POTENTIEL ÉVOLUTIF DE L'HIRONDELLE BICOLORE (*TACHYCINETA BICOLOR*): PLASTICITÉ PHÉNOTYPIQUE ET VARIATION GÉNÉTIQUE DANS UN CONTEXTE D'HÉTÉROGÉNÉITÉ ENVIRONNEMENTALE

par

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SOMMAIRE

Les changements environnementaux actuels entrainent des modifications importantes dans les pressions de sélection qui agissent sur les populations naturelles. Cependant, la capacité de réponse des populations à ces modifications et l'importance relative des différents mécanismes comme la plasticité phénotypique et les changements de la composition génétique des populations restent encore trop peu connus. L'objectif général de ma thèse était donc d'évaluer les rôles de la plasticité phénotypique et de la variation génétique sur le potentiel évolutif en population naturelle. Pour ce faire, j'ai utilisé comme modèle d'étude l'Hirondelle bicolore (*Tachycineta bicolor*), un passereau migrateur qui est suivi dans le Sud du Québec depuis 2004 dans un environnement hétérogène.

Dans un premier temps (chapitre 2), j'ai évalué les déterminants environnementaux de la date de ponte et évalué leurs effets à des niveaux individuels et populationnels de plasticité phénotypique. Comme observé chez de nombreuses espèces aviaires, la température avait un effet important sur la synchronisation de la ponte, similaire au niveau individuel et populationnel, avec les dates de ponte plus hâtive lorsque les températures étaient plus chaudes. Par contre, ces relations semblaient contraintes par la densité locale d'hirondelles, considérée dans ce système d'étude comme un indice de la qualité de l'environnement. Plus précisément, les réponses plastiques à la température étaient moins prononcées à faible densité, c'est-à-dire dans les habitats plus contraignants. Ces résultats suggèrent donc que malgré la présence de plasticité phénotypique chez une espèce donnée, son efficacité pour pallier les changements climatiques peut être inégale entre les populations.

Dans un deuxième temps (chapitre 3), je me suis intéressée à 4 gènes candidats liés à la phénologie (CLOCK, NPAS2, ADCYAP1 et CREB1) montrant de la variation de type courtes répétitions en tandem, et à leur relation avec deux traits phénologiques, la date de ponte et le temps d'incubation. Ces analyses ont montré plusieurs relations entre la variation observée à

ces gènes et celle des traits phénologiques étudiés, dans la plupart des cas en interaction avec des variables environnementales (densité locale, latitude ou température printanière). Par exemple, les femelles avec en moyenne des allèles plus courts au gène CLOCK pondaient plus tôt que celles avec des allèles plus longs, une relation plus marquée à densité locale élevée. Les différents résultats suggèrent l'importance que peuvent prendre les interactions génotype-environnement, qui sont rarement prises en compte dans les études de gènes candidats, et qui pourraient expliquer une partie des résultats discordants entre les celles-ci.

Dans un troisième temps (chapitre 4), j'ai vérifié la faisabilité d'une étude en génétique quantitative avec les données récoltées dans le système d'étude utilisée, caractérisé par un fort taux de reproduction hors couple et un faible recrutement des oisillons. Plus précisément, j'ai testé à l'aide de données empiriques et simulées la précision et l'exactitude des estimations d'héritabilité et de corrélations génétiques pour trois types de traits, morphologiques, reproducteurs et d'oisillons. Les résultats suggéraient un manque de précision important pour les traits morphologiques et reproducteurs, de même que des biais considérables lors de l'utilisation du pédigrée social plutôt que du pédigrée génétique. Ces analyses révèlent entre autres l'utilité des simulations pour tester adéquatement la faisabilité d'une étude en génétique quantitative sur une population donnée.

Dans une dernière étude (chapitre 5), j'ai documenté les effets de l'hétérogénéité environnementale et de l'utilisation de différentes approches de génétique quantitative sur les prédictions de réponses évolutives en population naturelle. Plus particulièrement, cette étude s'est concentrée sur trois traits morphologiques (masse, longueur de l'aile et du tarse) mesurés à différents moments au cours du développement des oisillons. Les différentes analyses ont montré une sélection plus forte à faible densité locale pour la masse à 12 jours ainsi que des variations dans les composantes de variances phénotypiques selon la qualité de l'environnement (densité locale faible ou élevée) pour la plupart des combinaisons trait-âge étudiées. Il en résultait une tendance à des réponses évolutives prédites plus grandes à faible densité locale. Par contre, les prédictions obtenues avec l'équation du reproducteur et le

second théorème de la sélection différaient fréquemment, et contrastaient grandement avec les tendances phénotypiques observées.

En somme, les résultats de ma thèse suggèrent que les possibilités d'ajustement aux changements environnementaux par la plasticité phénotypique et d'adaptation par des changements génétiques entre les générations peuvent varier selon l'environnement expérimenté par une population. Mes recherches contribuent à une meilleure compréhension des facteurs et mécanismes influençant la persistance à long terme des populations naturelles face aux modifications dans les pressions de sélection.

Mots clés : changements environnementaux, gène candidat, génétique quantitative, Hirondelle bicolore, héritabilité, plasticité phénotypique, réponse évolutive, sélection naturelle

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TABLE DES MATIERES

LISTE DES ABRÉVIATIONS

ASY	plus de deux ans | *after-second year*
BE	équation du reproducteur | *breeder's equation*
CV_A	coefficient de variance génétique additive | *additive genetic variance coefficient*
CV_R	coefficient de variance résiduelle | *residual variance coefficient*
h^2	héritabilité | *heritability*
LRT	test du ratio de vraisemblance | *likelihood ratio test*
MVBE	équation du reproducteur multivariée | *multivariate breeder's equation*
R	réponse évolutive | *evolutionary response*
r_A	corrélation génétique | *genetic correlation*
S	différentiel de sélection | *selection differential*
SNP	polymorphisme d'un seul nucléotide | *single-nucleotide polymorphism*
STS	second théorème de la sélection | *secondary theorem of selection*
SY	deux ans | *second-year*
UVBE	équation univariée du reproducteur | *univariate breeder's equation*
V_A	variance génétique additive | *additive genetic variance*
V_B	variance de l'identité de la couvée | *brood identity variance*
V_D	variance génétique de dominance | *dominance genetic variance*
V_E	variance environnementale | *environmental variance*
V_I	variance d'interactions génétiques | *interaction genetic variance*
V_P	variance phénotypique | *phenotypic variance*
V_{PE}	variance de l'environnement permanent | *permanent environmental variance*
V_R	variance résiduelle | *residual variance*
V_Y	variance liée à l'année | *year variance*

LISTE DES TABLEAUX

LISTE DES FIGURES

Chapitre 1

INTRODUCTION GÉNÉRALE

Cadre théorique

Les changements environnementaux

La variation environnementale est un élément central de l'histoire évolutive des espèces, entre autres par son rôle dans leur divergence et leur adaptation de même que par son influence sur leur persistance ou leur extinction (Schluter, 2001, 2009). Les effets de la variation environnementale ont donc, au cours du temps, grandement contribué à modeler la biodiversité observée aujourd'hui. Au cours des dernières décennies, l'environnement s'est grandement et rapidement modifié en raison des actions humaines. Ces changements environnementaux de causes anthropiques ont pris différentes formes ; changements climatiques, modifications, fragmentations ou destructions d'habitats naturels, surexploitation des ressources et introductions de nouvelles espèces en constituent quelques exemples (Vitousek *et al.*, 1997). Dans tous les cas, ces perturbations ont entrainé des modifications importantes dans les pressions de sélection agissant sur les populations naturelles, menaçant du même coup leur persistance (Hendry *et al.*, 2008; Palumbi, 2001). Par exemple, pour une espèce donnée, le taux d'évolution requis au niveau de sa niche climatique pour pallier le réchauffement global est en moyenne 10 000 fois plus rapide que ce qui est typiquement observé comme niveau de divergence entre les espèces (Quintero et Wiens, 2013). Dans ce contexte, il est donc impératif de prédire et comprendre les réponses possibles des populations face à ces changements environnementaux qui menacent maintenant la biodiversité (Allendorf *et al.*, 2008; Hendry *et al.*, 2008; Parmesan, 2006).

Pour répondre adéquatement aux changements dans les pressions de sélection, les populations naturelles ont trois options, soit la dispersion vers des environnements plus viables, l'ajustement par plasticité phénotypique ou encore l'adaptation par changements génétiques entre les générations (Gienapp *et al.*, 2008). Ces trois options sont non mutuellement exclusives et l'intégration de plus d'une réponse pourrait réduire le risque d'extinction (p. ex., Vedder *et al.*, 2013). Pour une population donnée, l'importance relative de chacune de ces réponses dépend de différents facteurs tels que l'intensité des changements environnementaux rencontrés, la capacité de dispersion et la disponibilité d'habitats alternatifs, les limites et les coûts associés aux réponses plastiques, l'architecture génétique des traits visés par la sélection et le temps de génération (Chen *et al.*, 2011; Gienapp *et al.*, 2008; Hoffmann et Sgrò, 2011; Reed *et al.*, 2011). D'autres options ont aussi été récemment mises de l'avant, comme les stratégies de minimisation de risques (c.-à-d., *bet-hedging*) qui pourraient permettre à plusieurs populations de s'accommoder des changements environnementaux (O'dea *et al.*, 2016; Simons 2011). Les populations qui ne parviennent que partiellement ou pas du tout à s'accommoder de ces changements subiront des déclins importants pouvant éventuellement entrainer leur disparition (Chevin *et al.*, 2010; Hoffmann *et al.*, 2010).

Le déplacement de la répartition spatiale vers les pôles ou en altitude a été observé chez de nombreuses espèces (p. ex., Loarie *et al.*, 2009; Parmesan et Yohe, 2003; Thomas, 2010), ce qui est attribué généralement à une stratégie pour contrer les effets des changements climatiques (revu dans Parmesan, 2006). Par contre, les vitesses de dispersion sont très variables d'une espèce à l'autre, limitant du même souffle l'utilisation de la dispersion comme réponse universelle aux changements environnementaux (Chen *et al.*, 2011). En général, bien que des changements phénotypiques attribués à la modification des conditions environnementales soient fréquemment observés en milieu naturel, la distinction entre les changements de causes plastiques ou génétiques demeure peu documentée (Gienapp et Brommer, 2014; Gienapp *et al.*, 2008; Hansen *et al.*, 2012; Hendry *et al.*, 2008; Merilä et Hendry, 2014). Une meilleure évaluation du rôle des composantes plastiques et génétiques dans la modification des traits reste donc essentielle pour prédire adéquatement le potentiel

évolutif des populations face aux changements environnementaux actuels (Gienapp et Brommer, 2014; Gienapp *et al.*, 2008; Merilä, 2012; Merilä et Hendry, 2014).

La plasticité phénotypique

La plasticité phénotypique est un terme très large qui peut englober toute variation phénotypique induite par l'environnement (Bateson, 2015; DeWitt et Scheiner, 2004; Stearns, 1989). Bien que cette composante importante de la variation phénotypique soit connue depuis plus d'une décennie, elle a longtemps été ignorée puisque perçue comme une nuisance, un bruit de fond non adaptatif limitant le potentiel évolutif des populations (Sarkar, 2004). Aujourd'hui, les études portant sur la plasticité phénotypique sont en hausse (Forsman, 2015) et elle est non seulement perçue comme pouvant être adaptative, mais aussi comme pouvant jouer un rôle actif dans l'évolution des populations (Ghalambor *et al.*, 2007; Pigliucci, 2010; Price *et al.*, 2003).

La plasticité phénotypique est généralement définie comme étant la capacité d'un génotype de produire différents phénotypes en réponse aux variations environnementales (Bradshaw, 1965; Stearns, 1989). Elle peut être représentée sous la forme d'une norme de réaction, décrite par une pente et une élévation, où chaque courbe représente un génotype dont le phénotype est décrit au travers d'un gradient environnemental (Figure 1.1; Nussey *et al.*, 2007; Via *et al.*, 1995). Un génotype qui exprime le même phénotype peu importe les conditions environnementales n'est pas plastique, du moins pas pour le trait et les conditions environnementales étudiées (Figure 1.1a). La plasticité phénotypique est présente dès que les pentes des normes de réactions sont différentes de zéro. Les pentes peuvent alors être similaires pour tous les génotypes, suggérant de la plasticité sans interactions génotype-environnement (GxE, Figure 1.1b). Lorsque les pentes des normes de réaction varient entre les génotypes, il y a présence de plasticité avec interactions GxE (Figure 1.1c). Comme n'importe quel trait, les différentes propriétés des normes de réactions peuvent répondre à la sélection si elles présentent de la variation génétique (Gienapp et Brommer, 2014; Pigliucci, 2005).

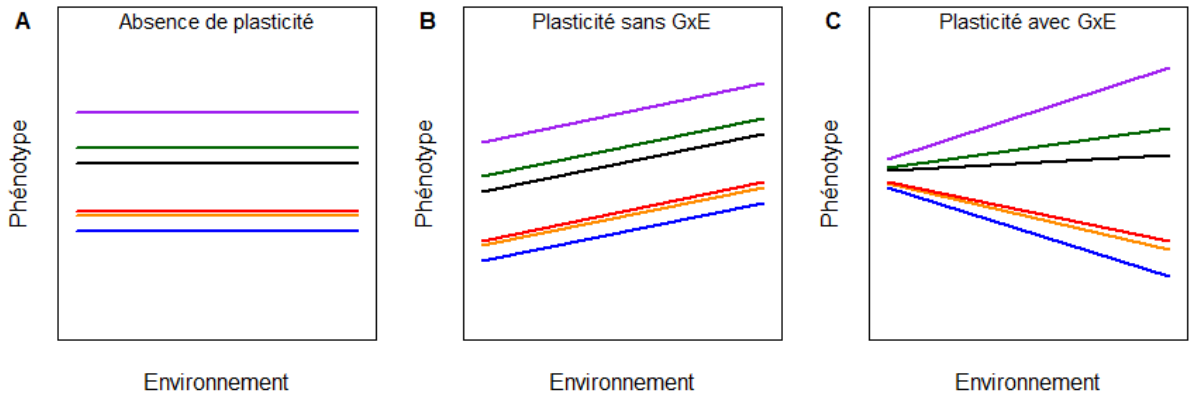

Figure 1.1 Patrons des normes de réactions possibles dans l'étude de la plasticité phénotypique soit a) en l'absence de plasticité, b) en présence de plasticité sans interactions GxE (génotype-environnement) et c) en présence de plasticité avec interactions GxE. Chaque courbe représente la gamme des phénotypes qu'un génotype peut exprimer sous différentes conditions environnementales.

Bien qu'elle soit définie au niveau du génotype, la plasticité phénotypique est souvent considérée à d'autres niveaux d'organisation du vivant, tels que l'individu, la population et même l'espèce (Forsman, 2015; Gianoli et Valladares, 2012; Nussey *et al.*, 2007). L'étude de la plasticité à ces différents niveaux peut permettre entre autres de mieux comprendre les déterminants environnementaux des réponses plastiques et d'évaluer le rôle de la plasticité dans la diversification des espèces (Forsman, 2015). Par contre, il est important de noter que la présence de plasticité à un niveau d'organisation donnée renseigne peu sur celle observée à un autre niveau (Figure 1.2; Forsman, 2015; Gienapp et Brommer, 2014; Nussey *et al.*, 2007; van de Pol et Wright, 2009). Par exemple, la présence d'une covariance entre les phénotypes observés et les conditions environnementales au niveau populationnel pourrait être causée par de la plasticité similaire au niveau individuel (Figure 1.2b) ou simplement une répartition non aléatoire des individus dans l'environnement (Figure 1.2ac). Conséquemment, certaines

précautions doivent être prises dans l'interprétation des patrons de plasticité d'un niveau d'organisation à l'autre.

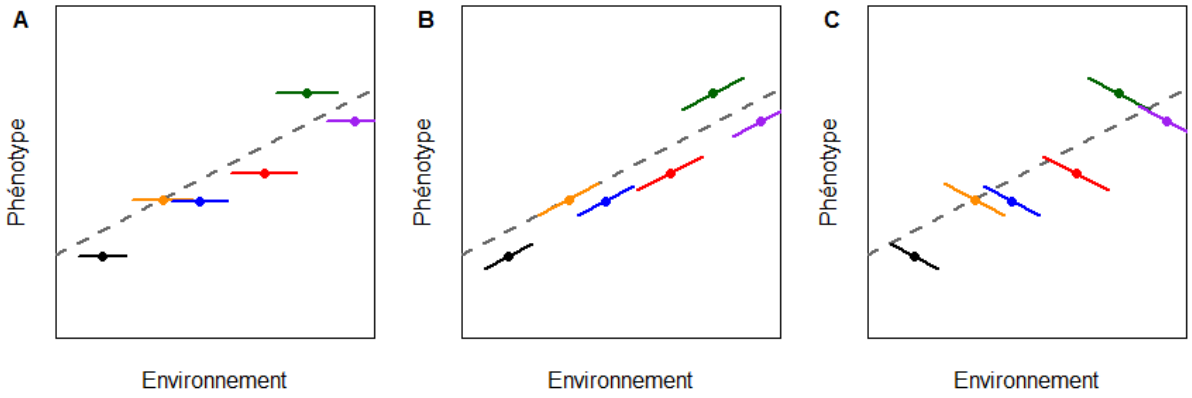

Figure 1.2 Exemples de patrons de plasticité observés à deux niveaux d'organisation différents, soit un niveau inférieur d'organisation (p. ex., individus; lignes pleines) et supérieur (p. ex., population; ligne grise pointillée; modifié de van de Pol et Wright, 2009). Des relations phénotype-environnement nulles (a), positives (b) ou négatives (c) au niveau inférieur peuvent toutes mener à une relation phénotype-environnement positive au niveau supérieur.

