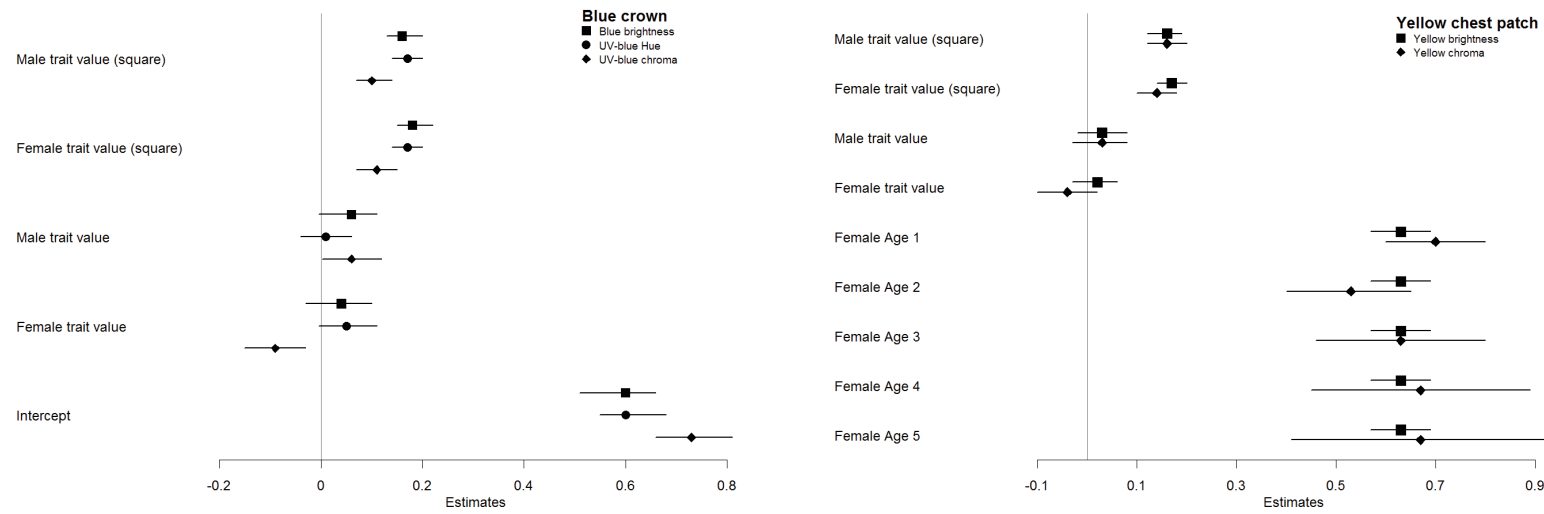


Figure 1. Forest plots corresponding to both patches.

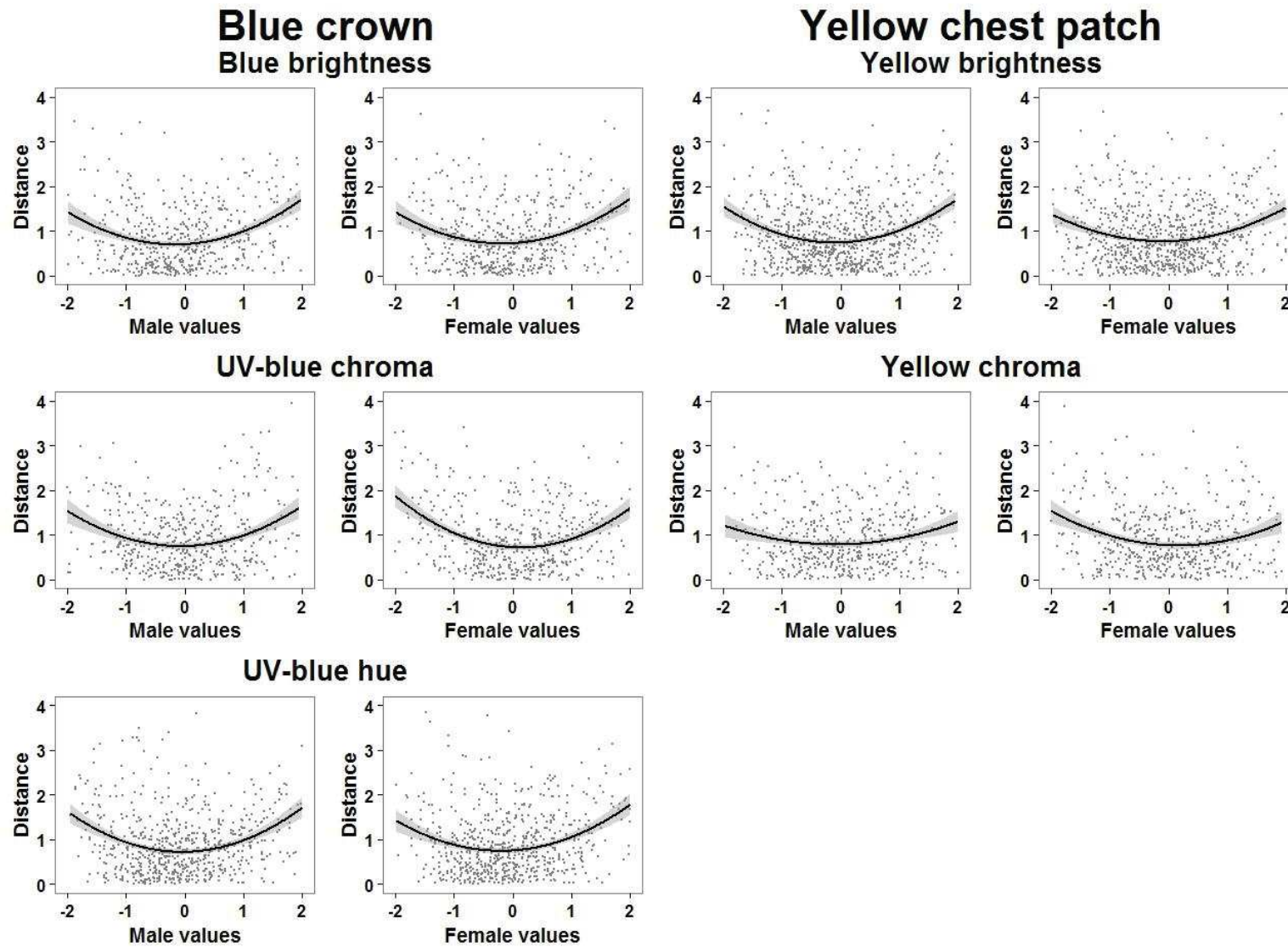


Figure 2. Correlation between individual values and distance in colouration within a pair for each colour variable and each sex.

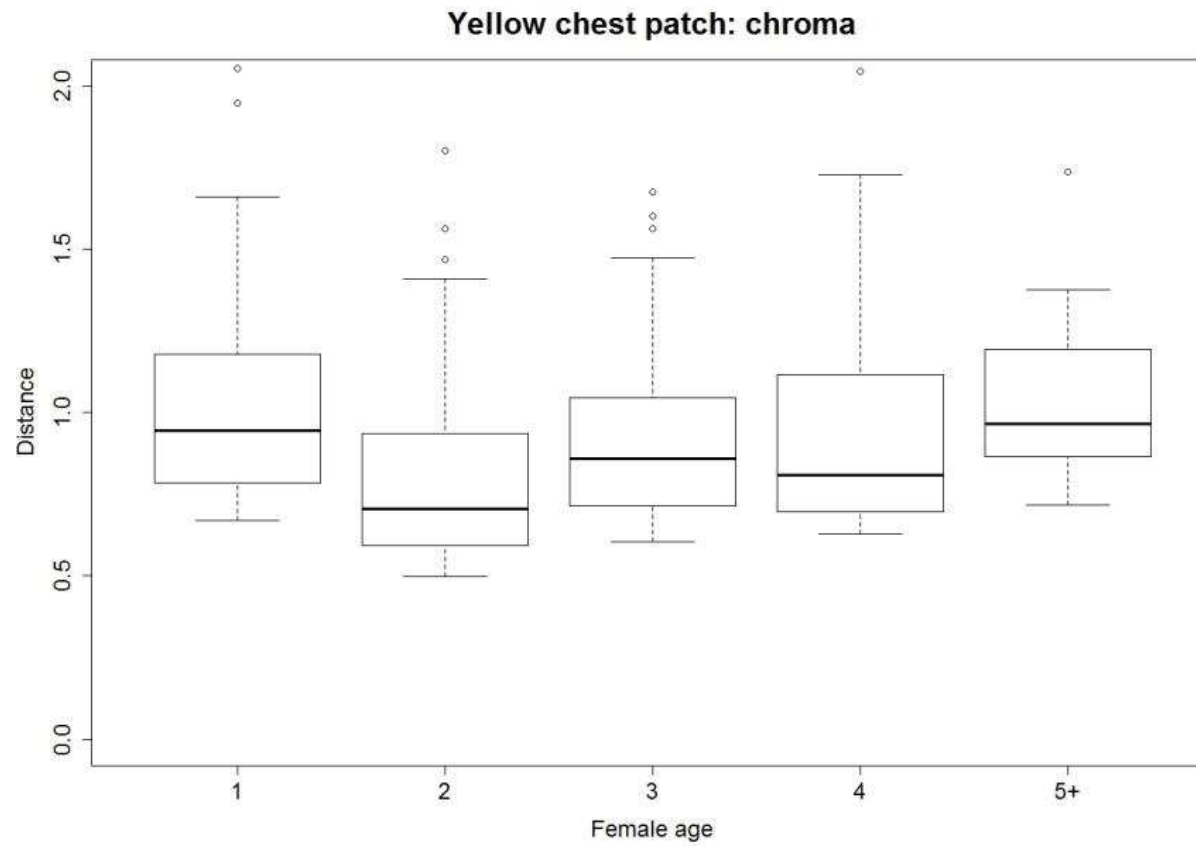


Figure 3. Box plots representing the distribution of distance of colouration within pairs depending on the minimal age of the female (see Methods for calculation). Female age “5+” represents females with a minimal age of 5 years old or more.

Table 1 Summary table representing years which have been selected in each population for each colour variable (see Methods for year selection).

Colour variable	D-ROUVIERE	D-MURO	E-MURO	E-PIRIO	Number of years	Number of pairs
Blue brightness (BB)	2010- 2014	2005-2006 2008-2010 2013-2014	2012-2014	2005	11	530
UV-blue hue (UVBH)	2005-2007 2011-2014	2009-2010 2011-2014	2012	2007-2009 2011-2013	13	663
UV-blue chroma (UVBC)	2007	2005-2006 2008-2009 2010-2011	2006-2014	2006-2007 2012	12	515
Yellow brightness (YB)	2005-2006 2008-2012 2014	2006-2008 2011-2012 2013	2010-2011 2012	2005-2009 2010-2011 2014	18	838
Yellow chroma (YC)	2009-2010	2006-2008 2009-2010 2012-2013	2011-2014	2011	11	506

Table 2 Estimates associated with each final model. NS means non-significant predictor not retained in the final model. Marginal r^2 corresponds to the ratio of variance explained by fixed effects; conditional r^2 to the ratio of variance explained by fixed and random effects (year and population as random effects). Significant effects in bold

Blue brightness ($r^2_{\text{marg}}=0.32$; $r^2_{\text{cond}}=0.32$)						
Predictor	Estimate	95% Confidence interval	Standard-error	t-value	Degrees of freedom	p-value
Intercept	0.58	0.51-0.66	0.04	15.75	515	<0.001
Squared male blue brightness	0.16	0.13-0.20	0.02	8.43	515	<0.001
Squared female blue brightness	0.18	0.15-0.22	0.02	10.08	515	<0.001
Male blue brightness	0.06	-0.003-0.11	0.03	1.83	515	0.07
Female blue brightness	0.04	-0.03-0.10	0.03	1.12	515	0.26
Laying date	NS	NS	NS	NS	NS	NS
Habitat quality	NS	NS	NS	NS	NS	NS
Male age	NS	NS	NS	NS	NS	NS
Female age	NS	NS	NS	NS	NS	NS
UV-Blue Chroma ($r^2_{\text{marg}}=0.16$; $r^2_{\text{cond}}=0.56$)						
Predictor	Estimate	95% Confidence interval	Standard-error	t-value	Degrees of freedom	p-value
Intercept	0.73	0.66;0.81	0.04	19.1	479	<0.001
Squared male blue brightness	0.10	0.07;0.14	0.02	5.22	501	<0.001
Squared female blue brightness	0.11	0.07;0.15	0.02	5.34	501	<0.001
Male blue brightness	0.06	0.003;0.12	0.03	2.05	499	0.04
Female blue brightness	-0.09	-0.15;-0.03	0.03	-2.97	502	0.003
Laying date	NS	NS	NS	NS	NS	NS
Habitat quality	NS	NS	NS	NS	NS	NS
Male age	NS	NS	NS	NS	NS	NS
Female age	NS	NS	NS	NS	NS	NS
UV-Blue hue ($r^2_{\text{marg}}=0.34$; $r^2_{\text{cond}}=0.34$)						
Predictor	Estimate	95% Confidence interval	Standard-error	t-value	Degrees of freedom	p-value
Intercept	0.62	0.55;0.68	0.03	19.4	696	<0.001
Squared male blue brightness	0.17	0.14;0.20	0.02	10.7	696	<0.001
Squared female blue brightness	0.17	0.14;0.20	0.02	11.2	696	<0.001
Male blue brightness	0.01	-0.04;0.06	0.03	0.38	696	0.70
Female blue brightness	0.05	-0.004;0.11	0.03	1.82	696	0.07
Laying date	NS	NS	NS	NS	NS	NS
Habitat quality	NS	NS	NS	NS	NS	NS
Male age	NS	NS	NS	NS	NS	NS
Female age	NS	NS	NS	NS	NS	NS

Table 2 (following)

Yellow brightness (r²marg=0.23 ; r²cond=0.29)						
Predictor	Estimate	95% Confidence interval	Standard-error	t-value	Degrees of freedom	p-value
Intercept	0.63	0.57;0.69	0.03	20.6	785	<0.001
Squared male blue brightness	0.16	0.12;0.19	0.02	9.51	803	<0.001
Squared female blue brightness	0.17	0.14;0.20	0.02	10.6	814	<0.001
Male blue brightness	0.03	-0.02;0.08	0.02	1.27	806	0.20
Female blue brightness	0.02	-0.03;0.06	0.02	0.63	813	0.53
Laying date	NS	NS	NS	NS	NS	NS
Habitat quality	NS	NS	NS	NS	NS	NS
Male age	NS	NS	NS	NS	NS	NS
Female age	NS	NS	NS	NS	NS	NS
Yellow chroma (r²marg=0.20 ; r²cond=0.29)						
Predictor	Estimate	95% Confidence interval	Standard-error	t-value	Degrees of freedom	p-value
Female age 1	0.70	0.60;0.80	0.05	13.6	486	<0.001
Squared male blue brightness	0.16	0.12;0.20	0.02	7.87	493	<0.001
Squared female blue brightness	0.14	0.10;0.18	0.02	6.60	482	<0.001
Male blue brightness	0.02	-0.03;0.08	0.03	0.79	493	0.43
Female blue brightness	-0.04	-0.10;0.02	0.03	-1.29	489	0.20
Laying date	NS	NS	NS	NS	NS	NS
Habitat quality	NS	NS	NS	NS	NS	NS
Male age	NS	NS	NS	NS	NS	NS
Female age 2	0.53	0.40;0.65	0.07	-2.65	491	0.008
Female age 3	0.63	0.46;0.80	0.09	-0.84	493	0.40
Female age 4	0.67	0.45;0.89	0.11	-0.24	491	0.81
Female age 5+	0.67	0.41;0.93	0.14	0.23	473	0.82

Table 3 Age ratio (number of birds in first year of reproduction divided by number of birds in second year of reproduction or more) in males and females every year in every population. n refers to the number of males or females used to calculate age ratio.

year	D-MURO				E-MURO				E-PIRIO				D-ROUVIERE			
	Male		Female		Male		Female		Male		Female		Male		Female	
	n	Age	n	Age	n	Age ratio	n	Age	n	Age	n	Age ratio	n	Age	n	Ag
2005	35	0.8	40	1.2	22	1.2	21	0.4	50	0.5	47	0.4	51	0.9	55	0.8
2006	39	1.6	42	0.7	26	0.6	32	0.3	39	0.7	41	1.1	49	0.8	51	0.6
2007	NA	NA	NA	NA	NA	NA	NA	NA	62	0.7	65	0.4	44	1.8	46	1.4
2008	44	0.4	50	0.4	25	0.6	25	0.6	40	0.3	40	0.4	54	1.3	66	1.1
2009	39	0.3	40	0.5	30	0.5	30	0.4	43	0.1	44	0.3	43	0.3	47	0.5
2010	55	0.9	63	0.9	33	0.6	33	0.7	52	0.2	58	0.3	70	0.7	72	1.3
2011	51	0.4	50	0.8	33	0.7	37	0.6	58	0.2	59	0.6	83	1.6	88	1.8
2012	61	0.8	63	1.3	31	0.7	33	0.5	34	0.2	34	0.4	59	1.3	77	1.7
2013	54	0.2	57	0.2	33	0.4	33	0.2	43	0.2	44	0.2	43	0.5	54	0.7
2014	56	0.6	57	0.5	41	1.0	41	0.6	40	0.5	44	0.5	85	1.4	88	1.0

Table 4. Estimate values associated with models on age ratio for each colour variable. Marginal r^2 corresponds to the ratio of variance explained by fixed effects; conditional r^2 to the ratio of variance explained by fixed and random effects (year and population as random effects). Significant effects in bold.