L'étude de la plasticité phénotypique peut prendre différentes formes dépendamment du trait d'intérêt et de l'espèce modèle. L'utilisation d'expériences en jardins communs ou de transferts réciproques peut s'avérer intéressante pour son étude puisqu'ils permettent de bien distinguer l'effet des gènes, de l'environnement et de leurs interactions possibles sur les phénotypes (Conover et Schultz, 1995; Conover *et al.*, 2009). Ces méthodes sont par contre limitées aux organismes qui peuvent être étudiés dans un milieu contrôlé, ou facilement transférables d'un milieu à l'autre. En milieu naturel, l'étude de la plasticité phénotypique est souvent limitée aux traits labiles, c'est-à-dire aux traits qui s'expriment plus d'une fois au cours de la vie d'un individu. Ces traits permettent de suivre l'expression phénotypique pour

un même individu – donc à génotype constant – dans des conditions environnementales variables (Brommer, 2013; Nussey *et al.*, 2007). Ces traits comprennent entre autres les traits phénologiques (p. ex., date de ponte, de migration) et de personnalité (p. ex., agressivité; revu dans Brommer, 2013). Dans le contexte des changements environnementaux, l'étude de la plasticité phénotypique à partir de traits labiles peut permettre d'évaluer l'ajustement des phénotypes des individus en réponse aux conditions environnementales, et de déterminer quelle part des changements phénotypiques observés peut être expliquée par cet ajustement (Gienapp et Brommer, 2014). L'étude des traits labiles peut aussi s'intégrer aux approches de génétique quantitative (voir la section suivante) afin de déterminer si la variation dans plasticité individuelle observée possède une composante génétique (Gienapp et Brommer, 2014; Nussey *et al.*, 2007).

Notre compréhension de la place que pourrait prendre la plasticité phénotypique dans les réponses des populations aux changements environnementaux actuels reste partielle pour différentes raisons. D'une part, les changements plastiques semblent la réponse la plus commune, et ils pourraient faciliter le maintien des populations à court et moyen terme (Gienapp et Brommer, 2014; Gienapp *et al.*, 2008). Théoriquement, il a été démontré que la plasticité phénotypique adaptative, c'est-à-dire dont la direction est similaire aux pressions de sélection, peut permettre un ajustement rapide des populations à un nouvel optimum phénotypique, et ce, sans nécessiter de changements génétiques (Price *et al.*, 2003). Dans le contexte actuel, une réponse plastique peut jouer un rôle de tampon, laissant plus de temps à la sélection d'agir et aux changements génétiques de survenir, réduisant du même coup la probabilité d'extinction (Chevin *et al.*, 2010; Vedder *et al.*, 2013). De l'autre part, la plasticité phénotypique adaptative rencontrée dans les populations naturelles n'est probablement optimale que dans les conditions environnementales dans lesquelles elle a évolué et conséquemment potentiellement inefficace si l'environnement subit des modifications trop importantes (Ghalambor *et al.*, 2007; Visser et Both, 2005). De même, l'importance de la plasticité peut varier entre les populations d'une même espèce (p. ex., Husby *et al.*, 2010; Porlier *et al.*, 2012), limitant potentiellement les extrapolations du rôle de la plasticité aux populations spécifiquement étudiées. Il faut aussi noter que la plasticité phénotypique peut être

incomplète, inadaptée ou simplement non adaptative, ne permettant alors pas l'atteinte de l'optimum phénotypique et l'adaptation immédiate qui en résulterait (Ghalambor *et al.*, 2007). Dans certains cas, la dégradation des conditions environnementales semble induire des réponses plastiques dans une direction opposée aux patrons de sélections, masquant potentiellement changements génétiques (Husby *et al.*, 2011; Teplitsky *et al.*, 2008). Le rôle de la plasticité phénotypique sur le potentiel évolutif des populations peut donc être, parfois facilitant, d'autres fois contraignant, mais l'importance relative de chacun reste peu documentée.

La variation génétique

Une condition essentielle pour qu'il y ait évolution dans une population, qu'elle soit adaptative ou non, est la présence de variation génétique entre les individus qui la composent. De manière générale, la variation génétique – la variation dans les séquences des nucléotides composant l'ADN – peut s'avérer neutre ou fonctionnelle, c'est-à-dire, avec ou sans influence sur les différentes fonctions de l'organisme. L'étude au niveau populationnel de la variation génétique neutre peut nous renseigner sur l'effet des différentes forces évolutives neutres (mutation, migration, dérive) sur la quantité totale de cette variation présente dans une population. Par exemple, une population dont la taille efficace diminue considérablement pourrait voir sa diversité génétique réduite également (p. ex., Hutchings et Fraser, 2008). Par contre, la quantité de variation génétique neutre présente dans une population est souvent peu liée à la variation génétique liée à des traits d'intérêts (Hartmann *et al.*, 2014; Reed et Frankham, 2001).

Les avancées techniques récentes en biologie moléculaire ont fait grandement avancer notre compréhension de la variation génétique fonctionnelle (Andrew *et al.*, 2013). Le séquençage du génome entier de divers organismes et les nouvelles technologies d'acquisition et d'analyses de données permettent de trouver facilement des gènes analogues entre les espèces, facilitant ainsi l'étude de leur fonction (Davey *et al.*, 2011). Les relations entre la variation génétique et les différents phénotypes peuvent s'établir par la cartographie de locus à

caractères quantitatifs (c.-à-d., *quantitative trait loci*), par les études d'associations pangénomiques (c.-à-d., *genome wide association study*, GWAS) ou à plus petite échelle par l'étude de gènes candidats (Ellegren et Sheldon, 2008; Stinchcombe et Hoekstra, 2007). Par exemple, la variation observée à des traits de personnalité (comportement exploratoire) est corrélée chez certaines populations de Mésange charbonnière (*Parus major*) avec les différents variants du polymorphisme d'un seul nucléotide (c.-à-d. *single-nucleotide polymorphism*, SNP) du gène *DRD4* codant pour un récepteur à la dopamine (Fidler *et al.*, 2007; Korsten *et al.*, 2010; Mueller *et al.*, 2013). L'accumulation de données provenant de scans génomiques de populations naturelles suggère par contre que les variations constatées à la plupart des locus étudiés n'expliquent qu'une faible proportion de la variance phénotypique observée (p.ex. Mouton de Soay (*Ovis aries*), Bérénos *et al.*, 2015; Gobemouche à collier (*Ficedula albicollis*), Husby *et al.*, 2015; Mésange charbonnière, Santure *et al.*, 2013, 2015). De plus, une récente étude sur deux populations de Mésange charbonnière suggère que très peu de locus ont des effets phénotypiques similaires entre les populations (Santure *et al.*, 2015). Les résultats issus des technologies récentes semblent donc appuyer les prédictions selon lesquelles les traits complexes sont sous l'influence de nombreux gènes à effets restreints et que leur architecture génétique peut être différente d'une population à l'autre (Falconer et Mackay, 1996).

Identifier directement les gènes responsables d'un phénotype n'est pas toujours essentiel pour répondre aux questions d'intérêts en biologie évolutive (Rausher et Delph, 2015; Rockman, 2012; Travisano et Shaw, 2013). Une approche alternative en génétique quantitative permet d'établir quelle fraction de variance phénotypique observée dans une population est causée par les différences génétiques entre les individus à partir d'informations sur leur apparentement, et donc sans nécessiter de connaissances sur le nombre et la fonction des gènes impliqués (Falconer et Mackay, 1996; Kruuk *et al.*, 2008). Dans sa forme la plus simplifiée (sans tenir compte des corrélations et interactions possibles entre les valeurs génétiques et environnementales), la variance phénotypique (V_P) observée au sein d'une population peut se décomposer comme suit :

$$V_P = V_G + V_E \quad (1)$$

soit V_G la composante de variance génétique et V_E celle de variance environnementale. La variance génétique peut elle-même se décomposer en quelques autres composantes supplémentaires reflétant l'action des gènes :

$$V_G = V_A + V_D + V_I \quad (2)$$

où V_A est la composante génétique additive, V_D la composante génétique de dominance, et V_I la composante d'interaction entre les gènes, ou d'épistasie. Le paramètre V_A est la composante génétique d'intérêt puisqu'il représente la cause première de ressemblance entre les individus apparentés et forme la plus large partie de V_G (Hill *et al.*, 2008). V_A ne représente pas uniquement la variance des gènes additifs, mais plutôt la variance de la partie additive de l'action des gènes (Falconer et Mackay, 1996). Pour leur part, V_D et V_I sont très difficile à estimer en milieu naturel en raison du niveau de complexité trop élevé dans la structure dans les données qu'ils requièrent, et conséquemment la majorité des études n'évalueront que V_A (Falconer et Mackay, 1996; Kruuk, 2004; Wilson *et al.*, 2010). La variance phénotypique sera alors plutôt étudiée comme :

$$V_P = V_A + V_R \quad (3)$$

où V_R est la variance résiduelle. V_R est souvent interprété comme représentant la variation liée aux effets de l'environnement en raison de la faible contribution de V_D et V_I à V_P, (Wilson *et al.*, 2010).

L'estimation des paramètres de génétique quantitative s'effectue traditionnellement par un schéma de reproduction contrôlé visant à générer des familles de demi-frères (c.-à-d., *half-sibling breeding design*) ou à l'aide d'une régression parents-jeunes (Falconer et Mackay, 1996). Alors que ces méthodes fonctionnent très bien en conditions contrôlées, la complexité des populations naturelles semble parfois biaiser les estimations qui en résultent (Åkesson *et*

al., 2008; Kruuk, 2004; de Villemereuil *et al.*, 2013). Ces difficultés peuvent être écartées par l'utilisation d'un modèle animal – modèle mixte intégrant une structure d'apparentement entre les individus – qui permet d'utiliser toutes les relations d'apparentement entre les individus, de fonctionner avec des données incomplètes ou non balancées et de contrôler l'effet de différentes causes environnementales de ressemblances entre les individus (revu dans Kruuk, 2004; Wilson *et al.*, 2010).

Le potentiel évolutif

L'évolution a longtemps été perçue comme un processus très lent, imperceptible à l'échelle d'une vie humaine. Pourtant, les exemples de microévolution s'accumulent maintenant dans la littérature et il est aujourd'hui plutôt bien accepté que de tels changements peuvent survenir relativement rapidement, sur une échelle dite écologique (Hendry et Kinnison, 1999; Kinnison et Hendry, 2001). Dans le contexte des changements environnementaux contemporains, les changements génétiques pourraient permettre une adaptation à long terme aux changements dans les pressions de sélection et ils semblent donc être la réponse ultime attendue des populations naturelles (Gienapp et Brommer, 2014; Gienapp *et al.*, 2008). Par contre, ils ont reçu beaucoup moins d'attention dans la littérature que les changements plastiques et ils sont généralement plus difficiles à la fois à détecter et à démontrer (Gienapp et Brommer, 2014; Merilä, 2012).

Pour qu'il y ait évolution adaptative, c'est-à-dire en réponse aux pressions de sélection, une population devra présenter de la variation phénotypique héritable pour le trait visé par la sélection. La réponse à la sélection d'un trait est communément estimée avec l'équation du reproducteur (c.-à-d., *breeder's equation*; Lush, 1937) :

$$R = h^2 * S \quad (4)$$

où R est le changement de la moyenne d'un trait entre deux générations, h^2 l'héritabilité (le ratio de V_A sur V_P) et S le différentiel de sélection (la covariance phénotypique entre l'aptitude

phénotypique relatif (c.-à-d., *relative fitness*) et le trait). Pour être adéquatement utilisée, cette approche nécessite la présence d'une relation causale entre le trait et l'aptitude phénotypique, un postulat qui a peu de chance d'être respecté milieu naturel. En effet, les conditions environnementales rencontrées par un individu peuvent avoir une influence directe à la fois sur son phénotype et son aptitude phénotypique, menant alors à des biais dans les réponses évolutives prédites avec l'équation du reproducteur (Kruuk *et al.*, 2003; Morrissey *et al.*, 2010). Une alternative serait l'application du second théorème de la sélection (c.-à-d., *secondary theorem of selection*; Price, 1970; Robertson, 1966, 1967) :

$$R = cov_A(\omega, x) \quad (5)$$

où $cov_A(\omega, x)$ est la covariance génétique additive entre un trait et l'aptitude phénotypique relatif. Cette approche est plus robuste que sa concurrente puisqu'elle reflète directement le changement évolutif entre deux générations pour un trait donné (Morrissey *et al.*, 2010). Appliquée en population naturelle, elle semble aussi mener à des estimations reflétant mieux les changements phénotypiques observés (Morrissey *et al.*, 2012). Par contre, elle n'est nullement informative sur les causes des changements prédits. Par exemple, l'action directe ou indirecte de la sélection sur le trait ne peut pas être inférée à partir de cette équation. En conséquence, il apparait justifié d'utiliser les deux méthodes, l'équation du reproducteur et le second théorème de la sélection, pour avoir une meilleure compréhension des réponses évolutives en milieu naturel (Morrissey *et al.*, 2010).

Documenter la variance génétique présente à un trait d'intérêt est souvent considéré comme la première étape pour établir son potentiel évolutif, c'est-à-dire sa capacité à répondre aux pressions de sélection (Falconer et Mackay, 1996). La plupart des traits étudiés en nature montrent de la variation génétique, mais son importance est toutefois très variable d'un trait à l'autre (Mousseau et Roff, 1987; Postma, 2014). Par exemple, les traits d'histoire de vie, plus fortement associés à l'aptitude phénotypique, présentent en moyenne une plus faible héritabilité que les traits morphologiques, physiologiques ou comportementaux (p. ex.,

McCleery *et al.*, 2004; Merilä et Sheldon, 2000; Postma, 2014; Teplitsky *et al.*, 2009). Ces différences sont généralement interprétées dans le cadre du théorème fondamental de Fisher sur la sélection naturelle (Fisher, 1930), où les traits sous l'influence d'une forte sélection directionnelle devraient voir leur diversité allélique réduite considérablement, les allèles conférant une meilleure aptitude phénotypique étant rapidement fixés. Par contre, l'interprétation des mesures d'héritabilité est parfois contestée puisque ces mesures donnent peu d'information sur la mesure absolue de V_A et sur l'évolvabilité d'un trait (c.-à-d., *evolvability*; Hansen *et al.*, 2011). Par exemple, les traits fortement associés à l'aptitude phénotypique ne montrent pas nécessairement moins de V_A, mais parfois plutôt une plus forte proportion de V_E (Kruuk *et al.*, 2000; McCleery *et al.*, 2004; Merilä et Sheldon, 2000). Le coefficient de variance génétique ($CV_A = \sqrt{V_A}/\bar{X}$, où $\bar{X}$ est la moyenne du trait, Houle, 1992) pourrait donc être une mesure plus appropriée pour refléter le potentiel évolutif d'un trait. En ce sens, les traits d'histoire de vies sont généralement ceux qui présentent de plus grandes valeurs de CV_A, suggérant que malgré leur forte association avec l'aptitude phénotypique, ils pourraient conserver un bon potentiel évolutif (Houle, 1992; Postma, 2014).

Les traits sont rarement génétiquement indépendants les uns des autres, et déterminer le potentiel évolutif d'un trait sans tenir compte des liens qui l'unissent aux autres caractéristiques phénotypiques peut être un raccourci trop simpliste (Walsh et Blows, 2009). Les corrélations génétiques entre les traits peuvent être causées par des effets pléiotropiques ou des déséquilibres de liaisons (Falconer et Mackay, 1996). La pléiotropie survient lorsqu'un gène affecte plus d'un trait, un phénomène qui semble commun dans le génome (p. ex., 78 % des gènes de la drosophile *Drosophila melanogaster* sont pléiotropiques, Fitzpatrick, 2004). Le déséquilibre de liaison pour sa part est défini comme la tendance statistique d'allèles de différents locus de se retrouver ensemble chez un individu, et ce type d'association peut être causé entre autres par une sélection pour l'association d'allèles. Les corrélations génétiques entre les traits peuvent modifier le taux d'adaptation d'une population en contraignant ou facilitant leurs réponses évolutives (Tableau 1.1.; Falconer et Mackay, 1996; Teplitsky *et al.*, 2014b). Par exemple, chez l'Hirondelle rustique (*Hirundo rustica*), la date d'arrivée suivant la

migration et le délai avant la reproduction sont génétiquement négativement corrélés entre eux (Teplitsky *et al.*, 2011). En raison de la sélection favorisant à la fois des dates d'arrivée plus hâtive et des délais plus courts (sélection négative sur les deux traits), le taux d'évolution prédit du délai avant la reproduction est réduit de moitié par rapport à l'estimation faite sans considérer les corrélations génétiques (Teplitsky *et al.*, 2011). Les corrélations génétiques peuvent aussi modifier la direction de la trajectoire évolutive des traits dépendamment de l'alignement entre l'axe majeur de matrice G de variance-covariance génétique additive et la direction de la sélection (Arnold *et al.*, 2001; Teplitsky *et al.*, 2014a). Dans le même ordre d'idée, une étude sur 4 traits morphologiques de 10 populations d'oiseaux (couvrant 7 espèces) a d'ailleurs récemment montré que la variance génétique présente dans l'axe de la sélection est souvent réduite par rapport à celles des autres axes, réduisant l'évolvabilité dans la direction favorisée par la sélection (Teplitsky *et al.*, 2014b). Des analyses multivariées, tenant compte de plus d'un trait, peuvent donc permettre d'avoir une meilleure idée de l'architecture génétique des traits et son impact sur leur potentiel évolutif (Walsh et Blows, 2009).

Tableau 1.1 Effets possibles (contrainte ou facilitation) de la sélection sur l'évolution de deux traits corrélés génétiquement (tiré de Conner et Hartl, 2000).

Corrélation génétique	Signe des gradients de sélection	
	Identique (+/+ ou -/-)	Opposé (+/-)
Positive	Facilitation	Contrainte
Négative	Contrainte	Facilitation

Finalement, l'architecture génétique des traits, et conséquemment leur potentiel évolutif, peuvent aussi varier suivant les fluctuations des conditions environnementales rencontrées, que ce soit dans le temps ou l'espace. Au niveau d'un seul trait, la tendance observée dans les populations naturelles consiste en une diminution de l'héritabilité dans les conditions environnementales les moins favorables (Charmantier et Garant, 2005; Hoffmann et Merilä,

1999). Cette variation peut bien sûr être causée par une diminution de V_A ou par une augmentation de V_E, ou les deux à la fois. L'environnement peut aussi modifier les corrélations génétiques qui existent entre ces traits, autant en intensité qu'en direction (Sgrò et Hoffmann, 2004). Par exemple, Robinson *et al.* (2009) ont montré que les conditions environnementales pouvaient affecter, en plus des composantes de variance, la stabilité des associations génétiques chez le Mouton de Soay. Chez les mâles, l'ampleur des corrélations génétiques pour la taille des cornes et le nombre de parasites était plus faible dans des conditions environnementales plus favorables. Ces effets de l'environnement, combinés aux variations qui peuvent aussi exister en termes de force et de direction de la sélection (Siepielski *et al.*, 2009, 2013), peuvent avoir des impacts divers sur les réponses évolutives des populations sauvages face aux modifications de leur environnement (Wilson *et al.*, 2006).