Blue brightness				Yellow brightness			
Predictor	Estimate	Standard-error	p-value	Predictor	Estimate	Standard-error	p-value
Male age ratio	0.38	0.13	0.01	Male age ratio	0.10	0.16	0.54
Female age ratio	0.07	0.16	0.69	Female age ratio	0.11	0.19	0.56
Male age ratio x female age ratio	-0.23	0.14	0.11	Male age ratio x female age ratio	-0.14	0.16	0.40
Marginal r^2	0.20			Marginal r^2	0.02		
Conditional r^2	0.21			Conditional r^2	0.02		
UV-blue chroma				Yellow chroma			
Predictor	Estimate	Standard-error	p-value	Predictor	Estimate	Standard-error	p-value
Male age ratio	-0.04	0.17	0.84	Male age ratio	0.20	0.15	0.19
Female age ratio	-0.27	0.20	0.19	Female age ratio	0.10	0.18	0.57
Male age ratio x female age ratio	0.16	0.18	0.38	Male age ratio x female age ratio	-0.22	0.16	0.17
Marginal r^2	0.04			Marginal r^2	0.08		
Conditional r^2	0.16			Conditional r^2	0.08		
UV-blue hue							
Predictor	Estimate	Standard-error	p-value				
Male age ratio	-0.22	0.13	0.12				
Female age ratio	-0.18	0.16	0.28				
Male age ratio x female age ratio	0.19	0.14	0.17				
Marginal r^2	0.08						
Conditional r^2	0.12						

Table 5. Estimate values associated with models for each colour variable with pairs mating together two successive years. “Pairing year” refers to “1” for first year pair was found mating together, “2” as second year pair was found mating together. Marginal r^2 corresponds to the ratio of variance explained by fixed effects; conditional r^2 to the ratio of variance explained by fixed and random effects (year and population as random effects). Significant effects in bold.

Blue brightness				Yellow brightness			
Predictor	F-value	df	p-value	Predictor	F-value	df	p-value
Pairing year	0.15	1;104	0.70	Pairing year	0.21	1;209	0.65
Marginal r^2	0.004			Marginal r^2	0.001		
Conditional r^2	0.004			Conditional r^2	0.001		
UV-blue chroma				Yellow chroma			
Predictor	F-value	df	p-value	Predictor	F-value	df	p-value
Pairing year	0.15	1;104	0.70	Pairing year	0.77	1;105	0.38
Marginal r^2	0.0006			Marginal r^2	0.003		
Conditional r^2	0.132			Conditional r^2	0.137		
UV-blue hue							
Predictor	F-value	df	p-value				
Pairing year	0.41	1;100	0.52				
Marginal r^2	0.002						
Conditional r^2	0.06						

Appendix

Table S1. Assortative mating values for each variable from the blue crown and the yellow chest patch, each year in each population. “r[CI]” refers to the corresponding value of Pearson correlation coefficient with its 95% confidence interval in brackets; n refers to the number of pairs corresponding to r[CI].

Blue brightness

year	D-MURO		E-MURO		E-PIRIO		D-ROUVIERE	
	n	r [CI]	n	r [CI]	n	r [CI]	n	r [CI]
2005	33	0.343 [0;0.614]	19	0.325 [-0.167;0.687]	46	0.346 [0.05;0.585]	62	0.074 [-0.179;0.318]
2006	39	0.344 [0.032;0.595]	24	0.28 [-0.15;0.621]	37	0.08 [-0.25;0.394]	47	0.1 [-0.193;0.376]
2007	0	NA	0	NA	58	0.043 [-0.225;0.304]	41	-0.009 [-0.316;0.299]
2008	39	0.267 [-0.062;0.544]	23	0.005 [-0.408;0.416]	33	-0.385 [-0.643;-0.048]	51	0.2 [-0.08;0.45]
2009	37	0.115 [-0.217;0.423]	26	-0.008 [-0.394;0.381]	37	0.011 [-0.314;0.334]	42	0.015 [-0.294;0.321]
2010	54	0.19 [-0.081;0.436]	30	-0.042 [-0.396;0.323]	50	-0.145 [-0.407;0.139]	65	0.343 [0.109;0.542]
2011	55	-0.146 [-0.396;0.124]	32	0.089 [-0.274;0.43]	51	-0.032 [-0.305;0.246]	76	0.096 [-0.132;0.315]
2012	58	0.098 [-0.166;0.35]	31	0.399 [0.045;0.664]	29	0.116 [-0.261;0.463]	56	0.013 [-0.251;0.275]
2013	50	0.283 [-0.005;0.527]	30	0.211 [-0.161;0.531]	38	0.193 [-0.155;0.499]	44	0.073 [-0.229;0.362]
2014	54	0.204 [-0.067;0.448]	39	0.305 [-0.012;0.566]	37	-0.008 [-0.331;0.317]	84	0.233 [0.018;0.427]

UV-blue chroma

year	D-MURO		E-MURO		E-PIRIO		D-ROUVIERE	
	n	r [CI]	n	r [CI]	n	r [CI]	n	r [CI]
2005	33	0.262 [-0.089;0.556]	19	-0.142 [-0.571;0.348]	46	0.069 [-0.236;0.362]	62	0.108 [-0.145;0.349]
2006	39	0.357 [0.046;0.604]	24	0.53 [0.151;0.773]	37	0.293 [-0.034;0.564]	47	0.109 [-0.184;0.384]
2007	0	NA	0	NA	58	0.218 [-0.05;0.457]	41	0.368 [0.068;0.607]
2008	39	0.42 [0.111;0.655]	23	-0.212 [-0.574;0.219]	33	-0.164 [-0.48;0.19]	51	0.064 [-0.216;0.333]
2009	37	0.597 [0.339;0.772]	26	0.154 [-0.248;0.511]	37	0.164 [-0.169;0.463]	42	0.191 [-0.124;0.471]
2010	54	0.237 [-0.033;0.474]	30	0.247 [-0.124;0.558]	50	0.175 [-0.109;0.432]	65	-0.143 [-0.374;0.104]
2011	55	0.295 [0.032;0.519]	32	-0.155 [-0.483;0.211]	51	0.078 [-0.202;0.346]	76	0.127 [-0.101;0.343]
2012	58	-0.166 [-0.409;0.099]	31	0.126 [-0.246;0.465]	29	0.321 [-0.052;0.615]	56	-0.043 [-0.303;0.222]
2013	50	0.006 [-0.282;0.293]	30	0.208 [-0.164;0.529]	38	0.043 [-0.299;0.376]	44	0.029 [-0.27;0.323]
2014	54	0.131 [-0.142;0.385]	39	0.333 [0.02;0.587]	39	0.354 [0.043;0.602]	84	0.076 [-0.142;0.287]

UV-blue hue

year	D-MURO		E-MURO		E-PIRIO		D-ROUVIERE	
	n	r [CI]	n	r [CI]	n	r [CI]	n	r [CI]
2005	33	0.07 [-0.28;0.404]	19	-0.204 [-0.613;0.29]	46	0.092 [-0.214;0.382]	62	0.212 [-0.04;0.439]
2006	39	0.041 [-0.278;0.352]	24	0.181 [-0.25;0.552]	37	0.067 [-0.263;0.383]	47	0.053 [-0.238;0.335]
2007	0	NA	0	NA	58	0.216 [-0.052;0.455]	41	0.444 [0.158;0.661]
2008	39	-0.002 [-0.325;0.323]	23	-0.097 [-0.489;0.329]	33	0.16 [-0.194;0.477]	51	-0.031 [-0.304;0.247]
2009	37	0.24 [-0.091;0.523]	26	0.016 [-0.373;0.401]	37	0.381 [0.065;0.627]	42	0.434 [0.146;0.655]
2010	54	0.282 [0.016;0.511]	30	0.182 [-0.19;0.509]	50	-0.051 [-0.325;0.23]	65	0.136 [-0.112;0.367]
2011	55	0.171 [-0.098;0.418]	32	0.084 [-0.279;0.426]	51	0.29 [0.015;0.524]	76	0.134 [-0.095;0.349]
2012	58	0.202 [-0.059;0.438]	31	0.277 [-0.092;0.58]	29	0.1 [-0.277;0.45]	56	-0.176 [-0.42;0.091]
2013	50	0.017 [-0.269;0.299]	30	0.233 [-0.139;0.548]	38	0.426 [0.097;0.671]	44	0.053 [-0.248;0.344]
2014	54	0.298 [0.033;0.524]	39	0.214 [-0.109;0.496]	37	0.193 [-0.14;0.486]	84	0.136 [-0.082;0.342]

Yellow brightness

year	D-MURO		E-MURO		E-PIRIO		D-ROUVIERE	
	n	r [CI]	n	r [CI]	n	r [CI]	n	r [CI]
2005	33	0.003 [-0.34;0.346]	19	0.314 [-0.163;0.672]	46	0.223 [-0.072;0.482]	62	0.27 [-0.009;0.51]
2006	39	0.228 [-0.094;0.507]	24	-0.14 [-0.515;0.279]	37	0.109 [-0.223;0.418]	47	0.374 [0.097;0.597]
2007	0	NA	0	NA	58	-0.103 [-0.352;0.16]	41	-0.04 [-0.344;0.271]
2008	39	0.397 [0.093;0.633]	23	-0.273 [-0.616;0.157]	33	0.112 [-0.24;0.439]	51	0.228 [-0.051;0.474]
2009	37	0.145 [-0.188;0.448]	26	0.163 [-0.24;0.517]	37	0.289 [-0.049;0.567]	42	0.156 [-0.163;0.446]
2010	54	0.097 [-0.176;0.355]	30	0.401 [0.041;0.669]	50	0.297 [0.021;0.532]	65	0.008 [-0.237;0.251]
2011	55	0.385 [0.134;0.59]	32	0.29 [-0.072;0.584]	51	0.183 [-0.097;0.437]	76	-0.05 [-0.276;0.18]
2012	58	0.199 [-0.062;0.435]	31	0.327 [-0.031;0.611]	29	-0.14 [-0.488;0.246]	56	0.305 [0.046;0.526]
2013	50	0.225 [-0.06;0.476]	30	-0.082 [-0.429;0.287]	38	0.14 [-0.189;0.44]	44	0.021 [-0.277;0.316]
2014	54	0.084 [-0.188;0.344]	39	-0.104 [-0.407;0.218]	37	0.303 [-0.023;0.571]	84	0.288 [0.076;0.476]

Yellow chroma

year	D-MURO		E-MURO		E-PIRIO		D-ROUVIERE	
	n	r [CI]	n	r [CI]	n	r [CI]	n	r [CI]
2005	33	0.138 [-0.215;0.46]	19	0.265 [-0.215;0.642]	46	-0.01 [-0.299;0.281]	62	0.042 [-0.239;0.317]
2006	39	0.354 [0.043;0.602]	24	0.261 [-0.159;0.601]	37	0.215 [-0.117;0.504]	47	0.118 [-0.175;0.392]
2007	0	NA	0	NA	58	0.106 [-0.157;0.355]	41	-0.004 [-0.311;0.304]
2008	39	0.38 [0.073;0.621]	23	0.227 [-0.205;0.584]	33	-0.147 [-0.467;0.206]	51	0.15 [-0.131;0.409]
2009	37	0.237 [-0.094;0.521]	26	-0.043 [-0.423;0.35]	37	0.193 [-0.15;0.494]	42	0.444 [0.153;0.664]
2010	54	0.205 [-0.067;0.448]	30	-0.024 [-0.387;0.345]	50	0.097 [-0.186;0.366]	65	0.289 [0.048;0.498]
2011	55	-0.057 [-0.317;0.212]	32	0.567 [0.266;0.767]	51	0.351 [0.083;0.571]	76	-0.096 [-0.317;0.136]
2012	58	0.211 [-0.05;0.445]	31	-0.205 [-0.522;0.161]	29	-0.14 [-0.488;0.246]	56	-0.027 [-0.288;0.237]
2013	50	0.331 [0.055;0.56]	30	0.124 [-0.247;0.464]	38	0.14 [-0.189;0.44]	44	0.186 [-0.117;0.458]
2014	54	-0.026 [-0.292;0.244]	39	0.281 [-0.038;0.548]	39	-0.001 [-0.325;0.323]	84	0.074 [-0.146;0.286]

Appendix: simulations

The positive quadratic effect found between male and female and distance in colouration in the pair revealed a disadvantage for extreme colourations compared to intermediate individuals. The large variance associated oriented us towards the idea of a limited choice of potential mates for individuals. We decided to run simple simulations in R to test for the limitation of choice among potential mates. To do so, we used a single population, D-MURO, and colour values for the ultraviolet blue chroma variable extracted from the blue crown. We used the scaled values for males and females across the nine years (2005 to 2014 except 2007, since no feathers were collected in the population). For each single male value, we assigned randomly two, four, ten or fifty female values. We then created a script which would assign the “best of n ” female (female value was closer to the male value). We then plotted male values against distance in the pair assigned by simulation for the four simulations (two, four, 10 or 50 potential mates) and we drew the predicted line associated with the plot, using function `smooth` in *ggplot2* (Wickham, 2009). Our results concurred with the idea that limited choice decreased assortative mating across pairs. When there was a larger choice of potential mates, distance in colouration within a pair was smaller, which meant assortative mating was stronger. Furthermore, the predicted line was flatter with a larger choice of potential mates, meaning any individual, independent of its colouration, was more able to find a mate with a colouration similar to itself. The extreme individuals were still disadvantaged with a weaker assortative mating (larger distance in colouration). Simulated plots looked closer to the real plots (Figure 2) when the choice of potential mates was limited to two or four. This concurred with the idea of a limited choice in the availability of potential mates.

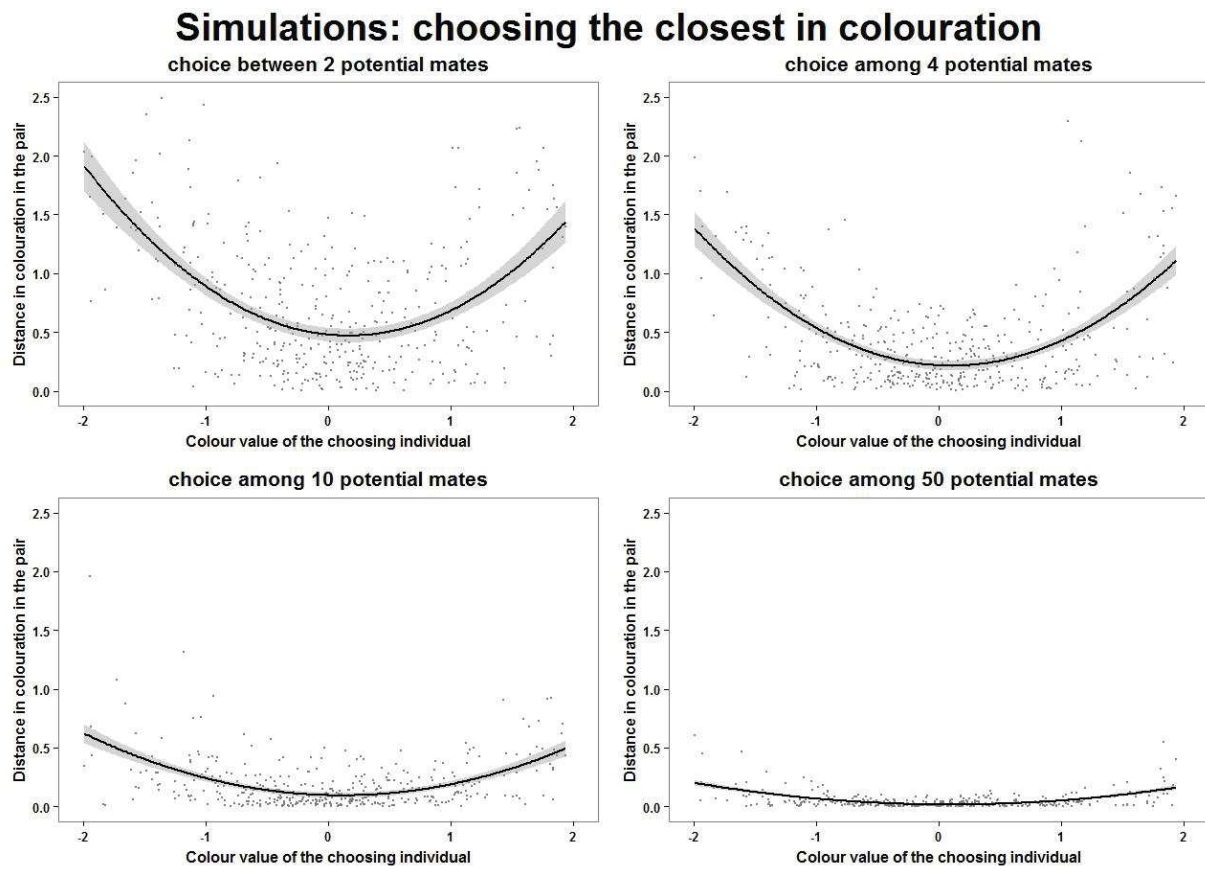


Figure S1 Plots illustrating results from simulations using all values from D-MURO populations and UV-blue chroma from the blue crown.

CHAPTER IV

DISCUSSION

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This thesis was aimed at assessing the role of sexual selection in the evolution and maintenance of female ornaments. The work performed was more particularly focused on the role of mate choice, either male or mutual in relation to plumage colouration. In a first part, the comparative methods on songbirds confirmed the importance of male investment in parental care as a key factor for the evolution of female plumage colouration, the mechanism being that parental care favours male mate choice which leads to the evolution of female ornamentation. This part also showed how female initial investment in reproduction may limit the development of colouration. The second part based on colouration in the Blue tit first revealed a wide spatiotemporal variation that needs to be considered when testing relations between proxies of reproductive success and colouration, and also when testing patterns of assortative mating on colouration. Despite this variation, mean values obtained through meta-analyses confirmed hints of selection gradients between female colouration and early proxies of reproductive success; there was also a weak but positive assortative mating for both coloured patches. Last results from quantitative genetics showed some heritability for blue ornamentation in both sexes and high levels of genetic correlations, both suggesting potential for evolution and that what occurs in one sex have consequences on the colouration of the other sex.

1. The interspecific level

In the 1990s, results of the comparative analyses on plumage colouration revealed the necessity to focus on female ornaments and to consider the factors supposed to drive their evolution (Irwin 1994; Burns 1998). To my knowledge, only one comparative study focused on female plumage colouration, tested and showed the effect of social selection (Rubenstein and Lovette 2009). The other comparative studies including the test of female ornaments were working on colouration in both sexes and tested ecological factors along with sexual selection (Dale et al. 2015; Dunn et al. 2015). Their scale of research could not test fine scale processes related to the effect of sexual selection on female ornaments. In particular, male mate choice which is supposed to occur only under restricted conditions (Edward and Chapman 2011) was not precisely tested. The comparative analysis conducted in my thesis offered to fill this gap. It offered in particular first answers on (1.1) the importance of taking into account phenotypic and genetic correlation, (1.2) the influence of male parental care and costs of reproduction and (1.3) the effect of breeding density for the evolution of female ornamentation. I will discuss these factors below, highlighting their limitation and the perspectives in these sections and in section 1.4.

1.1. Phenotypic and genetic correlation

Models were built to test factors related to male mate choice while considering costs of reproduction. However, since genetic correlation and other male correlated aspects could partly explain the evolution of female colouration (Lande 1980; Kraaijeveld et al. 2007), male colouration needed to be included to correct for its effect. This inclusion also allows being conservative in our results. Results revealed a strong phenotypic correlation between female and male colouration, either using colour volume or intensity of colouration. Phenotypic correlation is likely related to genetic correlation but may also be explained by other ecological factors such as a common diet in males and females (Jawor et al. 2003). In regards of these results, quantitative genetics seem a promising method to precisely assess genetic correlation (see MS2). Historically, genetic correlation has been the main factor developed to predict the evolution of female colouration (Lande 1980). Comparative studies using dimorphism or dichromatism considered genetic correlation in their approach (Rubenstein and Lovette 2009; Dunn et al. 2015; Hegyi et al. 2015). The recent study published by Dale and collaborators (2015) worked independently on male and female colouration, but they added an analysis to test the effect of genetic correlation. However to my knowledge no studies working exclusively on male ornaments corrected for the effect of female ornaments (e.g. Eaton 2006; Mason et al. 2014). The strong correlation found in our analysis and the fact that female ornaments are functional expressed the necessity to consider also female colouration when working on male colouration to test unambiguously the role in the focal gender. In a set of analyses more focused on the evolution of male colouration with data I collected, preliminary results show the same influence of female colouration on the evolution of male colouration (unpublished results) with equivalent values of correlation between male and female colouration.

Nonetheless, the strong phenotypic correlation between female and male colouration might be inflated. I used colouration on the whole plumage of the birds without accounting for placement on the body or the surface occupied by each colouration. One can think that males and females can develop the same colours but may not produce them on the same part of their bodies or may produce them in different quantities. Colour display on different parts of the body may be coded differently genetically, which may lessen genetic correlation. Mapping the colour placement in male and female in each species will then be a first step to assess how inflated phenotypic correlation (and underlying genetic correlation) could be in my study. Then, it will be useful to build a metric allowing the consideration of colouration

and placement on the body, which will offer a more accurate view of the true phenotypic correlation between males and females.

1.2. Male investment in parental care and costs of reproduction

In line with predictions of theoretical models (Johnstone 1996; Kokko and Johnstone 2002), results confirmed the importance of male investment in parental care as a driver of the evolution of female colouration. For both proxies of colouration used (colour volume and intensity of colouration), the effect of male investment in parental care was revealed. The strength of the metric used relied in the ability to account for relative investment of males compared to females (Webb et al. 2010). In both cases, a greater investment in parental care by males was positively correlated with a larger colouration in females. As predicted from theoretical models on the evolution of male mate choice (Kokko and Johnstone 2002; Edward and Chapman 2011), these results also revealed some complexity due to costs related with reproduction for both males and females.

In the case of colour volume, the large male investment in parental care was positively correlated with a greater colouration in females but this was driven by species encountering a strong vulnerability to predation. Vulnerability to predation was defined as a score including four factors already related to predation risk on females: the openness of the habitat, the placement of the nest, the openness of the nest and the migratory status of the species (Friedman et al. 2009; Soler and Moreno 2012). Colour volume was expected to be sensitive to predation as colour contrasts increase the visibility of a species (Bortolotti 2006; Caro and Stankowich 2010). Based on theoretical models on male mate choice, the interaction between mate investment and vulnerability to predation could be explained by the incurring costs and risks for males (Edward and Chapman 2011). When their investment in reproduction is strong and vulnerability to predation is high, males may have reduced likelihood of pursuing breeding events in the future and thus be more careful in choosing the good mate. Still, it would be important to understand the reasons underlying male investment in parental care when vulnerability to predation is high and it could be crucial to focus on researches working on the evolution of parental cooperation (Remes et al. 2015). It would also be important to understand why female colour volume is not affected by male parental care when vulnerability to predation is low. Researches more focused on the relation between survival probability and the evolution of ornaments in both sexes may help understand this result.

When working with the intensity of colouration, male investment in parental care was interacting with female initial investment in reproduction. This represents another type of cost of reproduction related in this case to female allocation of resources. As developed by theoretical models, there might be a trade-off between energy allocation to reproduction (clutch size and egg size in the case of birds) and the development of costly ornaments (Fitzpatrick et al. 1995). The results of the interspecific analysis on the intensity of colouration highlighted this trade-off. For species with low female initial investment in reproduction, there was a positive correlation between male investment in parental care and female colouration. On the contrary, the same correlation declined when female invested more in laying eggs. To my knowledge, this is the first analysis to reveal this pattern at the interspecific level. Furthermore, the interaction with male investment in parental care adds some complexity. For a low male investment in parental care, females seem to be more coloured when investing more in reproduction. It is not easy to conclude on this pattern. When starting the project, variation in female quality was thought to be tested (Johnstone 1996) and clutch volume, representing female fecundity, was first thought to be a proxy for this parameter. When males are not constrained by parental care, clutch volume may represent a proxy of female quality. However, it would still be intertwined with costs of reproduction for females. It would be necessary to find a way to disentangle both aspects of clutch volume. Another problem is related to the few number of species in our dataset with no or very small investment in parental care by males. To focus more on this pattern, it would be interesting to increase the sample size and to define more species with low or no male investment in parental care. There is a possibility to increase the species sample, while keeping the major restrictions imposed in the analysis; the species sample only covered 17 closely-related families from Motacillidae to Cardinalidae. It can be enlarged to less closely-related families of songbirds such as Sylviidae or Paridae. However, widening the species sample may not necessarily lead to a larger amount of species lacking male investment in parental care since biparental care is common in passerine birds.

As a step forward in understanding female colouration and the influence of female cost of reproduction, it will be interesting to consider patch size in relation to colour intensity. Our main interest in the analysis was to detect the presence of colouration in females, regardless of how much it may cover the body. Colour intensity was thus assessed on the whole plumage without using a weight index of patch size. By refining colour intensity, we may get a better understanding of the interaction between male investment in reproduction and cost of

reproduction for females. One strategy for females could be to display small patches of colouration, limiting thus the resources allocated to colour production. Considering the size of the patch may also give insights regarding costs of reproduction in both sexes, as it may also lessen visibility from predators (Bortolotti 2006 p. 29; Hegyi et al. 2008).

1.3. Breeding density

The last main result from the interspecific analysis was related to the encountering rate, which was a key factor developed by theoretical models and suggested to be positively related to the intensity of mate choice (Johnstone 1996). There was a negative quadratic effect between breeding density and female colour volume. If the first part of the hump-shape line reflects the prediction related to the encountering rate, the second one, the decrease, is more in line with studies on sociality. A very intense breeding density is related to very high levels of interactions and has already been related to territoriality associated to a decrease in colouration (Doutrelant et al. 2016). A way to better unravel the “social part” of the proxy of breeding density from its “sexual part” would be to consider sociality of our species samples, either on breeding and wintering sites. However, when working at the interspecific level, it is very difficult to score correctly sociality outside winter. Different terms in literature exist with blurry and overlapping definitions. So before including sociality in the proxy of breeding density, a necessary step will be to assess and compare these definitions before scoring accurately sociality.

Results found may also be related to the clade used. Theoretical models were developed to apply to every clade, from invertebrates to vertebrates. The three key factors used may have different involvement depending on the clade. For instance, variation in female quality is of large interest in fishes, insects or reptiles (Amundsen and Forsgren 2001 for fishes). Female body size has been reported to be directly related to fecundity and can be easily assessed by males. The encountering rate can have a major effect when working on species with limited means of transportation and facing ecological barriers (Johnstone 1996). In the case of birds, and in particular songbirds we used, the potential encountering rate is probably high, limiting the impact of this factor. Furthermore, when building the score for maximal breeding density, I realized the species I used were having larger breeding density than the species used by Bennett and Owens (Bennett and Owens 2002). By applying the exact same score they used (from 1 to 6 with 1 as the lowest breeding density score), I attributed the two highest scores to 80% of my species sample. Even though I adapted the

score to fit my species sample, it can nonetheless highlight the low impact of this key factor on my species sample.

This analysis was set to fill the gap existing regarding the role of sexual selection in the evolution of female ornaments. Focusing on male mate choice offered the possibility to answer Irwin's demand (1994) and to test theoretical models developed in the same period of time. The results revealed unambiguously that independent female colouration evolution through sexual selection was possible. They also highlighted the importance of male investment in parental care and costs of reproduction, without neglecting genetic correlation embedded in the phenotypic correlation. They also highlighted the complexity associated with the evolution of female ornaments, which was predicted by theoretical models. They outlined the necessity to consider the evolution of female ornaments with more details, which is a stepping stone in this area. Besides perspectives already developed above, there are other analyses which can be developed further.

1.4. Further analyses

Most of the perspectives I develop hereafter are related to refining colour metrics. I already mentioned the interest of considering the size of the patches. It could also be interesting to focus on the part of the plumage where these colours are produced. When female attendance at nest during incubation increases vulnerability to predation (Wallace 1889; Martin and Badyaev 1996), females seem yet to display colouration (Hegyi et al. 2008). As stated in the introduction and the analyses, most studies focused on areas on the body plumage where males display colouration, especially the top of the head, the throat and the chest. When looking at a bird in its entity, either male or female, the development of conspicuousness in areas such as wings, tails and rump is striking. Considering female body position when incubating, these areas are recovered by mantle, scapulars and wing coverts. Natural selection pressures may be stronger on these areas, conspicuous to predators and hidden zones may represent a safe area for colour expression. The specific areas of colouration could be displayed by females to attract males during courtship then hidden during incubation. Mapping the placement of colouration on female plumage as well as on male plumage could help understand the evolution of female ornaments. It would be interesting to confront this mapping to factors such as male investment in parental care and vulnerability to predation. While collecting measurements, I added zones where colours were quantified and have thus the information to map colouration on male and female body.

However, developing an analysis to compare mapping and to incorporate them in comparative analyses is still needed.

Another perspective which may be developed is related to the cost of producing colouration. The two indexes of colouration used in the analysis did not reveal the same interaction with female initial investment. While intensity of colouration concurred with theoretical models on female allocation to reproduction (Fitzpatrick et al. 1995), the analysis with colour volume did not. There was a tendency towards positive correlation between female investment in reproduction and colour volume, which may indicate female colouration as a visual cue for their quality. Colour volume represents the amount of colouration and contrasts an individual displays, whereas intensity in colouration represents chromaticity in colouration, regardless of the amount of colours used. Intensity in colouration is related to pigmentary-based colouration, especially carotenoid-based colouration. They are known to be costly to produce (McGraw 2006). To display colours while limiting costs of production, females may use contrasting colourations less costly to produce while still enabling the assessment of their quality. However, this implies two strong assumptions which need to be tested. First, it assumes contrasts and/or multiplying the number of colours represents an efficient visual signal. After a quick review of literature, I was surprised of the scarcity of researches on the subject in birds (Bortolotti 2006). Researches on contrast were mostly related to antipredator strategies and social communication (Bortolotti 2006). The second strong assumption refers to the cost of producing several colours compared to the cost of producing intense colouration. Assessing accurately the costs of colouration can be complex. Carotenoid-based colourations are costly to produce because of the necessity to rely on resource intakes (McGraw 2006). The white colouration due to incoherent scattering of light through plumage structure (Prum 2006) is probably not very costly to produce. Recent studies also focused on the costs of melanin-based colouration (Roulin 2016) and structural colouration (Maia et al. 2012). However, there is no comparison about costs of producing the different types of colouration.

Finally, having a whole data set with male plumage colouration and life-history traits, it is interesting to relate factors to male colouration, while considering female colouration. I already ran the exact same models used in females with male colouration as the variable to explain (unpublished results). Besides phenotypic correlation described above, there were also interesting preliminary results regarding the interaction between male investment in parental care and vulnerability to predation. Contrary to results with females, male colouration

decreased when both male investment in parental care and vulnerability to predation were high. However, the predictors used may not reflect costs incurred by males. For instance, the metric for vulnerability to predation was highly related to nest attendance, a cost which may be inadequate considering the lesser investment of male in nest attendance. A better definition of the predictors related to the evolution of male colouration may help understand better male colouration as well, and may also give new insights regarding the evolution of male colouration.