Objectifs

L'objectif général de ma thèse est d'examiner les rôles de la plasticité phénotypique et de la variabilité génétique sur le potentiel évolutif en populations naturelles. Pour atteindre cet objectif, j'utilise comme modèle d'étude une population d'Hirondelle bicolore (*Tachycineta bicolor*) que l'on retrouve dans un environnement hétérogène. Cet objectif est couvert par les quatre chapitres centraux de ma thèse, dont les objectifs spécifiques sont, respectivement :

1. Identifier les déterminants spatiaux et environnementaux d'un trait phénologique et déterminer leur importance à des niveaux individuels et populationnels de plasticité phénotypique;

2. Déterminer la variabilité de différents gènes candidats liés à la phénologie et établir leurs relations avec des traits reproducteurs, incluant les interactions possibles avec des facteurs environnementaux;

3. Vérifier la faisabilité d'une étude en génétique quantitative dans un contexte de fort taux de reproduction hors couple et de faible taux de recrutement;

4. Prédire les réponses évolutives de différents traits morphologiques à travers un environnement hétérogène et différents stades de vie.

Méthodes

L'hirondelle bicolore comme modèle d'étude

L'Hirondelle bicolore est un passereau migrateur insectivore aérien qui niche dans les cavités secondaires et les nichoirs au travers de l'Amérique de Nord (Winkler *et al.*, 2011). Cette espèce monogame socialement produit une seule couvée par année, contenant généralement de 4 à 6 œufs. Les deux parents prodiguent des soins parentaux et participent au nourrissage de jeunes, et les oisillons vont s'envoler du nid autour du 18-22^{ième} jour après l'éclosion. L'Hirondelle bicolore présente l'un des taux de reproduction hors couple les plus élevés parmi les passereaux (Griffith *et al.*, 2002), avec environ 50 % des oisillons qui sont issus de reproduction hors couple et au moins un jeune hors couple présent dans 80 % des couvées (Dunn *et al.*, 1994; Lessard *et al.*, 2014; Whittingham et Dunn, 2001).

Suivant le patron observé chez de nombreux insectivores aériens, les populations d'hirondelles subissent un déclin, particulièrement dans le nord-est de leur distribution (Michel *et al.*, 2016; Nebel *et al.*, 2010). Le changement des pratiques agricoles vers une agriculture plus intensive et la diminution de l'abondance des insectes aériens sont souvent évoqués pour expliquer leur déclin (Chamberlain *et al.*, 2000; McCracken, 2013).

Suivi à long terme dans le Sud du Québec

L'Hirondelle bicolore est suivie intensément dans le Sud du Québec depuis 2004, au sein d'un réseau de nichoir couvrant une superficie de 10 200 km² (voir Ghilain et Bélisle, 2008, pour plus de détails sur l'établissement du système). Ce réseau comporte 400 nichoirs, répartis également entre 40 exploitations agricoles diversifiées (p. ex., grandes cultures, agriculture biologique, exploitations laitières, etc.). L'intensité des activités agricoles varie selon un gradient longitudinal, avec des cultures généralement plus intensives à l'ouest (p. ex., monocultures de maïs ou de soya) vers des cultures plutôt extensives à l'est (p. ex., prairies). Depuis 2006, l'utilisation des terres dans un rayon de 500 mètres autour des nichoirs est établie annuellement par inspection visuelle au courant de l'été. Au niveau environnemental, des données de température journalière de même que de quantité de pluie sont récoltées sur chaque ferme du début mai à la mi-juillet. Les insectes sont aussi collectés sur chaque ferme au cours de la même période à l'aide de deux pièges passifs.

Durant la saison de reproduction (mai à juillet), les nichoirs sont visités aux 2 jours pour suivre les activités de reproduction des hirondelles : la date de ponte, le nombre d'œufs pondus, la date d'éclosion, le nombre d'oisillons produits et qui survivent jusqu'à l'envol, etc. Les adultes sont capturés directement au nichoir par un système de trappe, durant la période d'incubation pour les femelles et celle du nourrissage des oisillons pour les mâles. Durant leur capture, des mesures morphologiques sont prises (poids, taille de l'aile et du tarse) et les parasites sont comptés. Le sexe des adultes est déterminé visuellement, mais confirmé plus tard par des analyses moléculaires. Les oisillons sont capturés à quatre reprises au cours de leur développement (2, 6, 12 et 16 jours) pour permettre la prise de mesures morphologiques similaire à celle des adultes. Les adultes et les oisillons de 12 jours sont bagués pour permettre leur identification permanente et leur suivi. Avant 12 jours, les oisillons sont marqués de manière non permanente par la coupe d'une griffe.

Depuis 2006, pour effectuer des analyses moléculaires, du sang est collecté par la veine brachiale chez les adultes et les oisillons de 12 jours lors de leur capture et il est conservé à

température pièce sur un papier filtre (*qualitative P8 grade*, Fisher Scientific). Lorsqu'un individu est retrouvé mort avant qu'un échantillon de sang ait pu être collecté, un morceau de muscle est prélevé et conservé dans de l'éthanol à 95 %. L'ADN est extrait à partir de ces échantillons par une méthode d'extraction saline standard (Aljanabi et Martinez, 1997). Il permet le sexage moléculaire grâce au gène CHD (*Chromo-Helicase DNA binding*) situé sur les chromosomes sexuels (voir Lessard *et al.*, 2014). Chaque individu, adulte et oisillon, est aussi caractérisé à 6 locus microsatellites afin d'effectuer des assignations parentales et ainsi déterminer les pères génétiques des oisillons (Lessard *et al.*, 2014).

Chapitre 2

PLASTICITÉ PHÉNOTYPIQUE

Description de l'article et contribution

Bien que le rôle important de la température printanière dans la synchronisation de la ponte chez de nombreuses espèces aviaires soit généralement reconnu, il est moins clair si d'autres facteurs environnementaux ou spatiaux peuvent influencer ce trait phénologique. L'objectif premier de cet article était donc d'établir les déterminants environnementaux de la date de ponte de l'Hirondelle bicolore, ce qui n'avait jamais été effectué dans le système d'étude du Sud du Québec. Au départ, cet article ne devait constituer qu'une sous-partie du chapitre 3. Par contre, la lecture de van de Pol et Wright (2009) m'a inspiré à faire la distinction au niveau individuel et populationnel pour tous les facteurs environnementaux d'importance, avec comme résultat que ces analyses constituent un article complet en soi. Cette étude suggère notamment que la plasticité individuelle dans la date de ponte en réponse aux températures printanières est contrainte dans les habitats de moindre qualité, décrits ici comme les fermes présentant une faible densité locale.

Pour cet article, j'ai participé à l'élaboration des idées avec Dany Garant et j'ai effectué les analyses statistiques et l'écriture de la première version du manuscrit. J'ai aussi participé à la collecte de données sur le terrain au courant de deux saisons d'échantillonnages (2012-2013). Dany Garant a supervisé le tout et a corrigé plusieurs versions du manuscrit. Fanie Pelletier et Marc Bélisle ont contribué à l'interprétation des données et à la révision du manuscrit. Je tiens à remercier Gabriel Pigeon m'a aidé en me fournissant un exemple de code pour effectuer une analyse par fenêtres glissantes (c.-à-d., *sliding windows*).

Multidimensional environmental influences on timing of breeding in a tree swallow population facing climate change.

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Abstract

Most phenological traits are extremely sensitive to current climate change and advances in the timing of important life-history events have been observed in many species. In birds, phenotypic plasticity in response to temperature is thought to be the main mechanism underlying yearly adjustment in the timing of breeding. However, other factors could be important and interact to affect the levels of plastic responses between and/or within-individuals. Here we use long-term individual-based data on Tree swallow (*Tachycineta bicolor*) to identify the spatial and environmental drivers affecting plasticity in laying date and to assess their importance at both population and individual levels. We found that laying date has advanced by 4.2 days over 10 years, and that it was mainly influenced by latitude and an interaction between spring temperature and breeder density. Analyses of individual plasticity showed that increases in temperature, but not in breeder density, resulted in within-individual advances in laying date. Our results suggest that females can adjust their laying date as a function of temperature, but that this adjustment will be partly constrained in habitats with lower breeder densities. Such potential constraint is especially worrying for the broad array of species already declining as a result of climate change.

Keywords: climate change, density, laying date, phenology, phenotypic plasticity, temperature

Introduction

Effects of current climate change are ubiquitous and severely affect environmental conditions in wild populations (McCarty 2001; Parmesan and Yohe 2003; Walther 2010). Phenological traits are particularly sensitive to these environmental modifications and as a result, over the last decades, phenological changes have been observed in several taxa from plants to mammals (Root et al. 2003; Parmesan 2006; Menzel et al. 2006; Thackeray et al. 2010; Poloczanska et al. 2013). However, the processes underlying observed phenotypic changes remain largely unknown, mainly because the distinction between mechanisms such as genetic changes and phenotypic plasticity is often unclear (Gienapp et al. 2008; Gienapp and Brommer 2014; Merilä and Hendry 2014). Consequently, our predictions of species adaptations to the ongoing environmental modifications remain elusive.

Phenotypic plasticity – the variation in the expression of phenotypes by a genotype in response to the environment (Bradshaw 1965; Stearns 1989) – is usually accepted as the main process to cope with environmental changes in the short term (Gienapp et al. 2008; Charmantier and Gienapp 2014; Gienapp and Brommer 2014; Merilä and Hendry 2014). However, studies have suggested that the importance and magnitude of phenotypic plasticity might be variable among populations (Husby et al. 2010; Porlier et al. 2012) and that the quality of its inference is relatively weak (Gienapp and Brommer 2014; Merilä and Hendry 2014). Importantly, multiple potential environmental drivers of the observed phenotypic changes are rarely studied exhaustively, despite the fact that more than one environmental factor may be affecting or constraining the plastic responses observed in wild populations (Merilä and Hendry 2014). Yet, by choosing *a priori* a single environmental driver, one can miss important causes of the observed phenotypic change (e.g. climate change versus habitat degradation) and predict inaccurate species response and/or suggest ineffective conservation actions to undertake (Merilä and Hendry 2014; Charmantier and Gienapp 2014). Finally, phenotypic plasticity can also be under selection and contribute to adaptive evolution, either directly through an underlying genetic basis or indirectly by allowing survival of populations in new environmental conditions and maintain them relatively close to new phenotypic optimum

(Price et al. 2003; Brommer et al. 2005, Ghalambor et al. 2007; Nussey et al. 2007; Merilä and Hendry 2014). For all these reasons, investigating the importance of phenotypic plasticity, in terms of assessing individual and population variations, its environmental drivers and its influence in observed phenotypic trends, is a critical first step to obtain a more complete understanding of evolutionary processes underlying phenotypic changes caused by current climate change.

Different environmental and spatial drivers can affect plasticity of phenological traits, either directly by acting as cues of future environmental conditions or indirectly through population differentiation captured in space and/or by acting as constraints on plastic responses. Physiological regulation of phenological events in birds comes from the integration of diverse cues from which photoperiod is the most important because its perception allows an annual read of time passing (Sharp 2005; Bradshaw and Holzapfel 2007; Dawson 2008; Visser et al. 2010). Annual photoperiod variation increases with latitude and could explain most of the within-species latitudinal variation in life-history events (Lambrechts et al. 1997; Bradshaw and Holzapfel 2007; Dawson 2013). Finer adjustments (i.e. plasticity) are allowed by the integration of other environmental signals from the physical and social environments (Ball and Ketterson 2008; Dawson 2008). For instance, temperature is thought to be the main driver of timing of breeding in birds (Meijer et al. 1999; Visser et al. 2009; reviewed in Caro et al. 2013), but other factors such as rainfall, often a cue for food availability (Hau 2001; Saunders et al. 2013), and social interactions (Caro et al. 2007) have been reported to play a role in some populations. Knowledge of how these various cues are perceived by the circadian system is still scarce (Dawson 2008), as is appreciation of variation in the perception of these multidimensional cues among individuals (i.e. IxE) or populations (Visser 2008; Lyon et al. 2008; Visser et al. 2010). These cues may also interact with other environmental components and constrain the levels of plastic responses displayed between and/or within-individuals (Wilson et al. 2007). However, very few studies have addressed these possible interactive effects.

Here we use 10 years of data from a Tree swallow (*Tachycineta bicolor*) long-term study to investigate the role of multiple spatial (latitude, longitude and elevation) and environmental (spring temperature, rainfall and breeder density) determinants of laying date. We first assess the influence of potential factors and their interactions on laying date at the population level in our 10,200-km^2 study system. These factors were chosen based on previous knowledge of their potential influence on laying date in tree swallows and other bird species. We then examine the importance of these factors at both population (among-individuals) and individual (within-individuals) levels of plasticity. The tree swallow is a small migratory passerine, an aerial insectivorous, and it produces only one clutch per year, all characteristics of species more at risk under current climate changes (Both and Visser 2001; Møller et al. 2008; Dunn and Winkler 2010; Thackeray et al. 2010; Dunn and Møller 2014). In fact, tree swallow populations are severely declining in the eastern part of their distribution (Nebel et al. 2010; Shutler et al. 2012), including in our study area (Rioux Paquette et al. 2014). However, the causes of these declines are still unknown despite some indications pointing at agricultural intensification in breeding areas (e.g. Ghilain and Bélisle 2008; Rioux Paquette et al. 2013) or at cary-over effects from non-breeding areas (e.g. Rioux Paquette et al. 2014; but see also Dunn et al. 2011 and Dunn and Møller 2014).

The mean laying date of tree swallows has also advanced in most populations across the continent over the last five decades (Dunn and Winkler 1999, 2010; Rioux Paquette et al. 2014; but see Hussell 2003 for an exception). A previous analysis in our study system showed that selection favored earlier laying date in this population but that patterns of selection fluctuated in strength and direction through time (Millet et al. 2015). Also, the time lag observed in the studied area between spring arrival (eBird, http://ebird.org/) and reproduction suggests that further adjustments of laying date are possible. Latitude, spring temperature and breeder density (as a proxy of habitat quality) were suggested to influence tree swallow laying date at a large spatial scale (Dunn and Winkler 1999; Winkler et al. 2002), but we have little knowledge of other potential environmental and spatial factors, their influences at a small spatial scale, and their relative importance on population and individual levels of plasticity.

Methods

Study system and data collection

Between 2004 and 2013, during the breeding season (April to August), we monitored 400 nest boxes within 40 farms (10 nest boxes per farm, separated by 50 m, thus covering similar areas on each site) in southern Québec, Canada (covering an area of 10,200 km²) (Fig. 2.1; see Ghilain and Bélisle 2008 for more details on the study system). During this period, each nest box was visited every 2 days to record occupancy and laying date of the first egg (in Julian days; January 1=Julian day 1). Females were captured during the incubation period, while males were caught during the nestlings' food provisioning phase. All tree swallows were individually identified with an aluminium band (US Fish and Wildlife Service). Females were aged based on feather colour: brown females were assigned to second-year class (SY) and blue-green females to after-second-year class (ASY) (Hussell 1983). Since 2006, the sex of every individual was confirmed with a molecular technique following Lessard et al. (2014). In our analysis, we only considered first clutches, i.e. first breeding event in a nest box of both female and male (if known) within a reproductive season (n=2273; see Table S2.A1 for details on yearly sample sizes). Second clutches are rare (12.7% of all clutches) and mostly result from first clutch failures.

Spring temperature (°C) and rainfall (mm) data were obtained in two steps, using information collected from meteorological stations located within the study area (obtained from Environment Canada; http://meteo.gc.ca/; Table S2.A2; Fig. 2.1). First, a sliding windows approach was used to determine the most relevant time period suitable for all farms for these two meteorological variables and to guard against potentially misguided *a priori* choices (see Brommer et al. 2008 and Porlier et al. 2012 for similar approaches). For this analysis, we used a unique climatic variable value obtained by averaging values from the three meteorological stations nearest from the centroid of our study system (centroid: 45.57°N, -72.64°W; Table S2.A2). We tested windows varying from 5 to 91 days, from Julian days 60 to 151 (respectively March 1 and May 31 in non-leap year) for a total of 3828 windows. Pearson's

correlations between annual mean of averaged daily value for each window and annual mean laying dates were used to determine the most relevant period for each environmental variable. The strongest correlation between mean temperature and mean laying date was found between Julian day 96 and 129 (April 6 – May 9; r=-0.750, P=0.012), while for rainfall, this window was between Julian day 128 and 133 (May 8 – 13; r=-0.748, P=0.013). As a second step, we used these periods as our references for computing both annual mean temperatures and annual rainfalls (hereafter spring temperature and rainfall) from 10 meteorological stations near our farms (Fig. 2.1; Table S2.A2; distances range between each farm and the nearest meteorological stations: 1.6−20.1 km), allowing at the same time a fine resolution of the spatial and temporal environmental variation across the study system and a comparison of laying dates among farms in the plasticity analyses (see below).

Environmental determinants of laying date at the population level

We used the annual mean laying dates for each farm (n=392 since no birds were observed in 8 farm-years; r=0.92 between annual mean and median laying dates) to assess both the temporal (inter-annual) trend in laying and the environmental determinants of laying date. For the temporal trend, we used a linear mixed model to estimate the annual change in mean laying date over the study period (10 years), with farm identity included as random effect. Then, we fitted a linear mixed model to quantify the effects of different environmental variables on mean laying date. The full model included spring temperature, rainfall, breeder density (% of the 10 nest boxes on each farm occupied), elevation (m) and latitude (decimal degree) and all two-way interactions as fixed effects (see also Table S2.A3 for the range limit of each environmental component). We did not include longitude and distance from the St. Lawrence River as they were both highly correlated with elevation (r > 0.9; Fig. 2.1) (see also Porlier et al. 2009). All explanatory variables were standardized (zero mean, unit variance; Table S2.A3) to facilitate the interpretation of their relative influence on mean laying dates. Year and farm identity were tested as random effects using Likelihood Ratio tests (LRTs), but only year was significant and kept in analyses (but see Table S2.A4 for a model including both year and farm

identity as random effects – the selected final model and its effect sizes were similar in both cases).