2. The intraspecific level

Genetic correlation was historically the sole process thought to explain female ornamentation (Kraaijeveld et al. 2007). Mutual ornamentation through mutual mate choice has also been proposed to explain sexual monomorphism. A special case of mutual mate choice is assortative mating, which can also reinforce genetic correlation between the production of an ornament and preference for this ornament (Lande 1981). There are several empirical studies assessing assortative mating on colouration in birds and most of them share two major problems: they are based on a rather low sample size and they are tested in one population in a limited amount of time. However, several reviews on sexual selection, ornaments and preferences stated the important variation associated with those concepts (Svensson and Gosden 2007; Chaine and Lyon 2008; Cornwallis and Uller 2010; Kuijper et al. 2012; Cockburn 2014). There was a necessity to include the likely effect of spatiotemporal variation in analyses on ornaments and assortative mating.

2.1. Spatiotemporal variation

The most striking result when working on blue tit colouration was the amount of spatiotemporal variation in every analysis. Analyses on selection were preliminary. Based on early proxies of reproductive success (i.e. laying date and clutch size), there was a wide spatiotemporal variation associated with estimates of either male or female or their interaction. Regarding assortative mating, the first result also highlighted a large spatiotemporal variation, confirming the necessity to consider several populations in several successive years to conclude. Contrary to results on selection, the temporal variation pattern seemed to be quite the same in every population. Sexually selected signals can be affected by large-scale environmental effects (Garant et al. 2004). Climatic factors such as drought during moult can affect the four of the populations and may impact ornamentation in the same way (Hegyi et al. 2007). However, assortative mating was assessed with one Pearson coefficient correlation for each year in each population, which represents ten points replicated in four

populations. It may thus be importation to keep on collecting feathers, or to work on assortative mating within a pair, using distance in colouration within a pair as used in MS5. This data could then be confronted to values extracted from North Atlantic Oscillation (NAO) data to test whether there is the same effect among populations and/or among colour patches.

Large spatiotemporal variation has been found on different scales of analyses: heritability, selection and assortative mating. Working on each of these analyses with one population and two or three years of data, I may have found either strong values or no relation, a problem already mentioned by Parker in his meta-analysis on plumage colouration in blue tits (2013).

2.2. The evolution of female colouration in blue tits

The first set of analysis revealed the importance of considering the evolution of female colouration in blue tits. First, the analysis of phenotypic variation among populations (MS A1) confirmed variation in colouration in both sexes for each variable. In each population, for each variable studied, the amplitude of variation was equal between males and females. The presence of heritability (MS2) also confirmed the possibility for female colouration to evolve in our blue tit populations. The lack of heritability in some variables (brightness for instance) could be explained by a prevailing effect of environment on these variables. This second manuscript however also pointed the fact that female coloration could evolve by genetic correlation only and pointed the need to conduct analyses on selection gradients. Finally, regarding the preliminary results on selection gradients (MS3), within-study meta-analyses revealed that selection gradients on laying date and clutch size were more related to female colouration than male colouration confirming experimental study conducted on these early stages of reproduction (Doutrelant et al. 2008; Midamegbe et al. 2013). Significant effects associated with female selection were more common than what would have been expected by chance for both proxies tested (laying date and clutch size). I intend to pursue analyses and use other proxies of reproductive success, including number of hatchlings, number of fledglings and recruitment. These proxies may be more dependent on male investment in parental care and I expect thus more results related to male colouration. Still, all of these studies revealed female colouration can be selected and assessed by males.

2.2.1. Assortative mating

Analyses on assortative mating in the four Mediterranean populations were run with the idea to assess the possibility of mutual mate choice. The former analyses (Chapter III – A) revealed the likelihood of male assessment of female colouration. Within-study meta-analysis run on assortative mating on colouration helped in drawing conclusion despite the large spatiotemporal variation. A weak but positive assortative mating was found in each population for both colour patches. This result was solid since it was performed on ten successive years in four populations. However it opened avenues to many questions. First the values of assortative mating remained quite similar between patches in each population, despite the fact that when comparing their Pearson correlation coefficients each year, they seemed to vary differently. Furthermore, there was no correlation among variables. If both patches show the same overall level of assortative mating, this could mean individuals are assessing their mates based on multiple traits (Møller and Pomiankowski 1993; Hegyi et al. 2015). The dramatic variation in the strength of assortative mating by the two ornaments and their colour traits could be influenced by adaptive interannual plasticity in mate choice (Chaine and Lyon 2008; Kopp and Hermisson 2008). This plasticity could stem from the fact that the two ornaments are condition-dependent (in adults: Doutrelant et al. 2012, but see Peters et al. 2011; in nestlings: Jacot and Kempenaers 2007). They display differential abilities to respond to environmental conditions and are thus more or less informative depending on the main factors affecting signalling in any given year (Bro-Jørgensen 2010). For instance, variable carotenoid availability or parasite prevalence during the moult may mainly affect the reliability of the yellow chest patch as a signal while leaving no mark on the blue crown, which has a structural colouration (McGraw et al. 2002). Variation in mortality and density may also affect mate choice or competitive intensity, leading to a lesser degree of assortative mating during years when conditions are harsher (Crowley et al. 1991).

However, the values of assortative mating found could not guarantee the existence of mutual mate choice. Such a pattern may also be the results of other processes such as mate directional preference from one or both mates, convergence of the degree of ornaments within a pair, correlation of the ornaments with age, habitat choice or arrival on the breeding site (Kraaijeveld et al. 2007). A set of analyses was thus used to understand the processes underlying the values of assortative mating. The main result ensued expressed a similar pattern in each variable for females and males: pairs were better assorted in intermediate individuals. There was no effect of habitat quality, earliness in breeding season or age ratio on

assortative mating. The fact that more intermediate individuals were more assorted suggested that choice was limited in the populations. Simple simulations adapted from the “best of n ” strategies (Wittenberger 1983; Uy et al. 2001) were run. A set of potential mates (two, four, ten or fifty potential mates) were assigned to an individual. The individual was forced to choose the potential mate displaying the closest colouration to its own colouration. These “closest of n ” simulations confirmed the limited choice hypothesis; plots from simulations limiting potential mates to a panel of two or four were closer to what we found in our natural populations (see ESM MS5). However, these simulations could be improved especially by offering the possibility of using different strategies. Along with the prudent choice theory (Hardling and Kokko 2005) some individuals (e.g. the most coloured) may choose their mates while other (e.g. the least coloured) may mate indiscriminately. Furthermore, relations between colouration and territory quality need to be better assessed. As males and females are territorial, competing for access to a territory may be the key to understand assortative mating in these populations. Also, pairs seem to be formed during winter, at least two months before first egg is laid. Being able to use a more precocious proxy for timing of breeding (e.g. first stages of nest construction) can allow a better assessment of this factor. However, it is more difficult to catch and identify males during this period and sample sizes may decrease dramatically. Tests of mutual mate choice are also needed. Some experiments are already held on birds born in the D-Rouvière population and raised in aviaries in a project led by Samuel Caro, a permanent researcher in the team. They tried to assess whether mate choice was based on female own colouration. These experiments show so far no effect of reference to itself and they confirmed mutual mate choice might rather be the result of a common preference in male and female.

Finally, the use of visual models can give input to understand the relation between individual colouration and assortative mating. In the analysis, distance in colouration within a pair (as a proxy of assortative mating) was assessed with raw quantification of colouration. Using the “just noticeable difference” from visual models (Vorobyev and Osorio 1998) to assess distance in colouration within a pair may account for the ability of individual to detect differences among different potential mates. This may be more representative of actual assortative mating. Despite my will to use visual models in the study of blue tit colouration, this requires to rearrange the raw spectra to get the values through visual models. This represents a large amount of time I was lacking.

2.2.2. Genetic correlation and heritability

The analyses also revealed the importance of genetic correlation. Heritability on colour variables in both males and females reveals genetic correlation and may explain mutual ornamentation in blue tits. However, values on heritability remain rather low (varying between 0.10 and 0.25), probably due to the large environmental effects on colouration, something expected for condition-dependent traits (McGraw 2006; Hill et al. 2009). Some variables, especially achromatic ones were not heritable, a result also found in great tit by Evans and Sheldon (2012). This result suggests that brightness is less likely to evolve under selection although it can still give some indication on individual condition and be used in mate choice processes. Chromatic variables have higher heritability values than colours measured by spectrophotometry in this species (Hadfield and Owens 2006) but lower than what was found in other species for other colourations (Saino et al. 2013; Hubbard et al. 2015; Roulin and Jensen 2015).

2.3. *Further perspectives on selection*

As stated above, analyses on selection are preliminary and need to be completed. It would also be interesting to perform the same type of analysis using morphological traits such as tarsus length or wing length, which are also condition-dependent and may suffer from spatiotemporal variation (Charmantier et al. 2004). This would help to compare selection on signals to selection on other traits, an analysis which is essential to determine the evolutionary consequences of our results. Since meta-analysis provides average estimates of the slope associated with linear regression, comparing selection on morphological traits and selection on colouration could be possible with this statistic tool. Also it would be interesting to compare selection on colour known to evolve less under sexual selection. Claire Doutrelant has started to collect feathers from the mantle in the D-Rouvière population since 2013. As the Sparrowhawk (*Accipiter nisus*) is a Blue-tit predator, the olive-coloured patch on the back, easily seen from above, is supposed to be under high pressure of natural selection and neutral to sexual selection. It would be interesting to compare selection gradients relative to reproductive success on this camouflage colouration compared to the two sexual colourations. However, these samples cannot be used at the moment, since spectrophotometric measures have not been performed yet.

The strength of this study relies thus in the large dataset and the use of tools to consider variation. Despite this large spatiotemporal variation, there were significant results in each type of analysis. They suggested female ornamentation was functional and had the

potential to evolve independently or as a consequence of evolution on male ornamentation. Female ornamentation was variable, heritable with genetic correlation and related to selection and there was a pattern of assortative mating on both traits, which seemed related to mate choice. The large spatiotemporal variation documented open avenues for more studies to understand it.

3. Conclusion

Overall, in this thesis, I was interested in understanding the processes relating male or mutual mate choice from the micro-evolutionary to the macro-evolutionary scale. The results definitely drove avenues for further research. The comparative study revealed the necessity to consider detailed factors and conditions to estimate the evolution of female ornaments. It also revealed the utility of comparative analyses to test the predictions of mathematical models. It last pointed the necessity to consider phenotypic (embedding genetic) correlation by integrating male colouration. The intraspecific study revealed the large complexity associated with understanding the evolution of female colouration or male colouration, and the urge to work on different populations and several successive years as expressed by the wide spatiotemporal variation associated with the results.

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Ornamented Guinea fowl, known as «pintade» in French also refers to a shallow girl often giggling. Licenced-free picture.

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To be really unformal, I need to share a weakness: as afraid as I am to be unfair with anyone, I often decide to be unfair with everyone (getting thus a little bit back closer to fairness). So I won’t name my family, close ones, friends, and co-workers and stay very general: thank you for your large support and for all the great moments (even if you don’t know because you were not here). Hopefully, you’ll get the feeling I’m thinking about you (that includes you Nin, stop being upset). I may add a special thought to the stone-curlew which helped me indirectly in a key decision, to the spotted thick-knee on my computer desktop reminding me each day this decision and to the “pintades” (see Figure) who shared their office or coffee time with me. And to the leap year as it provided me with an extra day in busy year 2016 when every day was worth working.

Finally a bad poem for Nath because she thinks I still owe her a beer:

*Females are green
Males are blue...
Wait... Forget Eclectus parrots, let’s work on Roadrunners.*

APPENDIX

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REVIEW AND SYNTHESSES

Mediterranean blue tits as a case study of local adaptationAnne Charmantier,¹ Claire Doutrelant,¹ Gabrielle Dubuc-Messier,^{1,2} Amélie Fargevieille¹ and Marta Szulkin¹¹ Centre d'Ecologie Fonctionnelle et Evolutive, Campus CNRS, Montpellier, France² Département des sciences biologiques, Université du Québec à Montréal, Succursale centre-ville, QC, Canada**Keywords**

adaptive divergence, *Cyanistes caeruleus*, environment heterogeneity, genomic differentiation, genotype–environment association, heritability, isolation-by-environment, phenotypic differentiation, phenotypic plasticity.

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Abstract

While the study of the origins of biological diversity across species has provided numerous examples of adaptive divergence, the realization that it can occur at microgeographic scales despite gene flow is recent, and scarcely illustrated. We review here evidence suggesting that the striking phenotypic differentiation in ecologically relevant traits exhibited by blue tits *Cyanistes caeruleus* in their southern range-edge putatively reflects adaptation to the heterogeneity of the Mediterranean habitats. We first summarize the phenotypic divergence for a series of life history, morphological, behavioural, acoustic and colour ornament traits in blue tit populations of evergreen and deciduous forests. For each divergent trait, we review the evidence obtained from common garden experiments regarding a possible genetic origin of the observed phenotypic differentiation as well as evidence for heterogeneous selection. Second, we argue that most phenotypically differentiated traits display heritable variation, a fundamental requirement for evolution to occur. Third, we discuss nonrandom dispersal, selective barriers and assortative mating as processes that could reinforce local adaptation. Finally, we show how population genomics supports isolation – by – environment across landscapes. Overall, the combination of approaches converges to the conclusion that the strong phenotypic differentiation observed in Mediterranean blue tits is a fascinating case of local adaptation.

Introduction

Evolutionary biologists are primarily interested in understanding the processes that explain the origin and maintenance of biological diversity. Phenotypic differences within a given species can have at least three main origins: genetic drift, phenotypic plasticity or evolutionary divergence, also called adaptive divergence. For populations to adapt to their local environment, that is for local adaptation to occur, divergent selection between habitat patches should result in higher relative fitness of resident versus immigrant genotypes (Williams 1966). This process of divergent adaptation, although primarily triggered by opposing forces of natural selection, also involves a complex interplay between selection, gene flow, genetic drift and the presence of genetic variation in fitness traits (Hedrick 2000; Savolainen et al. 2013). Hence, demonstrating an adaptive process requires several steps that involve knowledge on the phenotypic variation, the populations' genetic properties and

dynamics, the quantitative genetic (co)variation of adaptive traits and the characterization of the shape, direction and strength of natural (social and sexual) selection acting on these traits.

The magnitude of adaptive divergence is theoretically expected to increase with the amount of genetic variation within populations (Fisher 1930; Lande 1980) and the level of environmental divergence between populations (Endler 1977). Adaptive divergence is also expected to be greater when genetic drift (Lande 1976; Hereford 2009) and gene flow between populations (Slatkin 1985; Lenormand 2002) are lower. However, the interplay between ecological and evolutionary processes can substantially complicate this general picture. For instance, although gene flow is generally predicted to have a homogenizing effect counteracting phenotypic diversification (Slatkin 1987; Garcia-Ramos and Kirkpatrick 1997; Lenormand 2002), gene flow can reversely promote adaptive divergence by counteracting inbreeding depression or by increasing genetic variation,

and thus adaptive potential (Garant et al. 2007). As a consequence of this complexity in the interactive processes, the study of local adaptation is ideally multifaceted and multidisciplinary (Kawecki and Ebert 2004), a challenge which is more realistically faced in experimental systems than in free-living populations. One of the best ways to investigate higher performance of resident versus immigrant organisms in a given habitat is to use reciprocal transplants or common garden experiments because these approaches allow maximizing the power to reveal genotype-by-environment interactions. Such experiments have provided so far the most extensive contributions to our understanding of processes involved in adaptive divergence (see Blanquart et al. 2013 for a review). However, they are not easily applicable in many organisms and do not always allow an accurate evaluation of the relative importance of selection versus gene flow or genetic drift, in particular in highly mobile organisms occupying a heterogeneous environment (see e.g. Merilä and Hendry 2014 for a review in the context of a response to climate change).

The study of the origins of biological diversity between species has provided several emblematic examples of adaptive divergence (Schluter 2000). Two prominent examples are the adaptive radiation in beak size and shape in the Galapagos Darwin's finches (e.g. Grant 1986) and the explosive diversification of cichlid fishes in the lakes of East Africa (e.g. Kocher 2004). Overall, studies of species complexes have provided sufficient examples to conclude that adaptive radiation is widespread (Schluter 2000; Gavrillets and Losos 2009). However, when it comes to understanding phenotypic variation at smaller temporal and spatial scales, there is much less compelling examples, in part because the above-mentioned complexity of processes makes it very difficult to demonstrate that phenotypic divergence is adaptive and that it has a genetic basis.