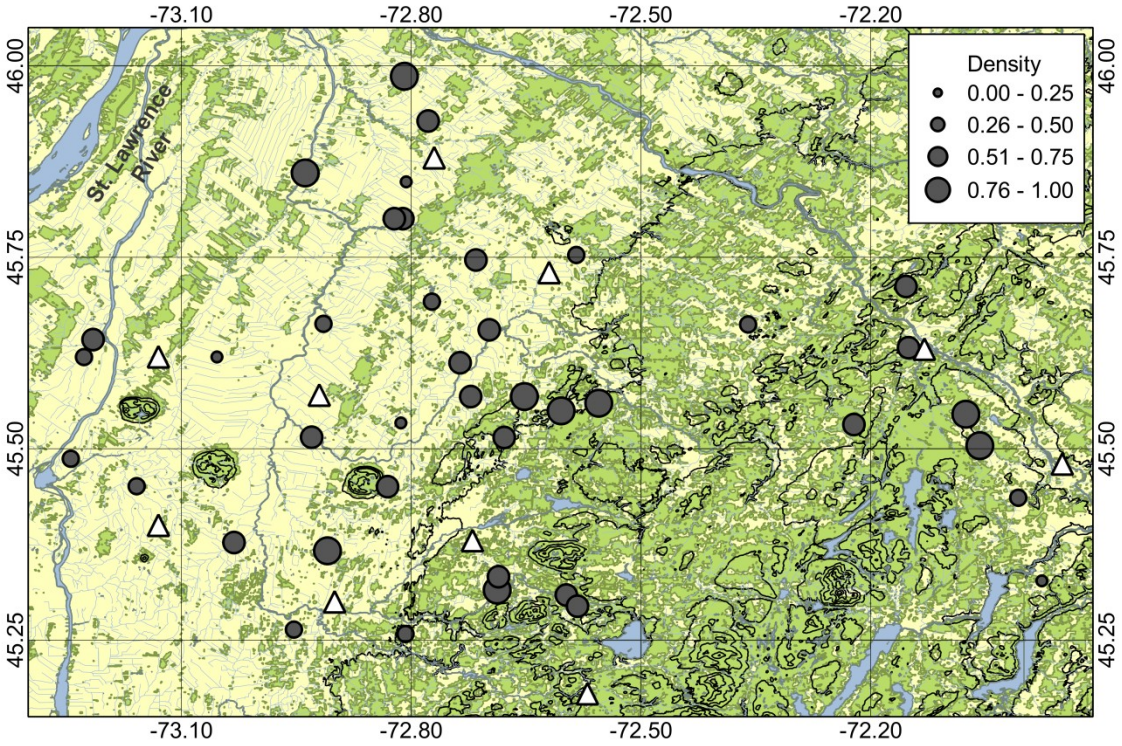

Figure 2.1 Distribution of the 40 farms (gray circles) and 10 meteorological stations (white triangles) in the study system in southern Québec. Mean density of breeders on a farm (% of occupied nest boxes) between 2004 and 2013 is represented by different circle sizes (see legend). Forest patches (green), rivers and lakes (blue), other land uses (mostly agriculture; yellow), elevation (100-m black isolines), latitude and longitude (in decimal degrees; thin black lines) are also represented. This figure was created with QGIS 2.0 (QGIS Team Development 2013).

Individual plasticity in laying date

Individual plasticity in laying date was modeled including only two out of three environmental variables that were significant in the population-level analysis (i.e. spring temperature, breeder density; see Results). Although latitude was significant at the population level (see Results), it was not an appropriate variable to assess individual plasticity because it has limited variation for a given individual over its lifetime. In fact, tree swallows can be considered philopatric to their breeding site in our study area as only 8.1% of our observations were indicative of females having dispersed between farms (n=1015 observations on 397 females, among different breeding events; see Lagrange et al. 2014). All environmental variables were standardized (zero mean, unit variance; Table S2.A3). Age was included as a covariate in our models because of its influence on laying date: older females reproduce earlier than younger ones (Stutchbury and Robertson 1988; Bentz and Siefferman 2013; this study, see Results), and thus females sampled in 2004 were excluded as we had no information about their age.

We first assessed the relationship between the difference in laying dates (laying date year 2 - laying date year 1) and the difference in environmental conditions between years (environmental value year 2 - environmental value year 1) for all females breeding in two consecutive years. This analysis was conducted using a linear model and was repeated for three datasets: 1) females observed as SY on the first year (n=63, refer to as the SY dataset), 2) females observed as ASY on both years (n=311, refer to as the ASY dataset) and 3) all females with age class on the first year as fixed effect (n=349, refer to as the total dataset). For females breeding in more than two years, we included only the first two consecutive observations in these analyses.

We then investigated individual plasticity and between-individual variation in plasticity (IxE) with a random regression analysis (Nussey et al. 2007) on females that were observed in at least two years between 2005 and 2013 (n=935 observations on 370 females). We compared increasing structure complexity of random effects (year, farm, female identity) with LRTs, including random slopes with environmental variables (IxE). Furthermore, because not all

individuals experienced the same set of environmental conditions, we used the within-subject centering technique for environmental variables to separate individual variation from the population trend (Kreft et al. 1995; Snijders and Bosker 1999; van de Pol and Wright 2009). Hence, each environmental variable (temperature and breeder density) was subdivided into a within-individual (β_W) and a between-individual (β_B) component. Briefly, for each female, we calculated a mean value of temperature and breeder density experienced (i.e. between-individual effect, reflecting the population trend), and for all observations, an individual deviation from these mean values (i.e. within-individual effect, reflecting individual plasticity). The full model included as fixed effects within-individual (β_W) and between-individual (β_B) components of both spring temperature and breeder density and also female age class and latitude to control for their effects. Best linear unbiased predictors (BLUPs) for each female (i.e. individual slope and elevation) were generated from the final model to graphically represent individual-specific plastic response.

All statistical analyses were conducted in the R statistical environment 3.0.2 (R Core Team 2014). Linear mixed model analyses were performed using the lme4 package (Bates et al. 2014). Degrees of freedom (Satterhwaite's approximation) and *P*-values of mixed models were calculated using the lmerTest package (Kuzetsova et al. 2013). Final models were determined by sequentially removing the least significant term from the model based on its *P*-value and comparing with a LRT this new model to the previous one, repeatedly until all remaining variables were significant ($\alpha = 0.05$) (Crawley 2007).

Results

Phenological changes and environmental determinants

Tree swallow annual mean laying date advanced by approximately 4.2 days over the 10-year study period (β=-0.419±0.076, t=5.50, P<0.001; Fig. 2.2a). Further analyses revealed an increase in spring temperature (β=0.183±0.017, t=11.09, P<0.001; Fig. 2.2b) and a decrease in

breeder density (β=-0.093±0.014, z=6.83, P<0.001; Fig. 2.2c) over the same period (linear mixed model and generalized linear mixed model (logit link and binomial error) were used, respectively, with farm identity included as a random effect). The final model of the environmental determinants of laying date included latitude and an interaction between mean temperature and breeder density as significant explanatory variables (Table 2.1). More specifically, farms at higher latitudes (northern locations) showed later mean laying dates than those at lower latitudes (Table 2.1; Fig. 2.3a). Laying date was also earlier when spring temperature increased; this relationship was steeper under higher breeder density (Table 2.1; Fig. 2.3b). Rainfall and elevation did not significantly affect laying date and thus were not kept in the final model.

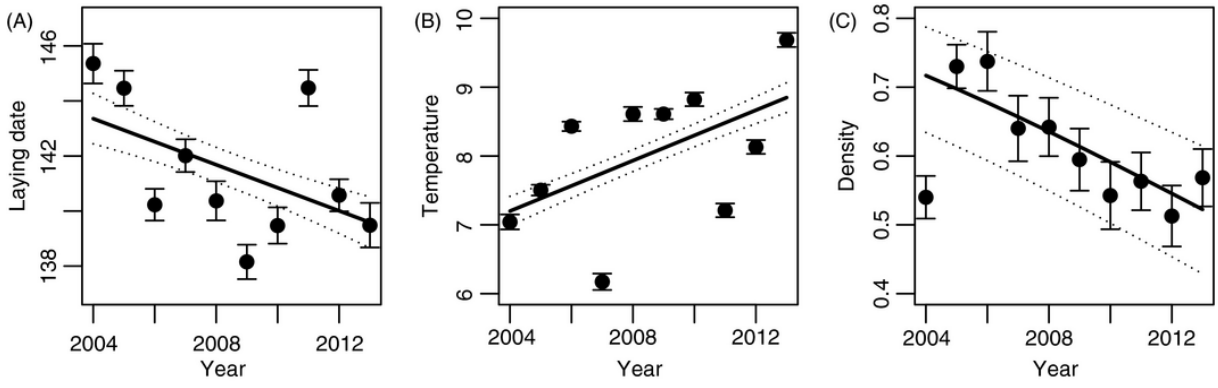

Figure 2.2 Temporal trend at the population level in A) mean laying date (Julian days) of tree swallows, B) spring temperature (°C) and C) density of breeders (% of occupied nest boxes) over the 40 farms monitored between 2004 and 2013. Black circles depict mean values (±SE) over all farms for each year, and black lines are model predictions (dotted lines: 95% CI).

Table 2.1 Final linear mixed model at the population level of the environmental determinants of mean laying date in tree swallows (n=392). Environmental variables have been standardized prior to the analysis. Year was included as random effect. Adjusted R^2 for fixed effects was 0.182

Variable	Estimate	S.E.	d.f.	t-value	*P*-value
Intercept	141.415	0.658	8.1	215.01	<0.001
Latitude	0.479	0.195	376.9	2.46	0.014
Breeder density	-1.469	0.205	383.4	7.16	<0.001
Temperature	-0.929	0.341	158.5	2.73	0.007
Temperature X Breeder density	-0.450	0.204	379.6	2.20	0.028

Individual plasticity in laying date

Our analyses showed evidence of individual plasticity as a function of spring temperature but not of breeder density. The first analysis of individual plasticity showed negative slopes for change in laying date as a function of temperature differential for all three datasets (i.e. SY, ASY and total datasets; Table 2.2). This result suggested that an increase in temperature between years resulted in earlier laying date over the same period. Contrastingly, analyses of change in laying date as a function of differences in breeder density revealed non-significant negative trends with earlier laying dates at higher densities for all the datasets (Table 2.2). Finally, SY females laid their eggs more than five days later than ASY ones (Table 2.2).

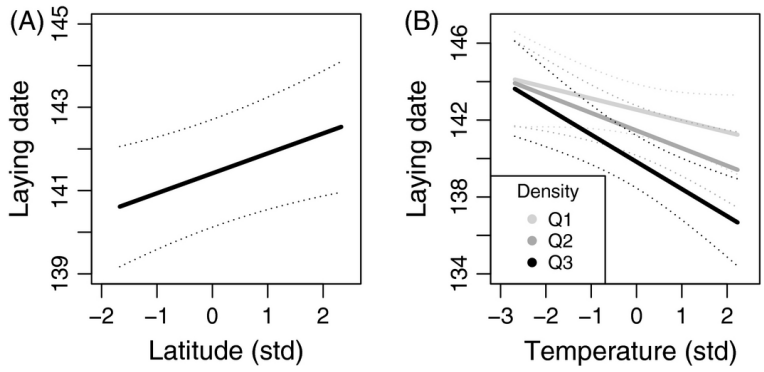

Figure 2.3 Predictions from the linear mixed model of environmental determinants of tree swallow laying date at the population level for A) latitude and B) the interaction between spring temperature and breeder density (first (Q1, lowest), second (Q2) and third (Q3, highest) quartile of density values presented). See Table 2.1 for details.

The random regression analysis first showed evidence for individual slopes variability in the relationship between laying date and breeder density in the random part of the model (i.e. IxE for breeder density; model 5: LRT=10.81, *P*=0.004; Table 2.3; Fig. 2.4b), but not for individual-by-temperature variability (i.e. no IxE for spring temperature; model 4: LRT=0.50, *P*=0.78; Table 2.3; Fig. 2.4a). Estimates of the within-individual (β_W) and between-individual (β_B) components of environmental variables showed different pattern for spring temperature and breeder density effects (Table 2.3; Fig. 2.4). For spring temperature, both β_W and β_B showed a significant negative relationship – with earlier laying date at warmer temperature. However, for breeder density only the between-individual component was significant and negative, suggesting that the earlier laying dates at higher breeder density reflected a difference at the population level but no individual plasticity. Finally, the comparison between estimates of within-individual and between-individual slopes within each environmental variable suggested no significant difference between temperature components ($\beta_W = \beta_B$, *P*=0.38) and a significant difference between breeder density components ($\beta_W \neq \beta_B$, *P*=0.039) (Table S2.A4; see equation 2 in van de Pol and Wright 2009 for more details on the technique used).

Table 2.2 Individual-based analyses of plasticity quantifying the change in laying date between two consecutive years by female tree swallows in relationship to change in spring temperature and breeder density for a) females observed as SY on the first year (n=63), b) females observed as ASY in both years (n=311), c) all females (n=349; age was included as fixed effect). Variables in bold characters were kept in final models and adjusted R^2 are presented.

Model	**Variable**	**Estimate**	**S.E.**	**t-value**	***P*-value**
SY	**Intercept**	**-8.159**	**1.246**	**6.55**	**<0.001**
R^2 = 0.113	**ΔTemperature**	**-3.742**	**1.256**	**2.98**	**0.004**
	ΔDensity	-1.111	1.262	0.88	0.38
ASY	**Intercept**	**-2.415**	**0.393**	**6.14**	**<0.001**
R^2 = 0.099	**ΔTemperature**	**-2.338**	**0.394**	**5.94**	**<0.001**
	ΔDensity	-0.629	0.394	1.60	0.11
TOTAL	**Intercept**	**-2.349**	**0.448**	**5.24**	**<0.001**
R^2 = 0.158	**Age**	**-5.683**	**1.056**	**5.38**	**<0.001**
	ΔTemperature	**-2.462**	**0.407**	**6.06**	**<0.001**
	ΔDensity	-0.764	0.406	1.88	0.061

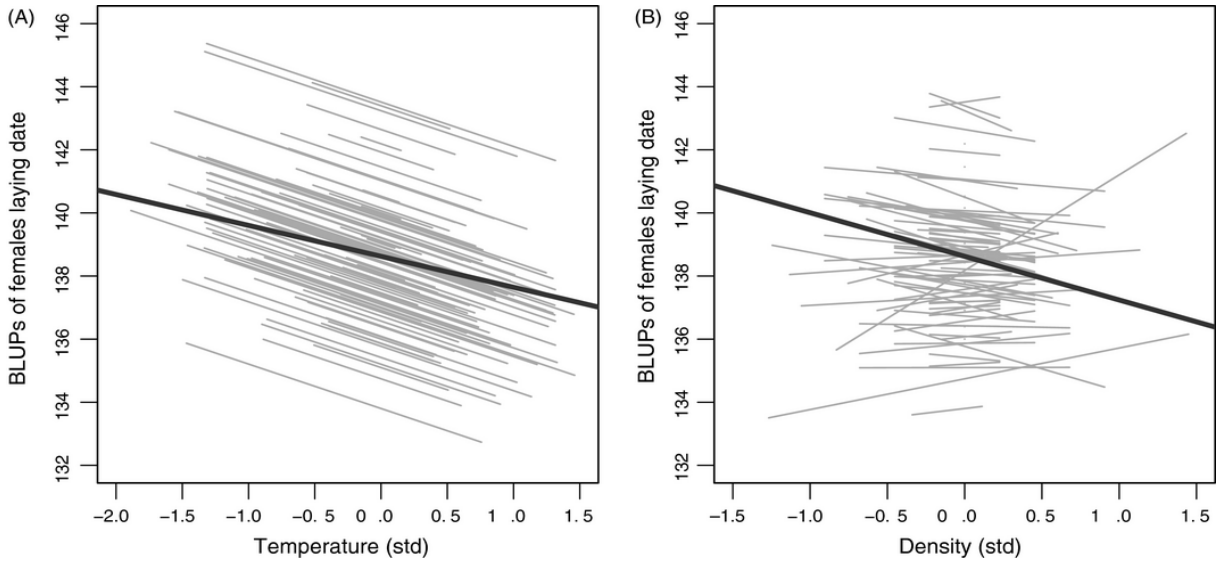

Figure 2.4 Best linear unbiased predictions (BLUPs; gray lines) for 100 female tree swallows (randomly chosen over a possibility of 370) from the random regression model (model 5, Table 2.3) of individual plasticity in laying date (Julian days), for within-individual component (β_W) of standardized A) spring temperature and B) breeder density. Bold black lines represent predictions from between-individual components (β_B).

Table 2.3 Random regression analyses of the effect within-individual (β_W) and between-individual (β_B) components of two environmental variables, spring temperature and density of breeders, on female tree swallow laying dates (n=935 observations on 370 females). Random structures of models 1 to 5 were compared with a LRT. Estimates of fixed effects and variance components of random effects of model 5 (random slope function of breeder density) are presented. Within-individual centering technique (β_W vs β_B) was applied as suggested by van de Pol and Wright (2009).

Models	**Log-L**	**Test**	**d.f.**	**LRT**	***P*-value**
1. Year	-2911.0		9		
2. Year + Farm	-2903.9	1 vs. 2	10	14.13	<0.001
3. Year + Farm + Female	-2885.9	2 vs. 3	11	36.12	<0.001
4. Year + Farm + Female X $Temperature_{within}$	-2885.6	3 vs. 4	13	0.50	0.78
5. Year + Farm + Female X $Density_{within}$	-2880.4	3 vs. 5	13	10.81	0.004

Fixed effects	**Estimate**	**S.E.**	**d.f.**	**t-value**	***P*-value**
Intercept (β_0)	138.605	0.752	7.7	184.41	<0.001
Age	7.274	0.646	838.7	11.25	<0.001
Latitude	0.638	0.303	26.9	2.11	0.045
$Temperature_{within}$ (β_W)	-1.408	0.468	76.5	3.01	0.004
$Temperature_{between}$ (β_B)	-0.995	0.470	88.4	2.12	0.037
$Density_{within}$ (β_W)	-0.347	0.421	175.0	0.82	0.41
$Density_{between}$ (β_B)	-1.386	0.297	105.0	4.67	<0.001

Random effects	**Var**	**Corr**
Female(intercept)	7.660	
$Density_{within}$ (slope)	7.488	-0.20
Year (intercept)	4.243	
Farm (intercept)	1.563	
Residual	19.092	

The observed population trend (i.e., β_B) as function of breeder density – without a significant within-individual component – and the observation of steeper laying date-spring temperature slope with increasing breeder density in the environmental determinant analysis suggested that females living on average at lower densities were possibly constrained in their plastic response. To further explore the hypothesis that lower density farms imposed a constraint on laying date plasticity (in response to spring temperature), we conducted additional individual plasticity analyses using datasets subdivided into high and low breeder densities (see Appendix B). We found that for both individual plasticity analyses (i.e. change in laying date and random regression analysis) plastic responses to temperature were slightly more negative in the high density than in the low density subset (Table S2.B1–3), which could potentially be explained a stronger plastic response at higher density of breeders.