Although historically, geographic isolation of populations was seen as a prerequisite for their adaptive divergence (Mayr 1963), it has now long been recognized that reproductive isolation can evolve even between populations connected by gene flow whenever divergent selection is strong relative to gene flow (Maynard Smith 1966; Rice and Hostert 1993). However, the realization that such divergence in spite of gene flow can occur at microgeographic scales, that is within the range of the organism's dispersal distance, is only just arising (Postma and Van Noordwijk 2005; Milá et al. 2010; Richardson et al. 2014). This is surprising given that some classical examples of fine scale adaptation, such as the adaptive variation in colour morphs of the Peppered Moth *Biston betularia*, date back several decades (Saccheri et al. 2008).

We aim here at showing how the Mediterranean blue tit *Cyanistes caeruleus* study system is an equally fascinating

example of phenotypic divergence. We will outline the story of a long-term project where we tested for phenotypic and genetic divergence as well as for possible local adaptation of bird populations using a diversity of complementary approaches – from the study of phenotypic differentiation to the recent development of ecological genomics. This project started with the erection of nest boxes by Jacques Blondel in 1975 in Mediterranean oak forests of Provence (Southern France) and on the island of Corsica, with the purpose of comparing breeding strategies between mainland and island populations (Blondel 1985). The long-term monitoring of blue tits in these forests led to the striking observation that populations as close as 24 km differed in their breeding phenology by up to 1 month, depending on the type of vegetation they use for reproduction (Blondel et al. 1999). Although the habitat-specific phenotypic differences were soon found salient (Blondel et al. 1993; Lambrechts et al. 1996, 1997a), understanding the processes that lead to this intraspecific biodiversity is still an ongoing objective. Indeed, if one is to avoid the Panglossian paradigm whereby adaptation is considered a fundamental assumption (Gould and Lewontin 1979), one needs to consider alternative (and nonexclusive) adaptive (including plasticity) and nonadaptive (including drift) processes when testing for an adaptive divergence. Moreover, the integration of phenotypic, genetic and fitness data is required to confidently confirm evolutionary adaptation at the genetic level (Barrett and Hoekstra 2011) and is at the core of current research in this study system. Within the abundant literature published from this study system, we will focus here on the main findings that provide tests for an adaptive origin to the strong phenotypic differentiation observed across heterogeneous Mediterranean landscapes.

After briefly presenting the model system, we first summarize the main phenotypic divergence observed in blue tit populations living in forests dominated either by deciduous or evergreen trees. For each divergent trait, we review the evidence obtained from aviary/common garden experiments regarding a possible genetic origin to the observed phenotypic differentiation as well as evidence for heterogeneous selection. Second, we argue that most phenotypically differentiated traits display heritable variation, a fundamental requirement for evolution to occur. Third, we review evidence for nonrandom dispersal, selective barriers and assortative mating as processes that could reinforce local adaptation. Fourth and finally, we show how population genetics and ecological genomics support isolation – by – environment (IBE) across habitats. As this study is published in a Special Issue that aims at highlighting women's contribution to basic and applied evolutionary biology, we have added some personal comments on women's position in this long-term project (Box 1).

Box 1: Personal reflections

Research presented here is based on the Mediterranean blue tit study system that was started forty years ago by J. Blondel. The project was originally developed by men only, in particular by J. Blondel, P. Perret and M. Lambrechts. In the past 10 years, the sex ratio of the team has largely become equal. We do not feel that this change in sex ratio led to any more changes in research questions than expected due to the inclusion of any new researcher in a team. However, more equal sex ratios may have resulted in more diverse ways of working with students and positively impacted group team working in the field and in consequent research work. We do not intend to draw general conclusions based on the progression of this single scientific group, yet we wish here to share some personal feelings as women working in the field of evolutionary biology.

Working in a country (France) where 80% of women claim to be victims of sexism at their workplace (2013 poll on 15 000 women by the Higher Council of Professional Equality (CSEP)), we feel privileged to state that the authors of this article have not experienced overt gender-based discrimination in their PhD, postdoctoral or senior research years. However, this does not exclude the occurrence of more subtle or hidden preconceived biases about the professional capacity of males and females that are still common worldwide. These are often reflected in recruitment outcomes when applying for permanent/tenure track positions (Reuben et al. 2014). In addition, the fact that tenured positions tend to be secured at an increasingly older age, often at a time when fertility curves in women are declining, only aggravates women's underrepresentation in science (Ceci and Williams 2011). While in-depth studies quantifying gender bias in science and identifying its causes and consequences are available elsewhere (Ceci and Williams 2011; Williams and Ceci 2012; Reuben et al. 2014; Leslie et al. 2015), here we highlight how, as women, we interacted with the requirements of scientific work involved in this long-term research project, and how we believe to have created a positive working environment from which the research programme, women and men researchers, and their children, have all benefitted from.

Conducting long-term research in ecology implies being committed to fieldwork for several weeks (if not months) away from home annually. As several female researchers reported beforehand (e.g. McGuire et al. 2012), we reconcile the two by occasionally bringing our children to the field and by relying on our partners and family for engaging in joint child rearing. This is true for both mothers and fathers working in our research group. At the same time, flexibility is an equally important aspect of running a large-scale field project with a larger number of fieldworkers. Thus, parental leaves of team members were never an issue as field logistics were always prepared well ahead of time and contingency plans were available. Importantly, special efforts were made to accommodate researchers with small dependents (*i.e.* their children), thereby substantially improving the suitability of the work environment for mother and father ecologists (for an excellent analysis of the challenges faced by women ecologists, see the study of McGuire et al. 2012). These steps not only incredibly benefitted each individual researcher at such special point in their life (Williams and Ceci 2012 and references therein), but it also strengthened the team of fieldworkers available each year, and allowed to foster a sense of continuity, inclusion and identity in promoting the long-term dimension of the programme.



Figure B1. Bringing children to the field. (A) The daily nest box monitoring in the Fango valley and in good company. (B) Allowing children to participate in fieldwork increases their awareness of the natural environment, which is often a stepping stone to set up science outreach projects. Photo credit: (A) F. Laurière; (B) M.-O. Beausoleil.

In terms of fieldwork practicalities, the only noticeable change following this sex-ratio transition was a lowering of the highest nest boxes to allow access to all, which made life on the Blue tit field easier for both men and women. With more PI women in the field, fieldwork became a more family friendly adventure (Fig. B1(A)), allowing mothers – and fathers – to find a new middle ground that focuses on fieldwork. From the child's perspective, coming to the field is an incredibly enriching experience (Fig. B1(B)), as they are exposed to wilderness we are increasingly separated from in our daily lives. Equally importantly, they can also witness their parents in their professional role, in a unique environment and in a setting void of gender roles. Including children into our fieldwork routines

Box 1: (continued)

often increased our participation in science outreach projects in kindergartens and schools while back at home, but also within the field site communities. Although quantitative data are needed to support this claim, we believe that transgressing traditional divides of work and life by welcoming children in the field sensitizes us to further participate in educational science outreach projects and thus directly impacts our communities. We hope this testimony will provide encouragement to young women and men and convince them that embarking in a field project is rewarding both professionally and personally and that it can be made compatible with a fulfilling family life.

A model species in a model environment

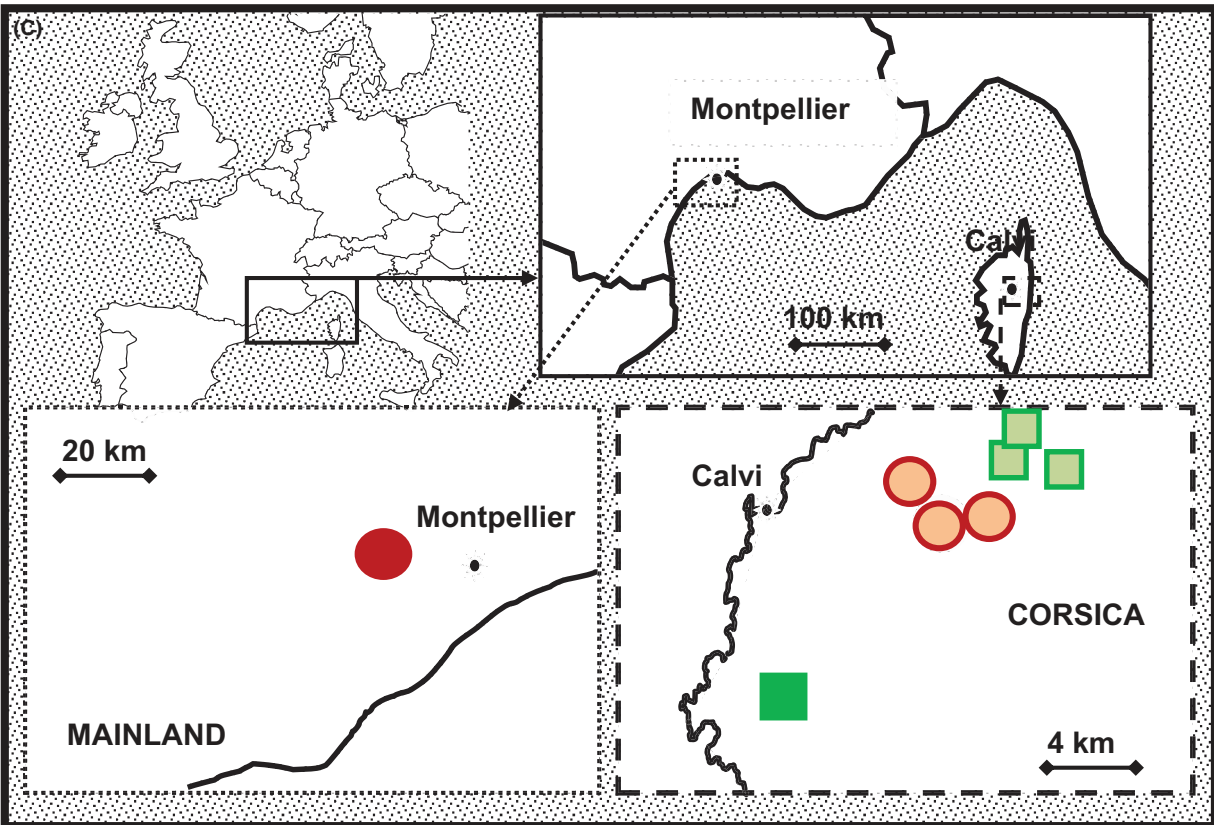
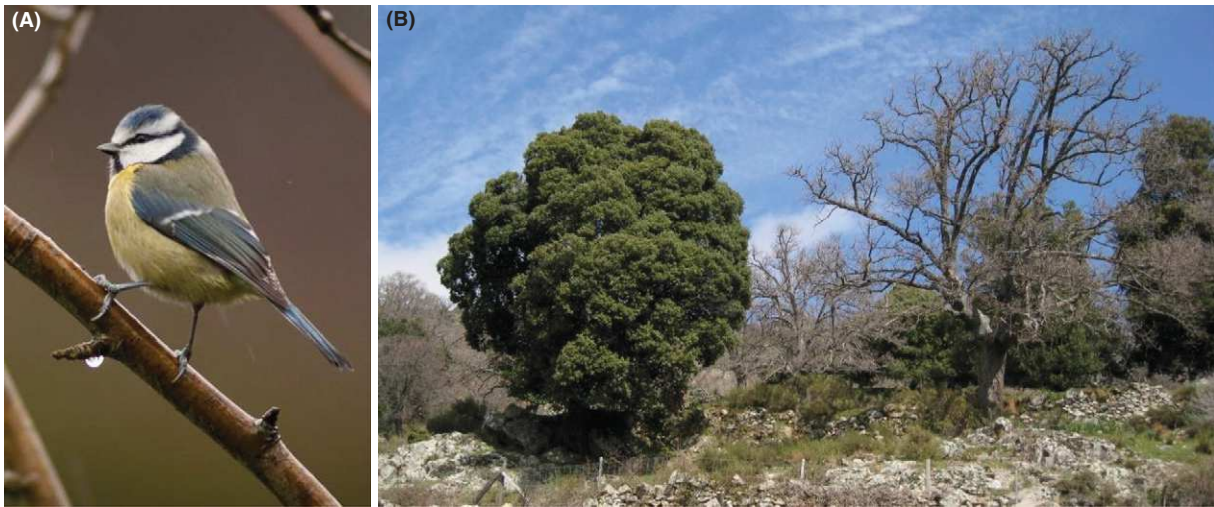
The geographic configuration of Mediterranean landscapes, characterized by a heterogeneous, fine-grained mosaic of habitats, provides an exceptional study system for investigating plastic and adaptive responses in spatially structured populations (Blondel and Aronson 1999; Blondel et al. 2010). In this system, we have focused on the variability expressed by a passerine bird, the Blue tit, whose ecological niche is preferentially linked to oak woodlands. The Blue tit is present in a narrower range of habitats compared to the Great tit *Parus major* (Snow 1954; Lack 1971), and yet is a common breeder throughout Europe and western Asia where it readily breeds in nest boxes, which makes it an ideal model to study adaptation to environmental heterogeneity. In our study area, the breeding activity of about 350 blue tit pairs is monitored each year in more than 1000 nest boxes erected across eight study sites, in a series of habitats that strongly differ in being dominated by patches of either deciduous (*Quercus pubescens*) or evergreen (*Quercus ilex*) oaks (Box 2). In deciduous forest patches, the leafing process occurs ca. 1 month (3–5 weeks depending on the scale of analysis) earlier compared to evergreen forests, and all leaves are renewed. This results in an earlier and more abundant caterpillar peak in deciduous patches (Zandt et al. 1990; Dias et al. 1994), which are considered as higher quality breeding habitats (Blondel et al. 2006). These differences between habitats are clearly visible to the human eye (Box 2) and can be mapped at a large scale using vegetation reflectance data from satellite sensors, the latter correlating remarkably well with the number of either type of oak quantified on the ground within a 50 m radius from each nest box (Szulkin et al. 2015). Apart from the notable heterogeneity of the Mediterranean landscape, these study populations are also situated at the species range-edge, a position known to be conducive to geographic differentiation (Mayr 1970; Enderler 1977). Hence, the study of these populations may be of particular conservation value because they represent significant components of intraspecific biodiversity (Hardie and Hutchings 2010) and potential sources of evolutionary innovation and persistence during rapid

environmental change such as global warming (Sexton et al. 2009).

Over the last forty years, the Montpellier blue tit group has ringed 6653 blue tit parents and 33 489 blue tit chicks in these study populations. This sustained hard work aimed at elucidating the phenotypic variation displayed by blue tits at three spatial scales (Blondel et al. 2006): mainland versus Corsica (min. distance of 85 km off mainland Italy and 170 km off the French mainland), a scale that is not the main focus of the present study (but more details about this inference level can be found in Dias and Blondel 1996; Lambrechts et al. 1997b; Blondel et al. 2001; Doutrelant et al. 2001); Muro versus Pirió valleys (24.1 km between D-Muro and E-Pirió, where 'D' stands for Deciduous and 'E' for Evergreen), and three deciduous plots (D-Muro) versus three evergreen plots (E-Muro) within the Muro valley (separated by 5.6 km). Although these distinct study plots within the Muro valley have been detailed in some previous publications (e.g. Lambrechts et al. 2004), we have pooled their blue tit populations here as they never display any significant differentiation, neither phenotypically nor genetically.

Because of the low rate of capture–recapture data using multisites, our knowledge on natal and breeding dispersal in the Blue tit remains approximate, with very different results in different studies. For example, while a maximum dispersal distance of 14.5 km was recorded in Belgium (Tufto et al. 2005), an earlier study over 1200 km² in Germany revealed that natal dispersal could reach distances of 24 km in males and 470 km in females (Winkel and Frantzen 1991). Note that in the same study, breeding dispersal (that is the distance between successive reproductive events) reached maximal distances of 0.75 km for males and 37 km for females. At the same time, the mode of the distribution is likely to be much smaller, especially in Corsican blue tits (Blondel et al. 1999), and most of our dispersal events are recorded within sites even when other populations are monitored close by. Over the last 17 years of ringing in Corsica, we observed 2 dispersal events between D-Muro and E-Muro (5.6 km), 2 reverse events between the same sites, and none between the Muro valley and E-Pirió (24.1 km).

Box 2



(A) The Blue tit *Cyanistes caeruleus* occupies habitats dominated by (B) the evergreen holm oak *Quercus ilex* or the deciduous downy oak *Quercus pubescens* (deprived of leaves in winter). (C) Locations of the study sites of blue tit populations monitored on the French mainland and in Corsica. Circles denote deciduous sites (*Quercus pubescens*) and squares evergreen sites (*Quercus ilex*). Phenotypic and pedigree data have been collected since 1979 in E-Pirio (Lat: 42.38; Long: 8.75 ■), 1991 in D-Rouvière (43.66; 3.67 ●), 1993 in D-Muro (42.55; 8.92, three sites of Avapessa, Feliceto, Muro ●) and 1998 in E-Muro (42.59; 8.96, three sites of Arinelle, Filagna and Grassa, ■). Photo credit: (A) S. Caro; (B) A. Charmantier.

Phenotypic divergence across blue tit populations

The phenotypic divergence measured across the four main study populations is detailed for life history, morphological, behavioural, vocal and colour traits in Table 1. Considering the massive amount of information regarding phenotypic differences, we only briefly discuss below the most remarkable results in the context of possible adaptive divergence. We also review attempts to test for the plastic/genetic origin in the described trait divergences as well as evidence for heterogeneous selection.

Life history traits

Differences in breeding phenology are particularly striking (Lambrechts et al. 1997a; Fig. 1). As leaf-eating caterpillars, the main prey collected for blue tit chicks, are available 1 month later in evergreen compared to deciduous forests (Blondel et al. 1999), the birds' breeding synchronization with local seasonal variation in food leads to divergent breeding phenologies even at a geographical scale much smaller than the dispersal range of the birds (Blondel et al. 1993): indeed, average laying dates are 1 month apart between D-Muro and E-Pirio, and 10 days between D-Muro and E-Muro (Table 1, Fig. 1). In fact, the breeding dates in E-Pirio are the latest described in the whole species distribution (see the striking fig. 1 in Visser et al. 2003), which was originally quite surprising considering this population is at the southern range of the species distribution and is under a much warmer climate than most other populations included in this comparison. Interrogations on the plastic versus genetic origin of such drastic differences in the onset of laying emerged in the mid-1980s (Blondel 1985) and led to common garden experiments in aviaries, including hand-reared chicks (Blondel et al. 1990). These experiments suggested that differences observed in the average onset of laying had a strong genetic basis when comparing birds from mainland deciduous and Corsican evergreen habitats (Blondel et al. 1990; Lambrechts and Dias 1993). Similar attempts to test for a genetically based difference in laying date between D-Muro and E-Pirio were less successful, presumably because D-Muro birds seemed to cope less well with novel and artificial environments than birds from other populations (Lambrechts et al. 1999). Concurrently to the evidence for a genetically based microgeographic variation in laying date, at least between the mainland and Corsican blue tits, plasticity was also revealed important. In these populations, as in many other bird species (Charmantier and Gienapp 2014), the timing of breeding is a plastic trait responding to various environmental cues. Early aviary tests confirmed that photoperiod is a key determining factor of timing of breeding (Lambrechts et al. 1997b; Lambrechts and Perret 2000). Further

experiments revealed the role of other factors such as aviary characteristics and social environment (Caro et al. 2007), and long-term series analyses revealed a relatively strong plastic response to spring temperature in all populations (Porlier et al. 2012a). In the context of elucidating proximate factors involved in the adjustment of the onset of breeding, our Mediterranean blue tit populations offered a valuable opportunity to test simultaneously the roles of food availability/temperature on the one hand (as historically favoured by ecologists, e.g. Perrins 1991) and photoperiod on the other (as favoured by physiologists, e.g. Silverin et al. 1993), respectively. They still offer promising perspectives for more refined aviary experiments (S. Caro, work in progress). Phenotypic differences in clutch size among the study sites are equally strong, with the E-Pirio population presenting the lowest average clutch size recorded for this species (Table 1, Blondel et al. 1993, 1998). This variation in the number of eggs laid is well related to the abundance of food supply (Lambrechts et al. 1997a; Blondel et al. 2006), that is caterpillars, during the breeding season, yet artificial food conditions in the aviaries make it difficult to test for a genetic mechanism, as has been done for laying date (Blondel et al. 1990). Overall, classic selection analyses showed that habitat-specific differences in the timing and abundance of food result in divergent selection for both laying date and clutch size (see detailed selection estimates in Porlier et al. 2012a) and thus confirm the possible role of habitat in driving phenotypic differences.

Morphological traits

Blue tits from the mainland belong to the nominal *Cyanistes caeruleus caeruleus*, and are 15% larger than blue tits from Corsica, which belong to the subspecies *C. c. ogliastrae* (Table 1, Dias and Blondel 1996). Additionally, within Corsica, birds are smaller and lighter (yet with longer bills) in the poorer evergreen habitat compared to the richer deciduous habitat, with once again birds from E-Pirio displaying extreme phenotypic values (Table 1). This habitat-linked morphological divergence has repeatedly been shown significant for adult birds (e.g. Blondel et al. 1999) as well as for nestlings (Charmantier et al. 2004b). Interestingly, there is strong divergent selection on adult size between habitats because small adult males have a higher breeding success compared to large males in the poor evergreen forest while the reverse is true in the deciduous environment (Blondel et al. 2002). This is in contrast with results of similar selection analyses for chick morphology, which show consistent selection for larger and heavier nestlings, although with differing selection force across the study populations (Charmantier et al. 2004b). Contrarily to what has been described above for laying date, the differ-

Table 1. Phenotypic divergence between blue tit populations in deciduous (D-) and evergreen (E-) patches on the mainland (D-Rouvière) and in Corsica (D-Muro, E-Muro, E-Pirio). Mean, sample size (*n*, number of individuals, except for survival probability where it is number of years), variance and coefficient of variance (CV) are provided for survival probability, four reproductive traits measured on first broods, four morphological traits measured on breeding individuals, four song traits recorded on breeding birds during the egg laying period, two behavioural traits measured just before or during the breeding period, and five colour traits measured on breeding birds during the whole reproductive period. For laying date, 1 = 1st march. For number of fledglings, all broods included in an invasive experiment (e.g. cross-fostering, increased cost) were removed. For tarsus length, only the twelve best measurers (of 70 in total, minima of 200 measures and a 90% repeatability) were retained. For life history and morphological traits, data were collected between the first year of monitoring, and 2014, except for annotated estimates driven from the literature. Data collection spanned 1998–2001 for song traits, 2011–2014 for personality traits and 2005–2013 for colour traits.

First year of monitoring		D-Rouvière 1991	D-Muro 1993	E-Muro 1998	E-Pirio 1976
Life history traits					
Adult survival probability*	Mean (<i>n</i>)	0.511 (8)	0.391 (6)		0.574 (14)
	Variance	0.005	0.016		0.005
Laying date	Mean (<i>n</i>)	39.12 (1773)	38.56 (1233)	48.21 (640)	70.08 (1920)
	Variance	58.89	65.36	57.39	52.70
Clutch size	Mean (<i>n</i>)	9.95 (1769)	8.50 (1235)	7.12 (638)	6.61 (1913)
	Variance (CV)	3.53 (0.19)	2.97 (0.20)	1.65 (0.18)	1.53 (0.19)
Incubation period (days)	Mean (<i>n</i>)	14.70 (1433)	13.62 (1161)	13.06 (587)	13.87 (1798)
	Variance (CV)	9.90 (0.21)	4.80 (0.16)	4.42 (0.16)	4.39 (0.15)
Number of fledglings	Mean (<i>n</i>)	6.24 (1445)	6.60 (1092)	4.14 (557)	4.15 (1273)
	Variance (CV)	16.48 (0.65)	8.67 (0.45)	9.15 (0.73)	6.56 (0.62)
Morphological traits					
Male Body mass (g)	Mean (<i>n</i>)	11.01 (1465)	9.82 (1032)	9.66 (455)	9.37 (1607)
	Variance (CV)	0.28 (0.05)	0.23 (0.05)	0.19 (0.04)	0.21 (0.05)
Female Body mass (g)	Mean (<i>n</i>)	11.01 (1713)	9.66 (1153)	9.47 (480)	9.23 (1616)
	Variance (CV)	0.57 (0.07)	0.28 (0.05)	0.22 (0.05)	0.31 (0.06)
Male Tarsus length (mm)	Mean (<i>n</i>)	17.00 (1227)	16.52 (578)	16.42 (198)	16.27 (789)
	Variance (CV)	0.17 (0.02)	0.23 (0.03)	0.18 (0.03)	0.20 (0.03)
Female Tarsus length (mm)	Mean (<i>n</i>)	16.44 (1432)	16.05 (614)	15.99 (224)	15.84 (798)
	Variance (CV)	0.18 (0.02)	0.18 (0.03)	0.25 (0.03)	0.18 (0.03)
Male Wing length (mm)	Mean (<i>n</i>)	67.21 (1418)	63.26 (1033)	63.32 (443)	63.61 (1527)
	Variance (CV)	3.24 (0.03)	3.08 (0.03)	3.00 (0.03)	2.46 (0.02)
Female Wing length (mm)	Mean (<i>n</i>)	64.44 (1647)	60.81 (1138)	60.83 (471)	60.70 (1503)
	Variance (CV)	2.83 (0.03)	2.55 (0.03)	2.52 (0.03)	1.85 (0.02)
Male Beak-nostril length (mm)	Mean (<i>n</i>)	6.54 (1310)	6.55 (965)	6.66 (415)	6.56 (1217)
	Variance (CV)	0.13 (0.05)	0.16 (0.06)	0.14 (0.06)	0.16 (0.06)
Female Beak-nostril length (mm)	Mean (<i>n</i>)	6.70 (1518)	6.82 (1060)	6.83 (446)	6.72 (1176)
	Variance (CV)	0.14 (0.06)	0.19 (0.06)	0.14 (0.05)	0.16 (0.06)
Behavioural traits					
Average male repertoire size reported in one morning†	Mean (<i>n</i>)	3.5 (14)	4.1 (20)		4.7 (12)
	Variance (CV)	1.25 (0.32)	1.46 (0.29)		3.84 (0.42)
Maximal song frequency‡ (Hz)	Mean (<i>n</i>)	7788 (93)	8339 (168)		8138 (133)
	Variance (CV)	29 2681 (0.07)	45 1584 (0.08)		33 5241 (0.07)
Song duration‡ (s)	Mean (<i>n</i>)	1.93 (93)	1.57 (168)		1.33 (133)
	Variance (CV)	0.58 (0.39)	0.69 (0.53)		0.38 (0.46)
Silence duration‡ (s)	Mean (<i>n</i>)	0.06 (93)	0.07 (168)		0.09 (133)
	Variance (CV)	0.0004 (0.33)	0.0004 (0.29)		0.0004 (0.22)
Male Handling aggression score (0–3)	Mean (<i>n</i>)	1.54 (81)	1.82 (339)	1.70 (223)	1.68 (282)
	Variance (CV)	0.84 (0.59)	0.83 (0.50)	0.85 (0.54)	0.96 (0.58)
Female Handling aggression score (0–3)	Mean (<i>n</i>)	0.96 (88)	1.58 (376)	1.25 (227)	1.31 (303)
	Variance (CV)	0.77 (0.92)	0.96 (0.62)	0.89 (0.75)	0.95 (0.74)
Male openfield speed (cm/s)	Mean (<i>n</i>)		15.00 (81)	13.14 (67)	11.69 (65)
	Variance (CV)		61.12 (0.52)	58.70 (0.58)	70.21 (0.72)
Female openfield speed (cm/s)	Mean (<i>n</i>)		13.09 (105)	11.11 (66)	9.84 (82)
	Variance (CV)		75.14 (0.66)	52.82 (0.65)	30.64 (0.56)

(continued)

Table 1. (continued)

First year of monitoring		D-Rouvière 1991	D-Muro 1993	E-Muro 1998	E-Pirio 1976
Colour ornament traits					
Male Blue Brightness	Mean (<i>n</i>)	16.6 (886)	15.4 (472)	16.1 (297)	15.4 (498)
	Variance (CV)	26.5 (0.31)	20.2 (0.29)	23.2 (0.30)	20.0 (0.30)
Female Blue Brightness	Mean (<i>n</i>)	14.1 (949)	13.13 (515)	13.4 (305)	12.4 (519)
	Variance (CV)	28.9 (0.38)	15.0 (0.30)	15.2 (0.29)	16.6 (0.33)
Male UV–Blue Hue	Mean (<i>n</i>)	376.3 (886)	370.1 (472)	377.0 (297)	377.6 (498)
	Variance (CV)	129.0 (0.03)	93.1 (0.03)	179.5 (0.04)	149.4 (0.03)
Female UV–Blue Hue (nm)	Mean (<i>n</i>)	387.9 (949)	381.2 (515)	384.3 (305)	384.2 (519)
	Variance (CV)	131.9 (0.03)	203.4 (0.04)	266.4 (0.04)	152.0 (0.03)
Male Blue UV Chroma (nm)	Mean (<i>n</i>)	0.38 (886)	0.39 (472)	0.37 (297)	0.38 (498)
	Variance (CV)	0.0013 (0.09)	0.0014 (0.10)	0.0007 (0.073)	0.0013 (0.10)
Female Blue UV Chroma	Mean (<i>n</i>)	0.34 (949)	0.35 (515)	0.34 (305)	0.34 (519)
	Variance (CV)	0.0011 (0.10)	0.0013 (0.10)	0.0009 (0.086)	0.0011 (0.10)
Male Yellow Brightness	Mean (<i>n</i>)	17.0 (854)	15.7 (472)	16.1 (297)	16.4 (500)
	Variance (CV)	12.0 (0.20)	11.3 (0.21)	12.5 (0.22)	11.1 (0.20)
Female Yellow Brightness	Mean (<i>n</i>)	17.1 (912)	16.5 (523)	16.7 (310)	17.0 (528)
	Variance (CV)	15.1 (0.23)	12.6 (0.22)	9.9 (0.19)	12.7 (0.21)
Male Yellow Chroma	Mean (<i>n</i>)	0.62 (854)	0.82 (472)	0.69 (297)	0.81 (500)
	Variance (CV)	0.03 (0.27)	0.03 (0.20)	0.02 (0.23)	0.02 (0.18)
Female Yellow Chroma	Mean (<i>n</i>)	0.61 (912)	0.74 (523)	0.65 (310)	0.70 (528)
	Variance (CV)	0.03 (0.28)	0.02 (0.20)	0.03 (0.26)	0.02 (0.18)

References for published results: *Grosbois et al. 2006; †Doutrelant et al. 2000a; ‡Doutrelant et al. 2001.

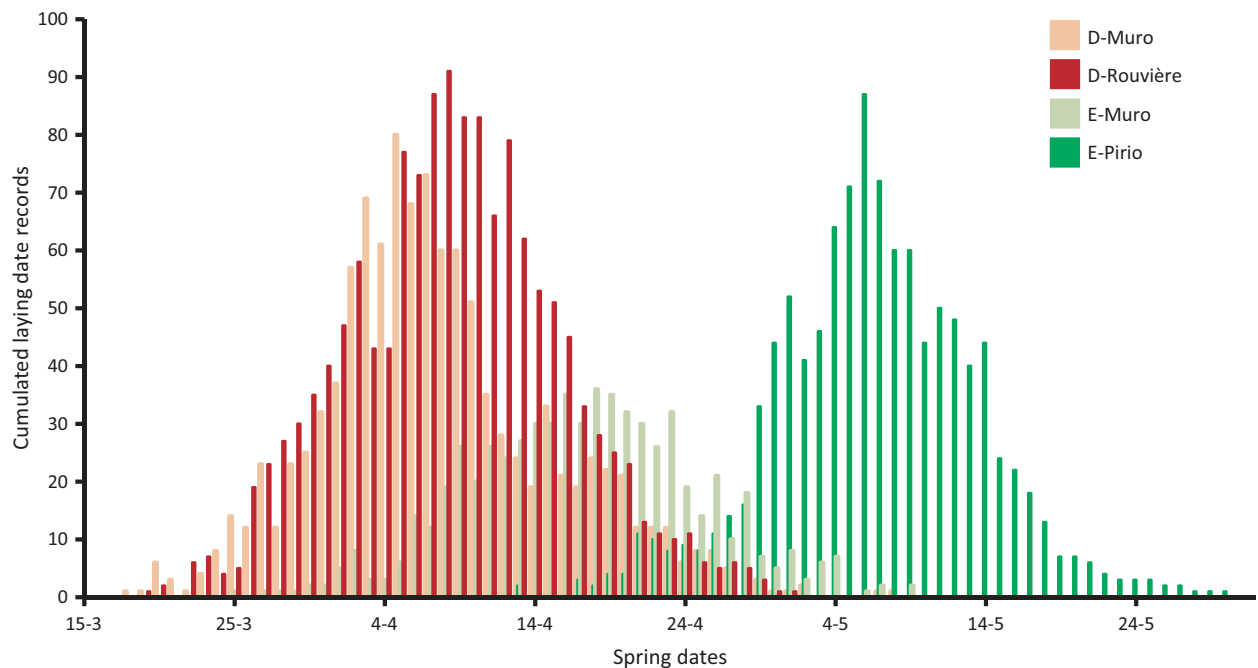


Figure 1 Differentiation in laying dates illustrated through the cumulated observations between 1998 and 2014 in four Mediterranean blue tit study sites.

ence in body mass (independently of adult size) measured between evergreen and deciduous habitats vanished in common garden aviary experiments (Braillet et al. 2002) as

well as in cross-fostering studies (Simon et al. 2005), suggesting a major role of phenotypic plasticity. Both caterpillar abundance and parasite prevalence were identified as

key factors driving the phenotypic plasticity in morphometrics and explaining the habitat-specific divergence with smaller and lighter birds in the poorer habitat (Blondel et al. 2006).