Discussion

In this study, we were interested in the multidimensional influence that environmental variation can have on phenological traits, even at a small spatial scale. Here, we have shown the importance of three environmental variables − latitude, spring temperature and breeder density − and found evidence of individual plasticity as a function of spring temperature but not of breeder density and no evidence of variation in individual slopes. Our results also suggested that females breeding on average in areas of lower breeder densities were possibly constrained in their adjustment of laying date in response to spring temperature.

Phenological change

Tree swallows in our population have advanced their annual mean laying date by about 0.42 day/year over the 10-year study period. This rate of advance is higher than the 0.28 day/year advance that was previously reported for this species throughout North America (study period: 1959 to 1991, Dunn and Winkler 1999). This difference can be explained by either an increase in this rate in the last two decades or by geographic variation in effects and/or responses to

climate change (e.g. Hussell 2003; Dunn and Møller 2014). However, these two potential explanations could only be distinguished by performing a new temporal trend analysis of tree swallow laying dates across their range. The observed advancement is also greater than the mean trend computed from several long-term studies on birds (mean advance of 0.13 day/year, n=68 species, Dunn and Winkler 2010), but is still comparable to observations from a few previous studies on migrant species (e.g. eurasian reed warblers (*Acrocephalus scirpaceus*): advance of 0.48 day/year, Crick and Sparks 1999; great reed warblers (*Acrocephalus arundinaceus*): advance of 0.55 day/year, Dyrcz and Halupka 2009).

Environmental determinants

Numerous previous studies in birds reported within-species latitudinal variation in phenology, reflecting different readings of photoperiod (e.g. Sanz 1998; Dunn and Winkler 1999; Gienapp et al. 2010; reviewed in Dawson 2013). However, the latitudinal variation in laying date documented here is particularly striking given the small spatial scale involved (80-km span in latitude) compared to previous studies (e.g. North American continent, Dunn and Winkler 1999; 700-km span in latitude, Gienapp et al. 2010). Our result may be partly explained by larger day length variation in space than in time during the breeding season in this region. For instance, on May 20th (the mean laying date across all observations in our study; Julian day 140 in non-leap years), the difference in day length between the most distant sites in terms of latitude in our study system was of approximately 5 minutes, while the difference between two consecutive days was around 2 minutes (calculated with the NOAA solar calculator, http://www.esrl.noaa.gouv). Considering that 30-60 minute changes in day length over an entire year can be perceived as cues for breeding and moulting in bird species distributed near the Equator (Hau 2001; Goymann et al. 2012), it is plausible that the latitude effect on laying date documented here in a region with a larger annual day length variation partly reflects a difference in day length captured by the circadian rhythm of individuals.

Variation in density of breeders is rarely studied as a potential determinant of timing of breeding in birds, but it showed the largest effect size on mean laying date. The negative

relationship we observed – later laying date at lower density – is similar to observations from other tree swallow populations (models using species abundance indices from the Breeding Bird Survey program, Dunn and Winkler 1999; Winkler et al. 2002), but contrary to expectations under intraspecific resource competition (e.g. Wilkin et al. 2006; Wilson et al. 2007; but see also Ahola et al. 2012 for a special case where intraspecific resource competition lead to earlier laying date). Dunn and Winkler (1999) suggested that differences in habitat quality should lead to an aggregation of individuals in areas with more food, while areas with fewer resources should limit and constrain laying date (e.g. food availability, Shorrocks et al. 1998; Robb et al. 2008). This is supported by the positive correlation usually observed between nest box occupancy rate and insect abundance (Hussell 2012), and by the negative correlation observed between timing of breeding and flying insect biomass during the laying period (Dunn et al. 2011) in tree swallows. Tree swallows do not follow an ideal-free distribution in our study area since birds nesting in low quality habitats have smaller clutch sizes and lower reproductive success (Ghilain and Bélisle 2008; Lessard et al. 2014). We could also speculate that the observed relationship is partly explained by the activity of the circadian system, where the density of breeders could act, similar to the effect of temperature, as an environmental cue (e.g. the presence of conspecific may be needed to initiate breeding events as in Caro et al. 2007) regulating timing of breeding in females (Dawson 2008). Nevertheless, our detailed analyses of individual plasticity do not support this last hypothesis (see below).

Temperature is usually proposed to be the most important environmental variable determining laying date in birds (Visser et al. 2009; Caro et al. 2013). In our sliding window analysis, the temperature during the month preceding the laying period was providing the strongest correlation. This period is similar to what has been observed in other bird species (e.g. common gulls (*Larus canus*), Brommer et al. 2008; great tits (*Parus major*), Husby et al. 2010; blue tits, Porlier et al. 2012) and corresponds to the period of increasing spring temperatures acting directly as a signal for the timing of breeding in birds (Visser et al. 2009; Schaper et al. 2012). Indeed, a tendency for earlier timing of breeding at higher spring temperature has been observed in several bird species (Dunn and Winkler 2010; Charmantier

and Gienapp 2014), including tree swallows (Dunn and Winkler 1999; Winkler et al. 2002; this study). The temperature-density interaction observed, with more negative laying date-spring temperature slope at higher breeder density, further supports the environment quality hypothesis, since at lower densities of breeders (lower quality habitats) it might be harder for individuals to respond to environmental cues and effectively adjust their laying date (see also discussion on individual plasticity below).

Individual plasticity and between-individual effect

Evidence of individual plasticity in laying date in response to spring temperature in both plasticity analyses suggests that this environmental variable may potentially act as a cue for timing of breeding in tree swallows. Our first observation that changes in temperature experienced by a female will lead to changes in its timing of breeding has been supported by the within-subject centering technique where individual plasticity (within-individual component, β_W) remained significant despite the heterogeneity observed in sampling (between-individual component, β_B). It is possible that different mechanisms drive the patterns observed at the population and individual levels even if the trends are similar in direction and magnitude. However, the similarity in coefficients for within- and between-individual spring temperature components potentially suggests that the population trend observed can be explained by individual phenotypic plasticity (see Brouwer et al. 2013 and Gienapp and Brommer 2014 for similar interpretations when $\beta_W=\beta_B$).

Density of breeders in our study system is probably not a social cue for reproductive timing, but could instead reflect a variation in individual capacity to initiate breeding linked with habitat quality. Our first individual plasticity analysis has shown no effect of variation in breeder density on individual laying date adjustment, and this finding was further supported by our second analysis showing that the within-individual component (β_W) was not different from zero (i.e. no individual plasticity). These results combined with the observed negative population trend (β_B) in laying date in our data suggested that changes in density a female will experience across breeding seasons will not affect her plastic response (i.e. not act as an

environmental cue for timing of breeding) and that all females living on average at higher densities laid their eggs earlier (and *vice versa*). The possible constraint on plasticity for females at lower densities (lower quality habitats) suggested from the steeper laying date-spring temperature slope with increasing breeder density in the population level analysis was further supported by the slightly stronger individual plastic response of laying date as function of temperature observed at high densities in our complementary analyses (Table S2.B1−3). Environmental constraints on phenotypic plasticity have also been described in song sparrows (*Melospiza melodia*) on Mandarte Island (British Columbia, Canada), where cohorts born in better environmental conditions showed higher plastic response in response to the El Niño Southern Oscillation (Wilson et al. 2007). While we believe that the pattern described here is likely to be non-adaptive, given that tree swallows breeding later show a reduced fitness in most years (Millet et al. 2015), further investigations are needed to clearly conclude on the effects of reduced plasticity in lower density habitats (e.g. compare selection gradients between low and high breeder density farms).

Variability in individual responses to the environment (IxE) is considered the raw material for phenotypic plasticity evolution (Nussey et al. 2007). In birds, IxE for laying date in response to temperature has been observed in most populations studied (reviewed in Gienapp and Brommer 2014). Here, the absence of IxE for spring temperature (i.e., no phenotypic variation in slopes), along with similar plastic responses at the population and individual levels, suggest that tree swallows can track temperature changes, probably as long as the observed variation is within the usual range of temperatures they are adapted to. The presence of IxE is usually tested by stepwise model building, where improvement in model likelihood when adding the IxE component is sufficient to suggest variation in the slope and thus individual variation in plasticity. Our results questioned this approach of assessing IxE. A first problem with this approach is the fact that an improvement to the model could be mainly due to the presence of a significant covariance between the slope and intercept rather than to a significant IxE interaction. Also, while we observed no variation in the slope for the spring temperature reaction norm, we observed individual variation in slope for breeder density (model 5, Table 2.3), but no direction or pattern in the way individuals respond to variation in breeder density

(i.e. β_W, individual plasticity). Previous studies argued that heterogeneity in residual variance could lead to an over-estimation of IxE (Brommer 2013; Nicolaus et al. 2013), a phenomenon that cannot be discarded here. For all these reasons, the presence of a significant IxE interaction involving breeder density as random individual responses may not be representative of variability in phenotypic plasticity at the individual level.

Applications of our study

Phenotypic plasticity in response to spring temperature can be an effective way for birds to keep adequate timing of life-history events in the face of climate change (reviewed in Charmantier and Gienapp 2014). For example, Vedder et al. (2013) have shown with a population persistence model that the actual level of individual plasticity in timing of breeding observed in great tits of Wytham Woods (UK) lowers their extinction risk by about 500-fold. However, the success of a population response to climate change via phenotypic plasticity can depend on many other environmental components. For example, degradation of environmental conditions in a Finnish population of pied flycatchers (*Fiducela hypoleuca*) is suspected to be a cause for the observed mismatch between breeding time and phenology of the environment (Laaksonen et al. 2006). Studying all potential factors influencing phenological traits is crucial for a more complete understanding of the potential of phenotypic plasticity to adequately track environmental changes. Here, our initial choice of environmental variables was based on factors previously shown to influence tree swallow laying date, but was also guided by data availability. Ideally, we should have used a measurement of habitat quality (e.g. food availability) rather than a proxy (i.e. breeder density) and also a finer measurement of climatic variables (e.g. temperature and rainfall for each farm). Yet, using the best proxy available is arguably a better option than not taking it into account when analysing plasticity.

Environmental conditions have changed over the study period in our system, with both an increase in spring temperature and a diminution in breeder density (see also Rioux Paquette et al. 2014). These changes influenced the phenological response to environmental cues in contrasting ways. While we found a phenotypically plastic response for changes in spring

temperature, the more limited capacity to respond to temperature cues (i.e. reduced individual plasticity) that we suspect in lower density habitats is worrying for tree swallow populations in the context of concurrent climate change, population decline and reduced fitness for individuals breeding later (Millet et al. 2015). Multiple environmental drivers of phenotypic changes can act in synergy and accelerate the rate of extinction (Brook et al. 2008). Unfortunately, models predicting species response to climate change rarely included phenotypic plasticity, population-level response and/or multidimensional environmental factors despite evidences of important bias caused by such omissions (Chevin et al. 2010; Reed et al. 2011; Bellard et al. 2012; Valladares et al. 2014). If plastic responses are constrained in lower quality habitats, and that several human-driven changes are occurring simultaneously, the ability of species to respond to climate change may be jeopardized and lead to further biodiversity loss. Studies such as this one are still necessary to improve our knowledge of the effects of important environmental factors, to understand how they interact together and to assess, rather than assume, the importance of plastic responses underlying observed phenotypic changes. Altogether, our results enlighten the complexity of phenotypic plasticity as a way for populations to cope with current climate change.

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Chapitre 3

GÈNES CANDIDATS

Description de l'article et contribution

Les approches par gènes candidats sont attrayantes puisqu'elles permettent de lier directement la variation génétique observée à un locus précis à la variation phénotypique, de même que suivre les changements dans fréquences alléliques entre les générations. Le gène CLOCK est celui qui est le plus fréquemment étudié en relation avec des traits phénologiques, mais récemment trois autres gènes ont aussi été mis de l'avant comme de bons candidats (NPAS2, ADCYAP1 et CREB1; voir tout particulièrement Chakarov *et al.* (2013) qui a inspiré leur inclusion dans cette étude). Cette étude quantifie dans un premier temps la variation présente à ces gènes chez l'Hirondelle bicolore, pour ensuite les mettre en relation avec deux traits reproducteurs, la date de ponte et le temps d'incubation. Cette étude innove par rapport à ce qui avait été fait précédemment par l'analyse de plus d'un gène, l'intégration des génotypes des mâles et l'inclusion d'interactions avec des variables environnementales, le tout dans une même étude. Les résultats obtenus suggèrent entre autres l'importance des interactions génotype-environnement dans l'expression des phénotypes en nature.

Pour cet article, j'ai effectué la majorité du travail de laboratoire, incluant la mise au point des conditions PCR et le génotypage des individus. Quelques stagiaires ont contribué travail de laboratoire, particulièrement Nicolas Bousquet qui m'a été d'une grande aide. L'élaboration des idées s'est effectuée en collaboration avec Dany Garant. J'ai effectué les analyses statistiques et j'ai écrit le manuscrit. J'ai également participé à la collecte de donnée sur le terrain durant deux saisons (2012-2013). Dany a révisé plusieurs versions du manuscrit. Merci à Fanie Pelletier et Marc Bélisle qui ont également révisé une version du manuscrit.

Candidate gene-environment interactions and their relationships with timing of breeding in a wild bird population

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Audrey Bourret and Dany Garant

Abstract

Monitoring and predicting evolutionary changes underlying current environmental modifications are complex challenges. Recent approaches to achieve these objectives include assessing the genetic variation and effects of candidate genes on traits indicating adaptive potential. In birds, for example, short tandem repeat polymorphism at four candidate genes (CLOCK, NPAS2, ADCYAP1, CREB1) has been linked to variation in phenological traits such as laying date and timing of migration. However, our understanding of their importance as evolutionary predictors is still limited, mainly because the extent of genotype-environment interactions (GxE) related to these genes has yet to be assessed. Here, we studied a population of Tree swallow (*Tachycineta bicolor*) over four years in southern Québec (Canada) to assess the relationships between those four candidate genes and two phenological traits related to reproduction (laying date and incubation duration), and also determine the importance of GxE in this system. Our results showed that NPAS2 female genotypes were non-randomly distributed across the study system and formed a longitudinal cline with longer genotypes located to the East. We observed relationships between length polymorphism at all candidate genes and laying date and/or incubation duration and most of these relationships were affected by environmental variables (breeding density, latitude or temperature). In particular, the positive relationships detected between laying date and both CLOCK and NPAS2 female genotypes were variable depending on breeding density. Our results suggest that all four candidate genes potentially affect timing of breeding in birds and that GxE are more prevalent and important than previously reported in this context.

Key words: ADCYAP1, candidate gene, CLOCK, CREB1, GxE, incubation duration, laying date, NPAS2

Introduction

Current environmental changes, such as climate warming, severely impact natural populations by generating new and/or modifying already existing selective pressures (Parmesan, 2006; Hendry *et al.*, 2008). To cope with these novel conditions, populations can disperse to more suitable habitats, exhibit phenotypic plasticity and/or an evolutionary adaptive response (Gienapp *et al.*, 2008; Hoffmann & Sgrò, 2011; Merilä, 2012). Over the long-term, a population evolutionary response to selection should involve genetic changes (Hoffmann & Sgrò, 2011). However, monitoring and predicting these changes have proved to be challenging. Recent approaches to achieve these objectives in natural populations include assessing the genetic variation and effects of candidate genes on traits indicating adaptive potential in the face of environmental fluctuations (Hoffmann & Willi, 2008; Hoffmann & Sgrò, 2011; Pardo-Diaz *et al.*, 2014).

A candidate gene approach tests statistical correlations between phenotypes and specific *a priori* relevant genetic components (i.e. identified/suspected from previous biochemical studies or of known influence in another species) to link phenotypic variations to gene variants (Fitzpatrick *et al.*, 2005; Hoffmann & Willi, 2008). More specifically, short tandem repeats (STR) are present in neutral (e.g. microsatellites) and functional genome regions but it is their variation in repeat numbers within functional genome regions (5'-UTR, exons, introns, 3'-UTR) that may modify gene functions (mainly the level of genic expression, see Elmore *et al.* 2012) and resulting phenotypes (Kashi *et al.*, 1997; Comings, 1998; Li *et al.*, 2004; Fondon III *et al.*, 2008) and thus represent potential candidate genes. For example, several STR length polymorphisms are associated with the presence of some human diseases (e.g. Huntington's disease) and variation in animal behaviours (e.g. vasopressin-dependent social behavior in prairie voles *Microtus ochrogaster*; reviewed in Fondon III *et al.*, 2008).

Recent studies in birds have highlighted four candidate genes showing STR length polymorphisms associated with phenological traits and thus relevant to study in the context of changing environmental conditions (Johnsen *et al.*, 2007; Steinmeyer *et al.*, 2009; see Table 3.1 for a summary). The most commonly studied gene so far is CLOCK, a highly conserved transmission factor central to the rhythmicity of the circadian oscillator (reviewed in Young & Kay, 2001). CLOCK possesses a poly-Q binding region that shows length polymorphism (in Q repeat number) which affects its binding affinity with its transmission factor (Darlington *et al.*, 1998). At the population level, a positive latitudinal gradient in the number of poly-Q repeats has been observed across blue tit populations in Europe (*Cyanistes caeruleus*, Johnsen *et al.*, 2007). This gradient was steeper than expected under neutral processes, thus suggesting an underlying functional basis to the genetic polymorphism (Kyriacou *et al.*, 2008). However, this gradient was not detected in the two other bird species where it was assessed (bluethroats (*Luscinia svecica*), Johnsen *et al.*, 2007; pied flycatchers (*Ficedula hypoleuca*), Kuhn *et al.*, 2013), raising doubts about the generality of this finding. At the individual level, length polymorphism in CLOCK was positively correlated with female laying date, hatching date and incubation duration in blue tits (Liedvogel *et al.*, 2009) and laying date in barn swallows (*Hirundo rustica*, Caprioli *et al.*, 2012). Nevertheless, such relationships were absent for the same traits studied in several other bird species (see Table 3.1). In a common buzzard (*Buteo buteo*) population, STR length polymorphism in three other candidate genes, NPAS2, ADCYAP1 and CREB1, has been recently reported for the first time in relationship to reproduction timing (Chakarov *et al.*, 2013, Table 3.1). The candidate gene NPAS2 shows length polymorphism in the same exon as its paralog CLOCK (Steinmeyer *et al.*, 2009) and is believed to overtake its functions (Debruyne, 2008). The two others, the neurotransmitter ADCYAP1 and the transcription factor CREB1, have shown STR polymorphism in their 3'-UTR region (Steinmeyer *et al.*, 2009) and both have a broad spectrum of functions related in part with the circadian rhythm core oscillator (Carlezon Jr *et al.*, 2005; Vaudry *et al.*, 2009). These three candidate genes have not shown significant relationship to reproductive timing in the only population studied (Chakarov *et al.*, 2013). However, relationships between length polymorphism at these genes and other phenological traits, such as dispersal and migration

behaviour, were reported in different bird species (Mueller *et al.*, 2011; Chakarov *et al.*, 2013; Peterson *et al.*, 2013).