Song

Blue tit song also shows marked divergence between populations (Table 1, Doutrelant et al. 2000a,b; Doutrelant and Lambrechts 2001; Doutrelant et al. 2001). The average repertoire size (number of song types produced by one male in one morning) varies among Corsican sites and is larger in these sites than in the D-Rouviere mainland population (Doutrelant et al. 2000a). In addition, across northern Europe, blue tit song is characterized by the presence of a rapid trill (a fast series of identical notes repeated at the end of song, Bijnens 1988; Doutrelant and Lambrechts 2001; Doutrelant et al. 2001). Our mainland population fits this description as about 70% of the recorded songs contain a trill. By contrast, in Corsica, the percentage of songs possessing a trill is lower, varying between 0% (E-Pirio) and 30% (D-Muro, Doutrelant and Lambrechts 2001; Doutrelant et al. 2001). Overall, local variation is much more pronounced in Corsica than on the mainland, revealing the existence of different song dialects (Doutrelant et al. 2001). In particular, blue tit songs in D-Muro and E-Pirio display significantly different frequency range, maximum frequency, number of phrases and total duration (Table 1, Doutrelant et al. 2001), and they share very few song types (around half the repertoire if we consider the whole range of song types produced but none in common if we only consider the song types sung by more than 10 individuals, Doutrelant et al. 2001). Founder effects, isolation or drift could drive a large part of the geographic variation observed because song is a cultural trait that needs to be learned to be correctly emitted and thus is very sensitive to stochastic factors (Catchpole and Slater 2008). However, playback experiments and correlative data suggest that adaptation to both local vegetation and local interspecific competition with great tits is also a good candidate to explain the described song differences (Doutrelant et al. 1999, 2000a; Doutrelant and Lambrechts 2001).

Personality

Very recently, the study of variation in personality using openfield protocols (Mutzel et al. 2013) and handling aggression (Class et al. 2014) scores (0–3) has revealed that these behaviours are repeatable and show strong differentiation among evergreen versus deciduous habitat patches in Corsica (G. Dubuc-Messier, A. Charmantier & D. Réale, in preparation). In short, individuals from the deciduous island habitat (D-Muro) show higher speed in the open-

field and higher handling aggression compared to individuals from the evergreen island habitats, with similar patterns in males and females (Table 1). At the same time, we did not find any personality difference between the two evergreen island habitats for both traits (openfield exploration speed and handling aggression). These results are consistent with the pace-of-life hypothesis proposed by Réale et al. (2010): individuals from the rich deciduous habitat (D-Muro) show life history characteristic of a fast pace-of-life (e.g. low adult survival, high reproductive investment, see Table 1) and display a personality phenotype classically associated with this kind of pace-of-life, that is fast exploration patterns in the openfield and higher handling aggression. The opposite is true for individuals from the evergreen habitats (E-Muro and E-Pirio), which are showing life history and personality phenotypes typical of a slow pace-of-life, demographically illustrated by lower fecundity (clutch size, number of fledglings) and higher survival rates. Similarly to the divergence observed in song characteristics, such behavioural differences across habitats could arise from restricted gene flow, and in turn could reinforce the isolation process (see more discussion on this in the mechanisms section below).

Colour ornaments

Table 1 illustrates in detail how each population of blue tits presented in Box 2 displays a unique colour phenotype (for detailed statistical results see A. Fargevielle, A. Grégoire & C. Doutrelant, in preparation) when combining measures on the blue crown patch and the yellow chest. Compared to Corsican blue tits, birds from the mainland have brighter yellow and UV–blue colorations and less dichromatic yellow chroma. Corsican populations also differ from one another. First, the chromatic values of the UV–blue coloration separate D-Muro from E-Muro and E-Pirio: deciduous birds display more UV (lower hue and higher UV chroma) and are more dichromatic (Fig. 2). Second, the chromatic values of the yellow coloration separate E-Muro from D-Muro and E-Pirio: the yellow chroma of E-Muro birds is lower and less dichromatic. Finally, the population of E-Pirio shows different brightness patterns, with duller UV–blue coloration and brighter yellow coloration. More studies are needed to understand the origin of these population differences and whether or not they are adaptive, but it is noteworthy that D-Muro and E-Muro birds differ so strongly in both UV–blue and yellow coloration. These differences may contribute to the observed genetic differentiation if mate choice preference for colour signal differs between these populations or if individuals do not settle randomly in respect to their coloration. In the face of a habitat-specific divergence at a micro-geographic scale, the observed differences in the UV–blue coloration between

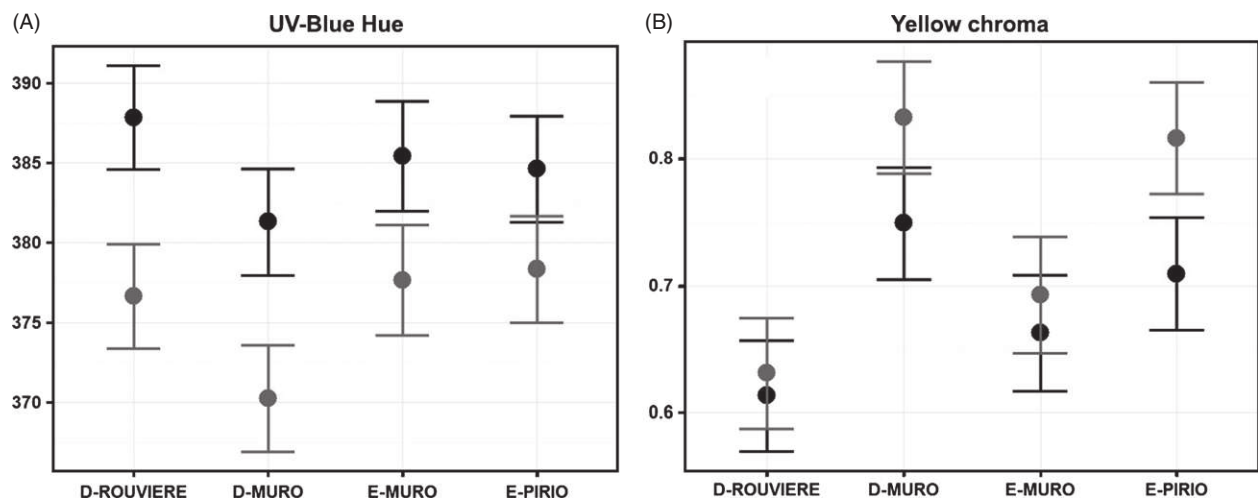


Figure 2 Differentiation in (A) UV/blue spectral hue of the crown patch and (B) purity (or chroma) of the yellow chest, for males (in grey) and females (in black) across our blue tit populations. Data were collected on breeding birds between 2005 and 2013. Within Corsican populations, D-Muro birds display lower UV–blue hue compared to E-Muro and E-Pirio birds (values for males: D-Muro: 370 nm, 95% CI = [367;374]; E-Muro: 377 nm, 95% CI = [374;381]; E-Pirio: 378 nm, 95% CI = [375;382]), whereas E-Muro birds display lower yellow chroma compared to D-Muro and E-Pirio birds (values for males: E-Muro: 0.69, 95% CI = [0.65;0.74]; D-Muro: 0.83, 95% CI = [0.79;0.88]; E-Pirio: 0.82, 95% CI = [0.77;0.86]). Means and confidence intervals are derived from linear mixed models (REML process) with age, sex and population as fixed effects and bird identity and year as random effects.

the two Muro populations are particularly interesting to illustrate this possible divergent intrasexual selection. Indeed, the blue crown patch is involved in social and intrasexual interactions (Rémy et al. 2010; Midamegbe et al. 2011), and as the deciduous forest of D-Muro offers a richer breeding habitat than the evergreen forests of E-Muro and E-Pirio, birds in D-Muro might display more UV because they are socially dominant over the birds breeding in evergreen patches (Braillet et al. 2002). Another hypothesis, involving plasticity in coloration, is that the stronger UV in D-Muro results from a lower cost of breeding in this population. We indeed experimentally demonstrated that increasing the cost of reproduction affects the parents coloration in the following year (Doutrelant et al. 2012).

The phenotypically differentiated traits are heritable

In the context of testing whether the strong phenotypic differentiation described in the previous section can be due to microevolution leading to local adaptation, an important albeit brief point we make here is that nearly all morphological and reproductive traits in Table 1 have been shown to be heritable, either in our populations, or in other blue tit studies. Based on an extensive review of heritability estimates published between 1974 and 2011 (see online supplementary material of chapter 2 in Postma 2014), average heritability in blue tit studies was 0.380 (SE = 0.353,

$n = 46$ estimates) for morphological traits, 0.261 (SE = 0.221, $n = 7$) for life history traits, -0.005 (SE = 0.078 $n = 2$, dispersal distance) for behavioural traits and 0.669 (SE = 0.505, $n = 7$) for physiological traits. When focusing on published estimates derived from the Mediterranean populations described in Box 2, we found evidence for significant heritability in laying date (range of heritability: 0.20–0.43, Caro et al. 2009), body mass (0.267–0.638, Charmantier et al. 2004b; Teplitsky et al. 2014b), tarsus length (0.418–0.597, Charmantier et al. 2004b,c; Teplitsky et al. 2014b), wing length (0.216–0.319, Teplitsky et al. 2014b) and nonsignificant heritability in adult survival probability (heritability = 0.018, CI = [0.000; 0.077], Papaix et al. 2010). As a note of caution, these heritability estimations can vary quite substantially depending on the environmental conditions (Charmantier and Garant 2005), as well as on the complexity of the models being fitted (Wilson 2008). Also, they were all based on animal models run on a social pedigree; hence, they might be underestimated because of frequent extra-pair paternities in our populations (Charmantier et al. 2004a; Charmantier and Réale 2005). Apart from survival, all other morphological and life history traits in Table 1 are classically shown to be heritable in avian species (Postma 2014), which does not preclude strong plasticity in many of these traits, as has been discussed above (see the roles of photoperiod and timing of food abundance for breeding phenology and clutch size). Although quantitative genetic estimates on behaviour and colour ornament traits

are much more recent and still scarce, there is now good evidence for strong additive genetic variance in blue tit and great tit personality traits such as handling aggression and exploratory behaviour (e.g. Dingemanse et al. 2002; Drent et al. 2003; Class et al. 2014), while much more moderate heritability for blue tit colour ornaments (Johnsen et al. 2003; Hadfield et al. 2006; Drobniak et al. 2013). To date, the cultural and genetic heritability of blue tit song has not been quantified and this remains an important step for future research in order to understand the observed differences (Wilkins et al. 2013).

Mechanisms involved in the onset and maintenance of phenotypic divergence

Adaptive divergence across populations has long been considered conditional on a) antagonistic selection across habitats and b) a physical isolation between divergent populations, thereby restricting gene flow (Wright 1943; Maynard Smith 1966). Both theoretical (e.g. Garcia-Ramos and Kirkpatrick 1997) and empirical evidence (e.g. Hendry et al. 2002) supported this view for almost half a century. Our overall understanding of adaptive divergence today includes much more complexity. For instance, it is now recognized i) that restricted gene flow can either be a cause or a consequence of adaptive diversification (Räsänen and Hendry 2008), ii) that in some instances, gene flow can induce rather than constrain phenotypic divergence (Guillaume 2011) and iii) that local adaptation can occur at a very fine spatial scale despite gene flow (see e.g. Muir et al. 2014; Langin et al. 2015; Moody et al. 2015). In a recent review, Richardson et al. (2014) discuss a set of mechanisms that can initiate divergence or reinforce it even in the presence of gene flow. Here we review evidence in our system for three of these nonexclusive mechanisms: habitat selection, selective barriers against migrants and positive assortative mating.

Habitat selection

There are several lines of evidence suggesting that a process of matching habitat choice (Edelaar et al. 2008) could cause directed gene flow between forest habitat types. The fact that birds from E-Pirio are smaller and lighter than birds from D-Muro allowed Blondel and colleagues (fig. 2 in Blondel et al. 1999) to provide the first evidence for nonrandom gene flow as immigrants showed more similar morphometrics compared to resident birds than expected by chance, suggesting that birds born in deciduous habitats would prefer to breed in deciduous habitats, and *vice versa* for birds from evergreen habitats. Later on, a study of feather isotopic signatures in the main study sites presented in Box 2 partly confirmed such habitat choice, while also

revealing in each area a set of immigrant birds with outlier isotopic signatures, presumably originating from oak wood habitats (Charmantier et al. 2014). Further investigations are underway to refine the definition of isoscapes within and around our study populations, in the aim of identifying the landscape origin of each immigrant bird (C. de Franceschi, work in progress). This would allow testing for a matching habitat choice process, for example to test the prediction that blue tits with fast exploratory behaviour preferentially disperse to deciduous forest patches while 'slow' birds prefer to breed in evergreen patches.

Selective barriers against migrants

Richardson et al. (2014) describe this mechanism as reduced fitness for migrants, acting before they reproduce through premating isolation. The habitat-specific breeding phenology could well work as a reinforcing barrier against maladapted dispersal if laying date is culturally or genetically inherited. Indeed, if two populations are breeding at different spring periods, they will obviously not mate with each other, which in turn will strengthen the reproductive barrier that may have arisen for other independent reasons. Anecdotal cases of birds born in deciduous habitats but breeding in evergreen ones support this view, as these birds initiated reproduction too early compared to the caterpillar abundance (Charmantier, pers. comm.), and vice versa for the reverse situation. Apart from this phenological mismatch between habitat types, it has also been suggested that differences in social dominance of birds originating from deciduous or evergreen habitats could contribute to restricting dispersal and maintaining population phenotypic differentiation at a micro-geographic scale (Braillet et al. 2002). Indeed, the larger size of male blue tits from D-Muro (and possibly stronger colorations) provides them with a systematic dominance over the smaller males of E-Pirio, thereby creating a potential barrier for the settlement of evergreen birds in deciduous areas. Finally, the dialects and differences in avian songs detailed previously could also contribute in driving population divergence, such as what has been shown in species where females prefer to mate with the males possessing the local versus a foreign dialect (e.g. Podos 2010). Hence, the existence of dialects in Corsica could contribute to explain the reduced gene flow between valleys, previously highlighted as part of an insular syndrome (Blondel et al. 1999). In the future, we aim at testing this crucial hypothesis using both mate choice experiments and playbacks.

Positive assortative mating

After they settle in a new habitat, maladapted immigrants can still have a restricted contribution to the local gene

pool because of sexual selection processes (Richardson et al. 2014). In particular, positive assortative mating may discriminate against rare phenotypes. In Corsica, we found support for a positive assortative mating in handling aggression scores (estimated correlation between male and female handling aggression: 0.156, 95% CI = [0.054, 0.260]; Fig. 3) and for exploratory speed in the openfield for young females of 1 and 2 years (estimated correlation: 0.280, 95% CI = [0.073, 0.473], $n = 41$ females, 38 males; 2011–2014). Similar assortative mating for personality has been identified in several bird species, including great tits (Groothuis and Carere 2005) and zebra finches *Taeniopygia guttata* (Schuett et al. 2011). Assortative mating for UV–blue and yellow coloration is also around 30%, while varying across years (A. Fargevieille unpublished data). Such assortative mating also suggests a potential role of sexual selection in the observed phenotypic differentiation, which needs further exploration in future work.