Despite the potential of these candidate genes to reflect, at least partially, the genetic basis of phenological traits related to reproduction, our understanding of their importance in natural populations is still limited for several reasons. First, apart from the study by Chakarov *et al.* (2013) on common buzzards, the four candidate genes have not been studied in the same population. Second, there are important discrepancies among studies in terms of sample sizes, reducing the detection probability of small to intermediate gene effect sizes (Manolio *et al.*, 2009). Also, previous studies generally focussed on female-specific analyses despite the potential importance of male genetic effects on phenological traits (e.g. Teplitsky *et al.*, 2010). Finally and importantly, despite some evidences of gene-environment interactions (i.e. GxE) being present across populations, very few studies assessed GxE within a population (but see Liedvogel *et al.*, 2009 and Liedvogel & Sheldon, 2010). At the individual-level, the presence of GxE could explain the lack of relationships between candidate genes and phenological traits documented in previous studies.

Here, we used four years of data from a Tree swallow (*Tachycineta bicolor*) long-term study in southern Québec (Canada) to investigate the relationship between length polymorphism at all four candidate genes (CLOCK, NPAS2, ADCYAP1 and CREB1) and phenological traits related to reproduction (laying date and incubation duration). Tree swallow is a small migratory passerine and while laying date for this species has advanced in North America during the last decades (Dunn & Winkler, 1999; Bourret *et al.*, 2015), we still know little about the underlying genetic basis of this trait. For example, a single study in this species analysed CLOCK variation in females within a population based in Ithaca (NY, USA) – no relationship was found between length polymorphism and laying date or incubation duration (Dor *et al.*, 2012). In this study, our objectives were to 1) describe variation at the four candidate genes in the southern Québec population, 2) assess the geographic and environmental variations in the genes and 3) examine the relationships between variation at

these genes for both males and females and phenotypic variation in laying date and incubation duration, while determining the importance of GxE in this system.

Methods

Study system and data collection

The study system in southern Québec (Canada) covers an area of 10,200 km^2 and includes 400 nest boxes equally distributed within 40 farms (Fig. 3.1; see Ghilain & Bélisle, 2008 for more details on the study system). Between 2010 and 2013, each nest box was visited every two days during the reproductive season to record nest box occupation, laying date of the first egg, incubation initiation and hatching date. Incubation duration was calculated as [hatching date - incubation initiation date] and was highly correlated with the incubation period defined from temperature variation obtained from thermocrons placed within a subset of nest boxes in 2013 (N=34, r=0.88, P<0.001; see Appendix A). Birds were individually identified with an aluminium band (US Fish and Wildlife Service) and females were assigned to an age class, second-year (SY) or after-second-year (ASY), based on feather colour (brown or blue-green, respectively; Hussell, 1983). DNA was extracted using a salt extraction method from blood samples collected from a brachial vein on a filter paper (Aljanabi & Martinez, 1997; Porlier *et al.*, 2009) and its quality and concentration was determined by electrophoreses on 1% agarose gel. The sex of each individual was confirmed with a molecular technique following Lessard *et al.* (2014). Meteorological data were extracted from 10 meteorological stations located within the study area (Environment Canada, http://meteo.gc.ca/). Time periods showing the strongest correlations with temperature were different for laying date (April 6 – May 9, Bourret *et al.*, 2015) and incubation duration (May 18 – June 8, Appendix A), and are referred hereafter to April and May temperatures, respectively. We only considered first breeding attempts in our analyses, i.e. first reproductive event that occurred in a nest box and first record of breeding attempt of both female and social male (if known) within a reproductive season (N=847, see Table S3.B1 for details on sample sizes).

Table 3.1 Summary of individual-based studies assessing relationships between candidate gene polymorphisms (CLOCK, NPAS2, ADCYAP1 and CREB1) and phenotypic variation at phenological traits related to reproduction. Number of years and individuals used (for both sexes if known), presence of a significant relationship (and the direction if significant) and of gene-by-environment (GxE) interactions (YES: tested and significant; NO: tested and nonsignificant; -: not tested) are reported.

Trait	Species	Localization	Gene	N Years	N individuals (F/M)	Relationship (direction)	GxE	Reference
Laying date	Barn swallow (*Hirundo rustica*)	Milano, Italy	CLOCK	4	922 (478/444)	YES (+)	-	Caprioli *et al.*, 2012
	Blue tit (*Cyanistes caeruleus*)	Wytham Woods, Oxfordshire, UK	CLOCK	2	950 (539/411)	YES (+)	NO	Liedvogel *et al.*, 2009
	Chilean swallow (*Tachycineta meyeni*)	Ushuaia, Argentina	CLOCK	3	88 (88/-)	NO	-	Dor *et al.*, 2012
	Great tit (*Parus major*)	Wytham Woods, Oxfordshire, UK	CLOCK	5	521 (521/-)	NO	NO	Liedvogel & Sheldon, 2010
	Mangrove swallow (*Tachycineta albilinea*)	Hill Bank, Belize	CLOCK	3	163 (163/-)	NO	-	Dor *et al.*, 2012
	Pied flycatcher (*Ficedula hypoleuca*)	La Hiruela, Spain	CLOCK	1	42 (26/16)	NO	-	Kuhn *et al.*, 2013
	Tree swallow (*Tachycineta bicolor*)	Ithaca, NY, USA	CLOCK	9	548 (548/-)	NO	-	Dor *et al.*, 2012
	Violet-green swallow (*Tachycineta thalassina*)	Mono Lake, CA, USA	CLOCK	2	48 (48/-)	NO	-	Dor *et al.*, 2012
	White-rumped swallow (*Tachycineta leucorrhoa*)	Chascomús, Argentina	CLOCK	2	169 (169/-)	NO	-	Dor *et al.*, 2012

Trait	Species	Localization	Gene	N Years	N individuals (F/M)	Relationship (direction)	GxE	Reference
Hatching date	Blue tit (*Cyanistes caeruleus*)	Wytham Woods, Oxfordshire, UK	CLOCK	2	950 (539/411)	YES (+)	NO	Liedvogel *et al.*, 2009
	Great tit (*Parus major*)	Wytham Woods, Oxfordshire, UK	CLOCK	5	521 (521/-)	NO	NO	Liedvogel & Sheldon, 2010
Incubation duration	Blue tit (*Cyanistes caeruleus*)	Wytham Woods, Oxfordshire, UK	CLOCK	2	950 (539/411)	YES (+)	NO	Liedvogel *et al.*, 2009
	Great tit (*Parus major*)	Wytham Woods, Oxfordshire, UK	CLOCK	5	521 (521/-)	NO	NO	Liedvogel & Sheldon, 2010
	Mangrove swallow (*Tachycineta albilinea*)	Hill Bank, Belize	CLOCK	3	163 (163/-)	NO	-	Dor *et al.*, 2012
	Tree swallow (*Tachycineta bicolor*)	Ithaca, NY, USA	CLOCK	9	548 (548/-)	NO	-	Dor *et al.*, 2012
	Violet-green swallow (*Tachycineta thalassina*)	Mono Lake, CA, USA	CLOCK	2	48 (48/-)	NO	-	Dor *et al.*, 2012
	White-rumped swallow (*Tachycineta leucorrhoa*)	Chascomús, Argentina	CLOCK	2	169 (169/-)	NO	-	Dor *et al.*, 2012
Timing of broods *	Common buzzard (*Buteo buteo*)	Eastern Westphalia, Germany	CLOCK	11	479†	-‡	-	Chakarov *et al.*, 2013
			NPAS2	11	479†	NO		
			ADCYAP1	11	479†	NO	-	
			CREB1	11	479†	NO	-	

* Timing of broods reflects timing of fledglings within a brood compared to the timing of fledglings in other broods within the same year

† Genotypes were defined as the average of nestling genotypes within a nest (N=976), thus reflecting both male and female genotypes

‡ CLOCK was monomorphic and thus no further analysis was made

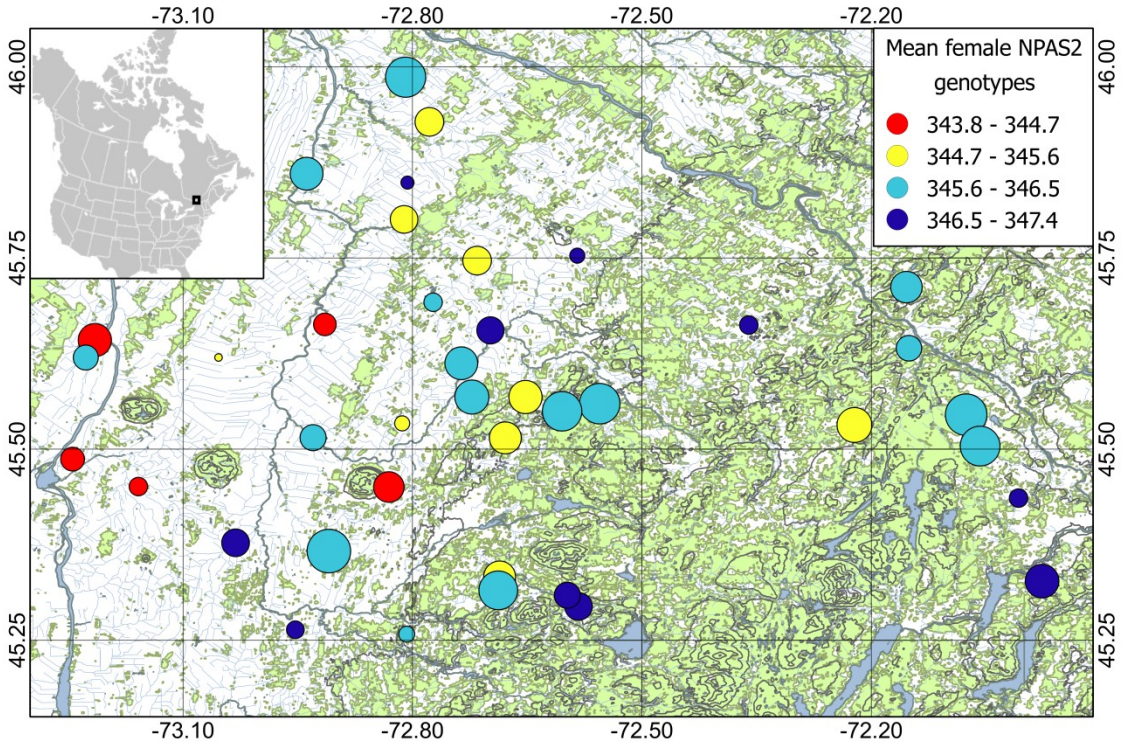

Figure 3.1 Mean female NPAS2 genotypes observed on the 40 farms (coloured circles, see legend) in the study system in southern Québec, Canada. Number of females observed between 2010 and 2013 is represented by different circle sizes (range: 2 – 45). Forest patches (green), rivers and lakes (blue), other land uses (mostly agriculture; white), elevation (100-m gray isolines), latitude and longitude (in decimal degrees; thin black lines) are also represented. This figure was produced with QGIS 2.0 (QGIS Team Development, 2013).

Candidate gene analyses

PCR conditions for CLOCK amplifications were performed following Johnsen *et al.* (2007) and for ADCYAP1, CREB1 and NPAS2 following Steinmeyer *et al.* (2009) (details can be found in Table S3.B2). We redesigned CREB1 reverse primer (5'-AGAATAACGCAGCC

CAGAGC-3') with Primer-BLAST (Ye *et al.*, 2012) to shorten the PCR product length from ~550 to ~280 base pairs and thereby eased PCR amplifications as well as fragments migration and visualization. PCR products were resolved on an AB3130xl automated DNA sequencer and allele lengths were established using GeneMapper 4.1 (Applied Biosystems, Foster City, CA, USA). Between 3 and 11 PCR products of each candidate genes (CLOCK: 3; NPAS2: 4; ADCYAP1: 11; CREB1: 7) were sent to a sequencing platform (Centre de recherche du CHUL/CHUQ, Québec, Canada; http://www.sequences.crchul.ulaval.ca) to assess the concordance between targeted genes and PCR products. CLOCK, NPAS2 and CREB1 showed sequences highly similar (>98% identical) to those previously published (Johnsen *et al.*, 2007; Steinmeyer *et al.*, 2009). However, as already reported by Steinmeyer *et al.* (2009), ADCYAP1 showed an increase of a single base pair between alleles outside of the repeat regions and thus was corrected to reflect the di-nucleotide repeat increase. A total of 60 individuals were replicated from DNA extraction to alleles scoring (6.5% of all individuals) to assess error rate, which was 1.9% on average for all loci (range: 0.0%−3.3%).

Deviations from Hardy-Weinberg equilibrium and heterozygosity were checked for all four candidate genes at different grouping levels (within years, sexes and age classes) with GenePop 4.0 (Raymond & Rousset, 1995; Rousset, 2008). Individuals observed more than once were randomly chosen in a single year to avoid pseudo-replication in these analyses. An AMOVA (analysis of molecular variance) was also conducted using Arlequin 3.5 (Excoffier & Lischer, 2010) to assess levels of differentiation among years and farms. An individual genotype at a given locus was defined as the sum of allele lengths to represent the additive effect of each allele. This definition of an individual genotype was used because it reflects the suspected effect of STR polymorphism within functional genome regions (Elmore *et al*. 2012) and it is more powerful statistically than defining distinct factors for each pair of alleles observed (see also Liedvogel *et al.* 2009 and Mueller *et al.* 2011 for the rationale behind this method and a comparison between different genotype definitions). Intra-individual correlations between genotypes at each locus, in both males and females, were assessed using Spearman's rank correlation.

Genetic variation distribution

To assess how genetic variation distribution was related to environmental and spatial variation, we used two approaches. First, we used a linear model implemented in the software R (R Core Team, 2014) to examine the relationship between individual genotypes and environmental components known to influence laying date and/or incubation duration in the study population (laying date: latitude, April temperature and breeding density (Bourret *et al.*, 2015); incubation duration: May temperature and longitude (Appendix A)). To avoid pseudo-replication, individuals were included only once in the analysis and explanatory variables were averaged for individuals observed in more than one year (N=220 individuals: 130 females and 90 males). The full model included latitude and longitude (decimal degree), breeding density (% of occupied nest boxes on each farm), temperature (°C), sex and all two-way interactions with sex as explanatory variables. As April and May temperatures were highly correlated within years (r>0.83), we decided to average these values to a mean temperature. Year was not included in this model since there was no difference in genetic structure among years (see AMOVA results). All explanatory variables were standardized (zero mean, unit variance; Table S3.B3) and the final model was determined by sequentially removing the least significant term from the model based on its *P*-value until all remaining variables were significant (α=0.05) (Crawley, 2007). In the second approach we looked for evidence of spatial autocorrelation in allele-frequency distribution with GenAlEx (Peakall & Smouse, 2012). We computed autocorrelation coefficients (r) for 10 distance classes of 10 km (covering important distance classes between farms; minimum: 1.9 km, maximum: 103.1 km, mean (S.D.): 42.2 (21.1) km) in three datasets: females only, males only and all individuals. Two-tailed 95% confidence intervals were obtained based on 999 permutations. Individuals observed on more than one farm (N=26, less than 3% of all individuals) were randomly assigned to a single location.

Genetic variation can also be non-randomly distributed between mating pairs. To assess the presence of non-random mating, we computed the distribution of pairwise genetic relatedness estimator (R_{XY}, Lynch & Ritland, 1999) between all observed mating pairs (N=485 pairs) for

all candidate genes separately (see Mainguy *et al.*, 2009). These distributions were compared with Mann-Whitney *U* tests to those of all possible male-female pairs within years (N=89,325 pairs), and differences between distributions would suggest non-random mating.

Reproductive parameters and genotypic variations

Laying date, incubation duration and hatching date are important reproductive parameters potentially correlated with variation in candidate genes (Table 3.1). However, as laying and hatching dates were highly correlated (r=0.96, P<0.001), we restricted our analyses to laying date and incubation duration (not correlated, r=-0.04, P=0.41). Using linear mixed models, we assessed the relationship between candidate genes and both laying date and incubation duration. As both females and social males showed significant adjusted repeatability for laying date (0.320 and 0.181, respectively) and incubation duration (0.195 and 0.070, respectively; Appendix C for more details on repeatability), only clutches with both parents known and genotyped were included in these analyses to disentangle their genotypes relative impact on these traits (Table S3.B1 for sample size of each analysis). Full models included as fixed effects: male and female genotypes (continuous), female age class (SY or ASY), relevant environmental variables (same variables as described above plus longitude from the genetic variation distribution analysis, see Results) and all two-way interactions between female age class or environmental covariates and genotypes (except for female age class and CREB1 as some genotype-age class pairs were not observed) to test GxE and genotype-age interactions. Female identity, male identity and year were included as random effects. Explanatory variables were standardized (zero mean, unit variance) and analyses were performed using lme4 package (Bates *et al.*, 2014) in R. We determined the final models by backward variable selection as explained previously and when a GxE interaction was included in a final model, the main effect of the concerned candidate gene was also assessed from a model without the interaction.

Results

Allelic and genotypic variation

We successfully genotyped more than 98.8% of the 925 breeders (554 females, 371 males) captured between 2010 and 2013 (Table 3.2). From the 4 alleles observed at CLOCK, the Q_8 allele (allele 182) was most frequent (61.6%), a result similar to the observation of Dor *et al.* (2012) in another tree swallow population. NPAS2 carried 7 different alleles in our study system (most frequent allele: 70.9%), ADCYAP1 was the most polymorphic candidate gene with an observed heterozygosity of 0.825 (13 alleles; most frequent allele: 24.0%) and CREB1 was the least polymorphic candidate gene tested with the most frequent allele accounting for 96.7% of allelic diversity and an observed heterozygosity of 0.064. None of the candidate gene overall allele frequencies deviated from Hardy-Weinberg equilibrium (P>0.39), neither within years (P>0.13) nor in female age classes (P>0.19). However, a closer look within sexes suggested a deviation in males at NPAS2 (F_{IS}=-0.019, P=0.042) that was not significant after Bonferonni correction for multiple comparisons.

Table 3.2 Characteristics of candidate genes analysed. Sample size genotyped (N), number of observed alleles (N alleles) and range, number of observed genotypes (N genotypes) and range, and observed heterozygosity (*Ho*) for adult tree swallows in this study. A genotype is defined as the sum of observed alleles within an individual.

Candidate gene	N	N alleles	Alleles range	N genotypes	Genotypes range	*Ho*
CLOCK	921	4	176 – 185	6	358 – 370	0.507
NPAS2	921	7	162 – 186	9	333 – 359	0.453
ADCYAP1	914	13	164 – 188	19	336 – 372	0.825
CREB1	921	3	261 – 265	3	524 – 528	0.064

CLOCK genotypes were weakly correlated with NPAS2 (r_s=0.092, P=0.041), ADCYAP1 (r_s=-0.114, P=0.011) and CREB1 (r_s=0.099, P=0.027) in females, however all results were not significant after Bonferroni corrections. No other correlations among pairs of genotypes for female or for males were significant (all P>0.21). Finally, in the AMOVA, more than 99% of the total genetic variance was due to individual differences, suggesting no genetic structure among years or farms.

Environmental effects on genetic variation distribution

The final model suggested an effect of the interaction between sex and longitude on the genotypic distribution of NPAS2 (Sex X Longitude: β=-0.428±0.203, t=2.11, P=0.035). A closer examination within each sex revealed a positive significant relationship with longitude in females (β=0.265±0.124, t=2.13, P=0.034; Fig. 3.1) but not in males (β=-0.164±0.163, t=1.00, P=0.32). No relationships between genotypic and environmental variations were observed for CLOCK, ADCYAP1 and CREB1. Spatial autocorrelation analyses revealed no spatial structure in any of the candidate gene allele distributions (Fig. S3.D1). Pairwise genetic relatedness between observed and random mating pairs showed no significant different distributions for all genes (all P>0.06; Fig. S3.D2).

Genotypic and environmental effects on reproductive parameters

Laying date was a function of the polymorphism at three candidate genes (Fig. 3.2a-d, Table 3.3). First, laying date showed a positive relationship with CLOCK female genotypes in interaction with breeding density – with a steeper slope at higher density (Fig. 3.2a) – and a positive relationship with CLOCK male genotypes, albeit marginally nonsignificant (P=0.084, Fig. 3.2b). As for CLOCK model, an interaction between NPAS2 female genotypes and breeding density was kept in the final NPAS2 model (Fig. 3.2c). However, the relationship with laying date in this case seemed null at higher density but turned positive at lower density. Finally, ADCYAP1 female genotypes also showed a relationship with laying date, but this time in interaction with latitude – with a negative slope at lower latitude turning positive at

higher latitude (Fig. 3.2d). None of the variables were kept in the final CREB1 model (Table D3.4). Main effects of CLOCK (β=0.510±0.294, t=1.74, *P*=0.08), NPAS2 (β=0.355±0.291, t=1.22, *P*=0.22) and ADCYAP1 (β=0.106±0.292, t=0.36, *P*=0.72) female genotypes were all non-significant.

Table 3.3 Final linear mixed models analyses of laying dates for a) CLOCK, b) NPAS2 and c) ADCYAP1 male and female genotypes. Female age class (SY or ASY) and environmental variables were included as fixed effects and tested for interactions with breeder genotypes. Year, female identity and male identity were included as random effects and all explanatory variables were standardized. None of the variables were kept in the final CREB1 model. Full models can be found in Appendix D.

Models	**Variables**	**Estimates**	**S.E.**	**t-value**	***P*-value**
CLOCK	Intercept	138.919	0.877	158.39	<0.001
	Density	-1.252	0.297	4.22	<0.001
	CLOCK male	0.493	0.287	1.72	0.087
	CLOCK female	0.556	0.296	1.88	0.061
	CLOCK female X Density	0.659	0.295	2.24	0.026
NPAS2	Intercept	138.957	1.216	114.29	<0.001
	Density	-1.136	0.295	3.86	<0.001
	NPAS2 female	0.309	0.290	1.07	0.29
	NPAS2 female X Density	-0.700	0.280	2.50	0.013
ADCYAP1	Intercept	138.935	0.873	159.17	<0.001
	Latitude	0.372	0.306	1.22	0.23
	ADCYAP1 female	0.103	0.293	0.35	0.73
	ADCYAP1 female X Latitude	0.697	0.316	2.21	0.028

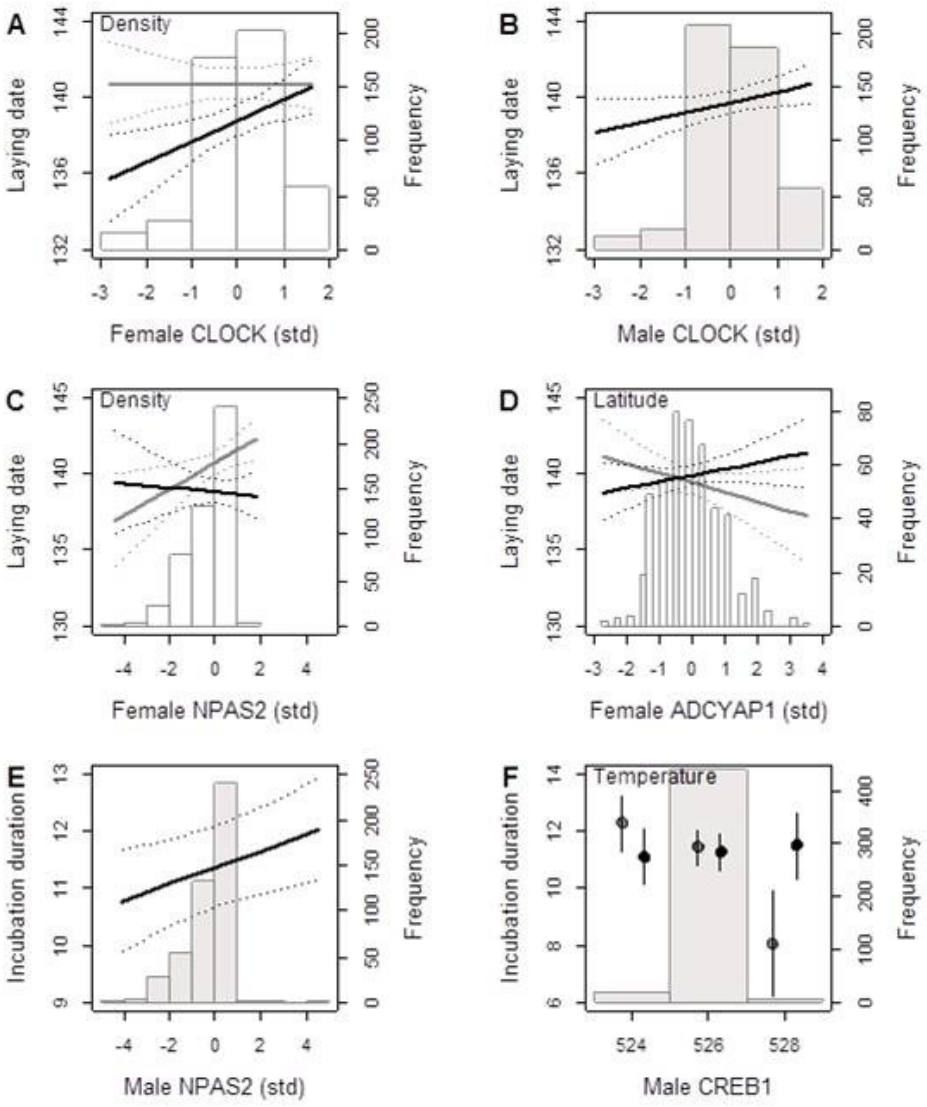

Figure 3.2 Predictions from the linear mixed models of tree swallow laying date (Julian days; A-D) and incubation duration (days; E-F) correlates with candidate gene genotypes (CLOCK: A, B; NPAS2: C, E; ADCYAP1: D; CREB1: F). Interactions with breeding density (A, C), latitude (D) and May temperature (F) are presented for the first (gray) and third (black) quartile of environmental values. Genotype frequency histograms for male (gray) or female (white) and 95% confidence intervals of predictions (from models with year included as a fixed effect) are also presented on each panel.

Table 3.4 Final linear mixed models analyses of incubation duration for a) NPAS2 and b) CREB1 male and female genotypes. Female age class (SY or ASY) and environmental variables were included as fixed effects and tested for interactions with breeder genotypes. Year, female identity and male identity were included as random effects and all explanatory variables were standardized. None of the variables were kept in the final CLOCK and ADCYAP1 models. Full models can be found in Appendix D.

Models	**Variables**	**Estimates**	**S.E.**	**t-value**	***P*-value**
NPAS2	Intercept	11.323	0.360	31.43	<0.001
	NPAS2 male	0.144	0.063	2.28	0.023
CREB1	Intercept	11.301	0.313	36.14	<0.001
	Temperature	-0.101	0.094	1.07	0.29
	CREB1 male	-0.109	0.062	1.76	0.079
	CREB1 male X Temperature	0.218	0.058	3.75	<0.001

Incubation duration varied as a function of male genotypes at two candidate genes (Fig. 3.2e-f; Table 3.4). NPAS2 male genotypes showed a positive relationship with incubation duration, with 1.2 day difference for most distant genotypes (Fig. 3.2e). CREB1 male genotypes in interaction with May temperature showed a negative relationship with incubation duration (Table 3.4; this last model was refitted with CREB1 genotypes defined as factorial variables and a similar interaction was observed, Fig. 3.2f), but the main effect was not significant after removing the GxE interaction (β=-0.076±0.621, t=1.22, *P*=0.22). No significant relationships between incubation duration and genotypes were found for CLOCK and ADCYAP1 models, although a marginally non-significant effect was observed for the interaction between CLOCK male genotype and both longitude (*P*=0.06) and May temperature (*P*=0.08) (Table S3.D5). Full models for laying date and incubation duration are detailed in Appendix D.

Discussion

We investigated the relationship between four candidate genes (CLOCK, NPAS2, ADCYAP1 and CREB1) and two phenological traits related to reproduction: laying date and incubation duration. We used four years of data for males and females and included in our statistical analysis gene-environment interactions (GxE) to account for potential confounding environmental effects. We observed relationships between length polymorphism at all candidate genes and laying date and/or incubation duration. Most of these relationships were affected by environmental variables (breeding density, latitude or temperature), emphasizing the presence and importance of GxE in our study system and its potential role in explaining divergent results among previous studies (see references in Table 3.1).

Polymorphism and spatial variation at candidate genes

Number of alleles and heterozygosity observed for CLOCK, NPAS2 and ADCYAP1 genes in this study were similar to those reported in other bird species (see Appendix E for a review of allelic diversity reported in other studies). Notably, CLOCK allele frequencies previously reported in a tree swallow population in Ithaca (NY, USA) by Dor *et al.* (2012) were almost identical to those observed in our study. In contrast, CREB1 number of alleles and heterozygosity were lower in our study system (N=3 alleles, *Ho*=0.064) than in most previous reports from other species (Table S3.E1, N=6−10 alleles, *Ho*=0.267−0.300), the only exception being the study by Chakarov *et al.* (2013) on raptors (N=1−3 alleles, *Ho*=0.093).

The presence of genetic variation in relation to space and/or environmental components can indicate underlying evolutionary processes, but also functional roles when observed at candidate genes (Fitzpatrick *et al.*, 2005). For example, latitudinal clines observed in CLOCK allele lengths suggested local adaptations to the photoperiodic gradient in some species and thus a functional role for its length polymorphism (e.g. Johnsen *et al.*, 2007; O'Malley & Banks, 2008; reviewed in Kyriacou *et al.*, 2008). Here, we did not observe a latitudinal cline in CLOCK genotypes within our study system. Also, the similarity of our population with the

tree swallow population in Ithaca in terms of CLOCK allele frequencies despite a latitudinal distance of approx. 3° suggests an absence of latitudinal cline at larger spatial scale for this species. However, the longitudinal cline observed for NPAS2 female genotypes could be in part linked to the genetic basis of timing of migration. A previous study using microsatellites in the same system found no strong genetic structure in space, but still a tendency for more genetically similar individuals to be more geographically distant, an observation that is contrary to any spatial cline or isolation by distance patterns (Porlier *et al.*, 2009). The pattern of allele distribution observed at NPAS2 was thus different from the pattern observed at putatively selectively neutral microsatellite loci, which suggests an adaptive role to the observed NPAS2 cline. Furthermore, the same study by Porlier *et al.* (2009) showed that settlement dates in nest boxes were positively correlated with farm distance to the St. Lawrence River, itself highly correlated with longitude (r=0.90), revealing a possible migration route from West to East within the study area. Taken together with our results, these observations suggest that earlier settlement dates could be related to shorter NPAS2 female genotypes.

Laying date vs candidate genes

We found a positive relationship between CLOCK female genotypes and laying date, which supports the results previously reported in blue tits and barn swallows of earlier laying dates at smaller allele length (Liedvogel *et al.*, 2009; Caprioli *et al.*, 2012). However, in our case, we found evidences of GxE as this relationship was influenced by breeding density with a steeper slope at higher densities. Our result also contrasts with those obtained in the Ithaca tree swallow population by Dor *et al.* (2012) where the relationship between CLOCK female genotypes and laying date was nonsignificant (β=0.494±0.612, $F_{1,462}$=0.65, P=0.42, Dor *et al.*, 2012) despite similar sample size. In that previous study, however, GxE was not considered which may explain the discrepancy. In fact, when applying the statistical model used by Dor *et al.* (2012) (i.e. linear mixed model with age and year as fixed effects, female identity as random effect and female genotype defined as CLOCK poly-Q average allele size) to our

dataset, we also found a non-significant relationship between CLOCK female genotypes and laying date (β=1.033±0.672, t=1.54, *P*=0.13).

The relationships between timing of reproduction and NPAS2, ADCYAP1 or CREB1 were previously tested only once in birds, in a study on the common buzzard that found no significant associations (Chakarov *et al.*, 2013, Table 3.1). To our knowledge, we thus provide the first evidence of relationships between variation at NPAS2 and ADCYAP1 and laying date in birds. Similarly to the results obtained for CLOCK, the relationship between NPAS2 female genotypes and laying date was affected by breeding density, but in a different fashion. In fact, while for CLOCK the laying date–genotype relationship was steeper at higher breeding density, for NPAS2 the relationship was steeper at lower breeding density. Despite the fact that CLOCK and NPAS2 are paralogs and have partially overlapping functions within the circadian system (Debruyne, 2008), they may not be affected in the same manner by a given environmental variable – which emphasizes the importance of considering several genes when assessing GxE.

We also found a correlation between ADCYAP1 female genotypes and laying date in interaction with latitude. In previous studies, ADCYAP1 longer genotypes were associated to greater migratory restlessness (blackcaps (*Sylvia atricapilla*), Mueller *et al.*, 2011; Oregon juncos (*Junco hyemalis thurberri*), Peterson *et al.*, 2013) and to a tendency to disperse earlier in the season (common buzzards, Chakarov *et al.*, 2013). In line with the NPAS2 cline reported here, we could speculate that the latitudinal difference found for the laying date-ADYCAP1 genotype relationship in females is linked to spatial variation in migratory patterns, but this should be further investigated. Again, for both NPAS2 and ADCYAP1, conducting analyses without including GxE effects would have resulted in non-significant relationships, emphasizing the importance of environmental interactions in the relationships observed here.

Finally, it is worth noting that the relationship observed between laying date and CLOCK male genotypes, despite being marginally nonsignificant, was similar in direction and effect

size to the equivalent relationship in females. This result is concordant with the small repeatability observed for this trait in males (Appendix C). The male component of genetic variation was rarely taken into account in previous studies of candidate gene variation effects on timing of reproduction (i.e. Table 3.1). This is somewhat surprising given the moderate male repeatability and/or heritability documented for laying date in some bird species (e.g. mute swans (*Cygnus olor*), Charmantier *et al.*, 2006 and Auld *et al.*, 2013; common gulls (*Larus canus*), Brommer & Rattiste, 2008; tawny owls (*Strix aluco*), Brommer *et al.*, 2015). In red-billed gulls (*Larus novaehollandiae*), for example, significant additive genetic variance component and non-zero heritability for laying date were reported in males (Teplitsky *et al.*, 2010). The authors suggested that this effect was due to the male influence on its partner through courtship feeding behaviour prior to the laying of the eggs. Here, the male CLOCK length polymorphism influence on laying date could be explained, for example, by individual differences in arrival date or by differences in capacity to select optimal site for breeding, which could in turn affect their partner timing of breeding.

Incubation duration vs candidate genes

Our knowledge of the genetic architecture underlying incubation duration in birds is minimal. Few studies have investigated the relationship between this trait and polymorphism at candidate genes (and for CLOCK only, see Table 3.1) despite limited but non-zero heritability in one out the three species studied so far (collared flycatchers (*Ficedula albicollis*): female h^2=0.040, Husby *et al.*, 2012; but see Liedvogel *et al.*, 2012 for zero heritability in great tits (*Parus major*) and blue tits). Repeatability, the superior limit of heritability (Boake, 1989; Falconer & Mackay, 1996; but see Dohm, 2002 for some limitations), was significantly different from zero here in both sexes (females: 0.195; males: 0.070; see Appendix C), suggesting potential for non-zero heritability in incubation duration, although a detailed quantitative genetics analysis will be required to verify this assessment. In our analyses, male NPAS2 and CREB1 genotypes were correlated with incubation duration. These results were somewhat unexpected given that the role of male tree swallows during incubation is hypothesized to be negligible: they do not incubate, nor do they feed their mates during this

period (Winkler *et al.*, 2011). However, males participate in nest building and line their nest with feathers, which could indirectly influence incubation duration (Lombardo *et al.*, 1995). Circadian components such as NPAS2 and CREB1 could also be related to male behaviour and indirectly influence female behaviour. For example, in great tits, a trait related to the circadian rhythm, the free-running period length, was suspected of being associated with male reproductive behaviour. This highly heritable trait (h^2=0.86) showed smaller value in extra-pair young than within-pair young (Helm & Visser, 2010), suggesting that extra-pair males had shorter free-running period length than the males they cuckolded. Behavioural influence related to candidate genes could possibly be indirectly acting here and this certainly deserves further investigation.