Isolation by environment revealed by population genetics and genomics

Throughout the duration of the project, it was established that an impressive number of phenotypic traits diverged between the mainland and the island of Corsica, but also at small spatial scales within Corsica (Table 1). Moreover, for a large number of these often uncorrelated traits, vegetation type was systematically identified as the driver for phenotypic differentiation. Given that significant addi-

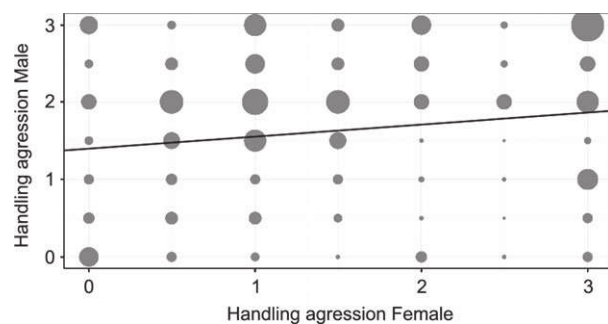


Figure 3 Relationship between handling aggression scores (0 to 3, with 0 no aggressive behaviour and 3 maximum aggressiveness) of male and female social partners in Corsican populations (E-Pirio, D-Muro and E-Muro) from 2011 to 2014; the slope (0.156, 95% CI = [0.054;0.260]) and intercept (1.400, 95% CI = [1.084;1.751]) of the line are derived from a linear mixed model (Bayesian framework) with females handling aggression score as response variable, partner handling aggression score and year as fixed effects and female identity, partner identity and handling aggression observer as random effects; $n = 336$ females, 345 males, 10 observers. The size of the points refers to the number of pairs with a given combination of handling aggression scores (min = 2, max = 24).

tive genetic variance was reported for several of these traits, it is pertinent to ask whether there is a habitat-driven genetic basis for the systematic differences observed at the phenotypic level. As discussed above, aviary and cross-fostering experiments provided evidence for a genetic basis in the habitat-specific differentiation of some of the traits (in particular laying date), but not others (e.g. morphology, clutch size), even though all traits were heritable. In this study system, testing whether blue tit genetic differences are driven by habitat type [defined as isolation by environment (IBE, Wang and Bradburd 2014), isolation by ecology (Edelaar and Bolnick 2012) or more broadly genotype–environment associations (GEA, Hedrick et al. 1976)] therefore becomes a necessary stepping stone before identifying the genetic basis of local adaptation (Barrett and Hoekstra 2011).

Gaining an in-depth knowledge of population genetic structuring has recently witnessed immense progress thanks to next-generation sequencing (NGS) methods (Davey et al. 2011). For our study system, we could thus transition from a panel of 6–10 highly polymorphic, neutral microsatellite markers (Porlier et al. 2012b) to up to *c.* 12 000 bi-allelic single nucleotide polymorphism (SNP) markers (Szulkin *et al.*, in press). SNP markers were generated through a RAD seq (restriction site associated DNA sequencing) approach (Baird et al. 2008) and positioned in both coding and noncoding regions of the genome. Thus, 197 resident birds from D-Rouviere and the three Corsican populations (D-Muro, E-Muro and E-Pirio) were genotyped using single-end RAD sequencing (Szulkin *et al.*, in press). The distribution of allelic frequencies revealed by the SNP marker data set differed between the mainland and Corsica and was characterized by a larger number of rare allelic variants in Corsica when compared to the mainland – a variability that will need to be addressed in future population genomic work. Using a series of complementary population genetics and landscape genetics analyses, both microsatellite and SNP-based studies found multiple evidence of habitat-linked genetic differentiation (Porlier et al. 2012b; Szulkin *et al.*, in press, see Table 2), which we refer to as isolation – by – environment (IBE, Wang and Bradburd 2014). Importantly, SNP-based analyses also found a small yet highly significant genetic differentiation, measured with pairwise *F_{st}* tests, between D-Muro and E-Muro (Table 2). This result was further corroborated by several complementary population genomic analyses. Given that the two study sites are only 5.6 km apart and that blue tit average dispersal distance is believed to range between 330 m and 4 km (depending on dispersal distance estimation method (Tufto et al. 2005; Ortego et al. 2011) while maximum dispersal values are known to range up to 470 km (Winkel and Frantzen 1991), the genetic distinctiveness of the two populations is remarkable. Overall, an

Table 2. Above the diagonal: F_{st} values from Porlier et al. (2012b, data averaged across several years), $n = 247$ Individuals, 6–10 microsatellite markers. Below the diagonal: F_{st} values for SNPs retained after filtering with 5% MAF and a 95% call rate. $n = 197$ individuals, 3159 SNPs (Szulkin et al., in press). Empirical P -values in brackets were computed using 500 permutations (lowest P -values are therefore bounded by 0.002).

	D-Rouvière	D-Muro	E-Muro	E-Pirio
D-Rouvière	–	0.049	0.042	0.041
D-Muro	0.0541 ($P \leq 0.002^{**}$)	–	0.007	0.004
E-Muro	0.0335 ($P \leq 0.002^{**}$)	0.0156 ($P = 0.004^{**}$)	–	0.005
E-Pirio	0.0520 ($P \leq 0.002^{**}$)	0.0099 ($P = 0.347$)	0.0102 ($P = 0.403$)	–

important question that thus needs to be further addressed is whether genetic IBE results from reduced dispersal through habitat choice, local selection against maladapted genotypes or a combination of both. Research in progress will focus on combining phenotypic and genetic data to improve our understanding of the ecological and evolutionary processes shaping observed patterns of genetic differentiation.

The possibility of combining pedigree-based quantitative genetics with genomic data is indisputably an exciting new analytical framework to study heritability (Stanton-Geddes et al. 2013; Berenos et al. 2014) and variation of phenotypic traits in our blue tit study system. Assuming a sufficiently dense marker density, the complementary use of SNP-based relatedness estimators and field-collected pedigrees should allow to accurately perform not only quantitative genetic analyses, but also to identify regions of the genome that have undergone selection (Jensen et al. 2014). Several methods have been developed to meet these goals: for example, QTL-focused approaches (recently identified as prone to type one error in the context of field-based studies with limited sample size, Slate 2013) are being replaced – or complemented by – new methods for phenotype-associated genomic prediction (Goddard and Hayes 2009), genomic partitioning (Robinson et al. 2013; Santure et al. 2013), as well as relatedness- and population structure-corrected genomewide association studies (GWAS) and candidate gene-based approaches (Savolainen et al. 2013).

With the decreasing costs of sequencing and the development of sequencing platforms capable of generating even longer sequence reads at lower cost, SNP data sets are likely to be replaced with sequence (haplotype) data and even whole genome sequencing in the future. While genotype-by-sequencing approaches will allow us to substantially improve the availability of reference genomes, alignment and ascertainment bias-free allelic frequency data, some challenges will also lie ahead: bioinformatics capacity in terms of processing power and know-how needs to be regularly updated. In addition, analytical lines of research will always have to be balanced with time spent in the field – a cornerstone stage necessary for the collection of pheno-

typic, fitness and environmental data relevant to our study species of interest.

Conclusions, limitations and perspectives on adaptive divergence

We have reviewed here evidence strongly suggesting that the striking phenotypic differentiation in ecologically relevant traits exhibited by natural populations of blue tits in their southern range-edge putatively reflects adaptation to the heterogeneity of their habitat. This is not exclusive to a concomitant role of plasticity (as has been discussed for laying date and body mass), bearing in mind that plasticity itself can vary across populations (Porlier et al. 2012a), can be adaptive and can evolve. The conclusions presented here are the result of 40 years of individual monitoring in contrasted habitats and of collection of data on a diverse set of phenotypic traits, including estimations of fitness, on individual relatedness and on genetic and genomic variation. This long-term effort has provided compelling evidence for a habitat-driven phenotypic variation that is most likely driven by local adaptation: laying date is a heritable trait, which shows contrasted distributions and divergent selection across evergreen and deciduous habitats, and these differences are partly maintained in aviary conditions. However, we acknowledge here that these clear conclusions come with certain limitations which will inspire our future research. First, our study system is dependent on the natural heterogeneity of the habitat, and as such, it has some inherent limitations. In particular, there is a lack of replication of deciduous landscapes in Corsica because of its rarefaction in the Mediterranean region. Second, studying a variety of traits in natural bird populations is not easily combined with common garden and/or reciprocal transplant experiments, which makes it more difficult to provide evidence that observed phenotypic differences have a genetic basis. Third, divergent selection has not yet been illustrated (nor explored) for all the traits presented in Table 1. Fourth, some of the phenotypic variation observed between our study sites (e.g. differences in yellow chroma across Corsican populations) is not clearly linked to habitat or geo-

graphic distance. In these rare cases where no ecological driver was yet identified, the most parsimonious explanation for this phenotypic variation remains that it is explained by nonadaptive processes such as drift (even if, in the specific case of the yellow chroma, the literature on the evolution of colours suggests that a finer exploration of factors such as parasites, food abundance or environmental stressors will probably reveal that some of these factors may explain the observed population differences). Two of the most relevant and promising perspectives to overcome these limitations are the multidimensional study of (natural, social and sexual) selection across habitats and the development of an ecological genomics approach.

In a recently developed theoretical model, the strength of local adaptation is shown to be correlated to trait dimensionality (Macpherson et al. 2015): local adaptation (or the fitness advantage of residents over immigrant individuals) increases with the number of traits under spatially variable selection, in other words the number of traits influencing adaptation to environmental heterogeneity. Although we have presented here a phenotypic divergence measured on many different traits, some of these traits, in particular the timing of breeding, display stronger differences across populations than others. A comparative analysis across traits could offer a comprehensive view on which traits are more strongly habitat related. Also, as many of these traits are correlated, further work on the evolution of habitat-specific trait combinations should integrate estimations of phenotypic and genetic covariances (Teplitsky et al. 2014a). Finally, the recent development of new approaches for the study of the dynamics of selection in space and time and its impact on adaptive evolution (Bell 2010; Siepielski et al. 2013; Chevin and Haller 2014) makes the estimation of spatial variation in selection for all traits in Table 1 a timely and exciting perspective which would greatly contribute to understanding differences in adaptive divergence rates.

It has long been acknowledged that loci involved in adaptation should show increased levels of genetic differentiation between populations (Lewontin and Krakauer 1973; Nielsen 2005). Benefiting from that idea, several methods have been proposed to detect loci under selection (e.g. Nielsen 2005; Excoffier et al. 2009; Narum and Hess 2011). Our next step in this project is to explore further the genomic differentiation across temporal, spatial or ecological boundaries and attempt to pinpoint genomic regions that have been involved in adaptation. While it will be impossible to identify all loci responsible for phenotypic variation (Manolio et al. 2009), new tools are being developed to most accurately capture loci with larger effects. Based on the strong phenotypic divergence described in this article, we are particularly excited at the perspective of examining whether outlier loci co-localize with candidate genes that

have been described for the timing of breeding (Johnsen et al. 2007; Caprioli et al. 2012; Liedvogel et al. 2012) and for personality (Savitz and Ramesar 2004; Fidler et al. 2007; Mueller et al. 2014). Such data will also allow to answer the crucial question of whether these focal traits are affected by the same molecular mechanisms in different populations and different ecological contexts.

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RESUME

Les traits ornementaux sont classiquement vus comme un attribut des mâles chez les espèces animales. Cette vision est liée à un rôle considéré historiquement comme très asymétrique des sexes, avec les mâles qui entrent en compétition (sélection intra-sexuelle) pour attirer les femelles qui choisissent (sélection intersexuelle) le meilleur partenaire. Cette idée fut développée en liaison avec l'asymétrie dans la production des gamètes mâles et femelles. Les femelles, qui produisent un nombre réduit de gamètes de grosse taille, maximisent la chance de survie de leurs descendants en investissant plus dans les soins parentaux ; elles deviennent ainsi le sexe limitant et choisissent les mâles qui entrent donc en compétition pour avoir accès à la reproduction. Tout trait ornemental qui augmente le succès d'appariement sera donc avantageux pour les mâles conduisant à des traits sexuels secondaires plus développés chez ce sexe. Si les traits ornementaux sont fréquents chez les mâles, il existe également de nombreux exemples chez les femelles, notamment chez les espèces socialement monogames à soins biparentaux. C'est seulement récemment que les biologistes évolutifs ont cherché à tester les processus expliquant l'apparition et le maintien des ornements femelles. Si le rôle de la corrélation génétique dans cette évolution est incontestable, et que la sélection sociale est aussi majeure, plusieurs études empiriques ont montré un choix mâle pour les ornements femelles et des modèles théoriques ont déterminé les paramètres conduisant à l'évolution du choix mâle. Par ailleurs, les approches phylogénétiques retraçant l'évolution des ornements ont montré une forte labilité des traits femelles, avec des apparitions et disparitions de traits ornementaux plus fréquentes chez les femelles que chez les mâles. Afin de mieux comprendre la relation entre la sélection sexuelle et l'évolution des ornements femelles, cette thèse s'est construite sur ces résultats précédemment acquis et a mené plusieurs approches pour mieux préciser le rôle de la sélection sexuelle dans l'évolution et le maintien de la coloration chez les femelles. Une approche comparative à l'échelle des passereaux a testé les paramètres déterminés comme conduisant à l'évolution du choix mâle par des modèles théoriques. En accord avec les modèles théoriques, les résultats mettent en avant l'importance de l'investissement du mâle dans les soins parentaux dans l'évolution de la coloration du plumage femelle. Ils montrent également comment l'investissement initial des femelles dans la reproduction limite l'évolution de la coloration femelle. Un autre axe de la thèse s'est focalisé sur la coloration chez une espèce monogame, la Mésange bleue *Cyanistes caeruleus*, en utilisant un vaste jeu de données à long terme avec 10 ans de données dans quatre populations pour tester notamment (i) la force de la corrélation génétique, (ii) les liens entre indices de succès de reproduction et coloration et (iii) l'existence d'un appariement par homogamie chez cette espèce. Si les résultats principaux montrent une forte corrélation génétique et soulignent une très forte variation spatiotemporelle, l'application d'outils méta-analytiques a permis de déceler une relation entre les colorations des femelles et les indices de succès de reproduction ainsi qu'un patron faible mais positif d'appariement par homogamie pour les deux patches étudiés (couronne et bavette). Les deux volets de la thèse représentent de nouveaux apports en faveur de l'évolution des ornements femelles. Ils soulignent la complexité associée à leur évolution et l'importance de prendre en compte la variation spatiotemporelle pour une compréhension étendue et une possibilité de généralisation.

Mots-clés : coloration du plumage femelle – sélection sexuelle – choix de partenaire par le mâle – appariement par homogamie – variation spatiotemporelle – coûts de la reproduction

ABSTRACT

Ornamental traits are classically associated with males in animal species. The asymmetrical view is related to sex roles, in which males are competing (intra-sexual selection) to attract females which chose the best mate (intersexual selection). This idea was developed with the concept of anisogamy, the asymmetry in the production of male and female gametes. Females producing few but large gametes maximize their offspring survival rate by investing more in parental care; they become the limiting sex and chose males which are thus competing for access to reproduction. Then, any ornamental trait increasing pairing success would become advantageous for males, leading to more developed secondary sexual traits in this sex. If ornamental traits are more frequent in males, there are also many examples with females, especially in socially monogamous species with biparental care. Evolutionary biologists have only started recently to test processes explaining the outbreak and maintenance of female ornaments. Genetic correlation is an unquestionable process involved in this evolution, and social selection is also a major process. Several empirical studies have also related male mate choice to female ornaments and theoretical models have defined key parameters driving the evolution of male mate choice. Furthermore, phylogenetical studies retracing the evolution of ornaments have showed a high lability in female traits, with more frequent gains and losses of ornamental traits in females compared to males. In order to link sexual selection to the evolution of female ornaments, this thesis was based on these previous achievements to develop different approaches to better understand the role of sexual selection in the evolution and maintenance of female colouration. Comparative methods in songbirds tested the key parameters defined by theoretical models as driving the evolution of male mate choice. In line with theoretical models, results highlight the importance of male investment in parental care in the evolution of female plumage colouration. They also show how female initial investment in reproduction limits this evolution. Another thesis axis focused on colouration in a monogamous species, the Blue tit *Cyanistes caeruleus*, using a large dataset across 10 years in four populations and tested in particular (i) the strength of genetic correlation, (ii) relations between proxies of reproductive success and colouration and (iii) the existence of assortative mating in this species. The main results highlight a strong genetic correlation and a wide spatiotemporal variation and the use of meta-analyses revealed correlation between female colouration and proxies of reproductive success as well as a weak but positive pattern of assortative mating on the two measured patches (crown and chest). Both sides of the thesis represent new insights in favour of the evolution of female ornaments. They also highlight the complexity associated with their evolution and the importance of considering spatiotemporal variation for extensive understanding and generalisation.

Keywords: female plumage colouration – sexual selection – male mate choice – assortative mating – spatiotemporal variation – costs of reproduction