Importance of GxE

Despite the importance of genotype-environment interactions in evolutionary biology (Via & Lande, 1985; Fitzpatrick *et al.*, 2005; Saastamoinen *et al.*, 2009; Bourret *et al.*, 2013), GxE involving candidate genes have been little studied in the wild in relation to phenological traits related to reproduction (see Table 3.1). In previous avian studies, GxE interactions were only tested for CLOCK in blue tits (Liedvogel *et al.*, 2009) and great tits (Liedvogel & Sheldon, 2010) of Wytham Woods, UK. In these studies, all ecological variables known to influence reproduction timing for these populations (i.e. altitude, oak richness and breeding density) were included but no GxE was detected. The GxE interactions reported at all candidate genes considered in our study suggest that their importance may vary depending on the environmental context and/or species. For example, migratory species, including Tree swallow, are likely to be subjected to selective pressures on their circadian system that are distinct from those affecting resident species such as the Blue tit and Great tit of Wytham Woods (Liedvogel *et al.*, 2011). Flexibility to adjust to local conditions could evolve through GxE in migratory species and this could explain the discrepancy between previous studies and this one.

Importantly, some precautions are needed when interpreting GxE interactions because of potential publication bias favoring significant results, high false-positive rate when assessing multiple comparisons and rare replications of previous findings (Little *et al.*, 2009; Duncan & Keller, 2011). The multiple comparisons problem is particularly important in studies assessing GxE interactions using numerous SNPs because of the massive increasing of type-2 errors that need to be accounted for, but this problem can still hold in this study at a smaller scale since we used four different candidate genes. However, applying a strict Bonferroni correction to our analyses (i.e. reduce the alpha level for a significant *P*-value to 0.013) still suggests the importance of one candidate gene-environmental interaction for laying date and incubation duration (NPAS2 female genotype−breeding density and CREB1 male genotype−May temperature interactions, respectively). Nonetheless, we believe that replicates should be obtained from other tree swallow populations and more species for a better understanding of the importance of GxE involving these phenological candidate genes.

Finally, the detection of relevant GxE interactions is also dependent on the underlying neutral population genetic structure and the validity and reliability of the environmental variables used when testing for such effects. Here, a previous study on this tree swallow population showed no important genetic structure at neutral microsatellite loci (Porlier *et al.* 2009, see *Polymorphism and spatial variation at candidate genes* discussion above), suggesting that observed patterns at candidate genes were not due to spurious associations caused by population structure. Moreover, we only assessed the effect of environment variables known to have a direct influence on the trait of interest or on the genotype spatial distribution to reduce potential bias in our candidate gene analyses (see Little *et al.*, 2009; Saïdou *et al.*, 2014). Indeed, the environmental variables (i.e. breeding density, latitude, and spring temperature) interacting with at least one candidate gene in this study were all previously known to influence bird phenology. First, breeding density was previously identified as the main determinant of laying date in this population and is hypothesized to be a good proxy of environmental quality (Bourret *et al.*, 2015). In fact, differences in habitat quality are thought to lead to aggregation of tree swallows in habitats with more food (Dunn & Winkler 1999), a situation that can be transposed in our nest box system given both the preference of tree

swallows for nest boxes over natural cavities and the presence of empty nest boxes in most farms (reviewed in Shutler *et al.* 2012). As previously reported, lower breeding density farms were associated with later laying dates (see Dunn & Winkler 1999 and Bourret *et al.* 2015), but our results also suggest that the effect of this environmental constraint varied depending on female CLOCK and NPAS2 genotypes. The second environmental variable, latitude, is tightly linked to photoperiod (Dawson, 2013) and can influence laying date even at a small spatial scale (Gienapp *et al.*, 2010; Bourret *et al.*, 2015). However, the antagonistic interaction observed here cannot be easily interpreted given the current sparse knowledge of ADCYAP1 influence on phenological traits in other tree swallow populations and other species. The last interacting environmental variable, spring temperature, influences incubation duration and partly controls male gonad maturation (Dawson, 2008). The GxE interaction observed at CREB1 in males seems due to the presence of the genotype 528, which showed variable incubation duration depending on temperature. This result is even more interesting given the low frequency of the genotype 528 in our population (i.e. 2.1%), which suggests that underlying evolutionary processes such as selection could have affected this particular genotype.

Conclusion

Candidate gene approaches provide complementary information to quantitative genetic studies (Liedvogel *et al.*, 2012) and offer a direct window on the potential for evolutionary changes. Most phenotypic traits are hypothesized to be under the control of numerous genes with small effect sizes (Manolio *et al.*, 2009). As a result, the power to detect relationships between phenotypes and variation at candidate genes rely on gene effect sizes, heritabilities, allele frequencies and sample sizes (Manolio *et al.*, 2009; Liedvogel *et al.*, 2012; Saïdou *et al.*, 2014). In the wild, variable environmental conditions and interacting ecological and genetic components complicate candidate gene studies. Despite all these constraints, we managed to document relationships between variation at four candidate genes and phenological traits and provided evidences of GxE. Altogether, our results suggest that CLOCK, NPAS2, ADCYAP1

and CREB1 can be good candidate genes to monitor and to predict future adaptation to changing environmental conditions if the environmental context in which they are expressed is taken into account.

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Conflict of interest

None declared.

Data accessibility

Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.f7t25.

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Chapitre 4

PRÉCISION ET BIAIS

Description de l'article et contribution

Le modèle animal est un outil formidable pour répondre à de nombreuses questions en biologie évolutive. Par contre, pour adéquatement y parvenir, le modèle animal nécessite beaucoup de données et un pédigrée de qualité. Cet article se voulait donc un prérequis essentiel au chapitre suivant afin de connaitre les limites du jeu de données disponible. À l'aide à la fois de données empiriques et simulées, cet article fait ressortir les problèmes liés à la faible connectivité dans le pédigrée utilisé, à la surparamétrisation des modèles et à l'utilisation d'un pédigrée social chez une espèce monogame socialement avec un fort taux de reproduction hors couple.

L'idée de cet article a germé lors de ma première réunion de conseillers, à la suite d'une suggestion de Fanie Pelletier d'intégrer une analyse de puissance à ma thèse comme préalable aux chapitres utilisant une approche par génétique quantitative (ils étaient au nombre de deux alors). J'ai peaufiné l'élaboration des idées avec Dany Garant. J'ai construit les pédigrées, effectué les analyses en génétiques quantitatives et j'ai bâti le code R des simulations à partir d'un premier code fournis par Melody Porlier. J'ai également participé à la collecte de donnée sur le terrain au cours de trois saisons d'échantillonnage (2012 à 2014). Dany m'a initié aux analyses en génétique quantitative et a révisé plusieurs versions du manuscrit.

An assessment of the reliability of quantitative genetics estimates in study systems with high rate of extra-pair reproduction and low recruitment

en révision pour le journal *Heredity*

Audrey Bourret and Dany Garant

Abstract

Quantitative genetic approaches, and particularly animal models, are widely used to assess the genetic architecture of key fitness related traits and infer adaptive potential of wild populations. Despite the importance of precision and accuracy of genetic variance estimates and their potential sensitivity to various ecological and population specific factors, their reliability is rarely tested explicitly. Here we used simulations and empirical data collected from an 11-year study on Tree swallow (*Tachycineta bicolor*), a species showing a high rate of extra-pair paternity and a low recruitment rate, to assess the importance of identity errors, structure and size of the pedigree on quantitative genetic estimates. Our simulations revealed an important lack of precision in heritability and genetic correlation estimates for most traits, a low power to detect significant effects and important identifiability problems. We also observed a large bias in heritability estimates when using the social pedigree instead of the genetic one (deflated heritabilities) or when not accounting for an important cause of resemblance among individuals (e.g. permanent environment or brood effect) in model parameterizations for some traits (inflated heritabilities). We discuss the causes underlying the low reliability observed here and why they are also likely to occur in other study systems. Altogether, our results re-emphasize the difficulties of generalizing quantitative genetic estimates reliability from one study system to another and the importance of reporting simulation analyses to evaluate these important issues.

Key words: animal models, extra-pair paternities, quantitative genetics, pedigree errors, pedigree simulation, power

Introduction

Understanding the genetic architecture underlying important fitness related traits is essential to infer the adaptive potential of wild populations (Lynch and Walsh, 1998; Hendry *et al.*, 2011). However, since most traits are likely under the control of numerous genes with small effects (Lande, 1981; Falconer and Mackay, 1996; Husby *et al.*, 2015), potentially interacting with the genetic components of other traits (Lande and Arnold, 1983; Blows and Hoffmann, 2005) and/or with the environment in which they are expressed (Via and Lande, 1985; Hoffmann and Merilä, 1999), this task remains challenging despite rapid advances in whole genome analysis methods (Vinkhuyzen *et al.*, 2013). For these reasons, quantitative genetics approaches – statistical methods using known relationships between individuals to assess the genetic and environmental components of a population phenotypic variance − remain efficient ways to infer the underlying genetic variation of focal traits (Falconer and Mackay, 1996; Kruuk *et al.*, 2008). For this purpose, animal models are particularly suitable for wild population datasets because they allow the use of all relationships between individuals and can account for missing data, unbalanced designs and other potential biases (e.g. environmental causes of phenotypic similarity among individuals rather than genetic ones) (Kruuk, 2004; Kruuk and Hadfield, 2007; Wilson *et al.*, 2010). Moreover, heritability estimates from wild populations obtained with animal models are generally more accurate than those estimated with traditional parent-offspring regressions (reviewed in Postma, 2014).

Several factors will influence the validity of the genetic variance estimates obtained in the wild, which in turn will affect our capacity to infer population responses to selective pressures (e.g. through breeder's or Robertson-Price equation, see Morrissey *et al.*, 2010). Precision – the reproducibility of a measurement – is highly dependent on sample size both in terms of pedigree depth and completeness but also on the complexity of the genetic architecture underlying the focal trait (Morrissey *et al.*, 2007; de Villemereuil *et al.*, 2013). For example, given two pedigrees with identical sample sizes, a pedigree with a weak degree of connectivity between individuals, e.g. in populations with strong natal dispersion and/or immigration resulting in few related individuals, will be less precise than a pedigree with known

relationships between all individuals (Wilson *et al.*, 2010). However, precision is rarely tested *per se*, but rather deducted from the size of standard errors around estimates (e.g. Charmantier and Réale, 2005). Accuracy – the proximity between the estimated value and the true value – is affected mainly by pedigree errors (i.e. erroneous links between individuals in the pedigree; Charmantier and Réale, 2005; Morrissey *et al.*, 2007; Firth *et al.*, 2015) and by model parameterizations (Kruuk and Hadfield, 2007; Wilson, 2008; Wolak *et al.*, 2015). Pedigree errors could be particularly common in study systems where extra-pair copulations are important and parental links in pedigrees constructed solely based on social pair observations (i.e. social pedigree). Theoretically, the presence of extra-pair paternity (EPP), if not accounted for, can bias downward the heritability estimates, even though evidences so far suggest that this bias is generally small in wild populations (Charmantier and Réale 2005; Bérénos et al. 2014; Firth et al. 2015; but see Lee and Pollak 1997 for reports of higher bias in the animal breding litterature).

Despite previous knowledge of what can affect precision and accuracy of genetic variance estimates, extrapolating the reliability of these estimates from one study system to another is difficult given the large diversity of life history observed across species that modified inevitably dataset and pedigree structures. Therefore, simulation analyses were recommended for testing the limits of a particular dataset, pedigree and model to answer specific biological questions (Morrissey *et al.*, 2007; Wilson *et al.*, 2010). Simulation framework allowed the assessment of power – the probability of detecting an effect given that this effect is true – of a particular dataset or model. In animal models, power is affected by the same factors as precision (Quinn *et al.*, 2006; Morrissey *et al.*, 2007; Bérénos *et al.*, 2014). However, performing a power analysis alone could provide an incomplete picture of the validity of genetic variance estimates. In fact, a particular model applied on a dataset could have a high power to estimate a given effect, but the model itself might be missing a critical variance term, leading to precise but inaccurate estimates (Kruuk and Hadfield, 2007). Similarly, even if all important variance terms are included in a particular model, the model variance structure might not allow to discriminate between two variance terms being included, a problem of statistical models related to its low 'identifiability' (Bolker, 2008). Confounded parameters

could be frequent when applying animal models to wild populations (Wilson *et al.*, 2010), but the extent of identifiability problems is still unknown.

Here, we used an 11-year study on Tree swallow (*Tachycineta bicolor*) to assess the effects of identity errors, structure and size of a pedigree on reliability of quantitative genetic estimates. Tree swallows are small migratory passerines and their breeding grounds are widely distributed across North America (Winkler *et al.*, 2011). Similarly to other migrating species, tree swallow shows high natal dispersion, high mortality within the first year and low nestling recruitment rate (Hosner and Winkler, 2007), which reduce kinship among individuals in monitored populations. This socially monogamous species also displays one of the highest rate of EPP documented in birds (Griffith *et al.*, 2002), with more than 80% of nests containing at least one extra pair offspring and overall around 50% of nestlings resulting from extra-pair copulations (Dunn *et al.*, 2001; Lessard *et al.*, 2014). In the context of building a pedigree for quantitative genetics analyses, this means that half of paternal links would be erroneous if using a social pedigree. This represents a higher proportion of EPP than those tested in previous studies to assess their impacts on heritability estimates (e.g. in Charmantier and Réale 2005; Firth et al. 2015), but it is a proportion that will be typically found in other similar study systems (see Griffith *et al.*, 2002 and references therein). Furthermore, reliability of genetic variance estimates for this and other similar species is difficult to predict from previous knowledge since the scarce pedigree structure typical of migratory species could be compensated by the natural half-sib design caused by their high rate of EPP.

In this study, we used a social and a genetic pedigree to estimate and compare genetic additive (co-)variances and heritability of traits differing in their completeness through the pedigree structure. More specifically, we first used empirical data to assess the bias resulting from using a social pedigree over a genetic one. Then, we used simulated data to assess precision and accuracy of quantitative genetic estimates obtained with both pedigrees, as well as power of datasets and models and identifiability among variance terms.

Methods

Study species, system and phenotypic data

Tree swallows breed in tree cavities or nest boxes, they produce only one clutch per year, containing on average 5 eggs and both parents provide care to nestlings (Winkler *et al.*, 2011). Since 2004, we intensively follow their activities during the breeding season through 400 nest boxes equally distributed within 40 farms in an area covering 10,200 km^2 in southern Québec, Canada (detailed in Ghilain and Bélisle, 2008). Nest boxes were visited every two days from May to July to record nest box occupancy and important brood characteristics (e.g. laying date, clutch size, and hatchling date). Adults and nestlings were individually marked with an aluminium band (US Fish and Wildlife Service). Adults were captured directly in their nest box by a trap system, during the incubation period and food provisioning period for females and males, respectively. Morphological measurements of body mass (±0.01 g), non-flattened wing length (±0.5mm) and tarsus length (±0.02 mm) were taken on adult tree swallows during captures. Females were classified based on their plumage colour as second-year (brown) or after-second-year (blue-green) (Hussell, 1983) and a minimal age was determined for all adults based on the year they were first observed in the study system. Nestlings were captured before they fledged at 16 days old (fledged around 18-22 days) to record body mass (±0.01 g), primary length (hereafter wing length; ±0.02 mm) and tarsus length (±0.02 mm). Blood samples of adults and nestlings were collected since 2006 on a qualitative P8 grade filter paper (Fisher Scientific) for further molecular analysis (see below).

To reflect the large differences that can exist between traits in terms of sample size and amount of standing genetic variance, we focused on nine phenotypic traits grouped in three categories: 1) morphological traits, which included wing length, body mass and tarsus length of all adults; 2) reproductive traits, which were restricted to females, and included laying date (i.e. date of the first egg laid), clutch size (i.e. number of eggs laid within a nest box) and incubation duration (defined as hatching date – [laying date + clutch size – 1]); and 3) nestling traits which included wing length, body mass and tarsus length measured at the age of 16 days.

Most traits were measured since the beginning of the research program (i.e. in 2004), but some traits were first measured later (nestling body mass: 2005; nestling wing length: 2006; adult/nestling tarsus length: 2007) creating differences in data completeness among traits (sample size per traits can be found in Table S4.A1).

Molecular analysis and pedigree construction

DNA extraction, molecular sexing and microsatellite data analyses are detailed in Lessard *et al.* (2014). Briefly, DNA was extracted from blood samples following a standard salt-extraction method and DNA concentration was determined by migration on 2% agarose gels with a molecular weight standard. A molecular sexing technique was used to determine nestling sexes and to validate adult field observations. All DNA samples were characterized at six microsatellites loci using an AB3130xl automated DNA sequencer and allele lengths were determined using GeneMapper v4.1 (Applied Biosystems).

We constructed a social pedigree using social male identities (i.e. males caught in nest boxes while feeding the young) and a genetic pedigree using genetic father identities (i.e. males assigned as fathers using genetic analyses – see below). In both pedigrees, dam identities were first determined by female captures during egg incubation and then verified molecularly based on locus mismatches with nestlings (2.1% of broods with 2 females captured within the same nest box, from which only 3.6% were from mixed maternity; 11 nestling genotypes (0.15%) mismatched at more than 2 loci with their social mother genotype). Genetic fathers were determined by parental assignations with CERVUS v3.0.7 (Kalinowski *et al.*, 2007; Lemons *et al.*, 2015) in a three step procedure. First, we proceeded to father assignments each year separately following a method slightly modified from Lessard *et al.* (2014). Candidate fathers considered in analyses included all males captured during a given breeding season within 15 km of the nest box of interest (see Lessard *et al.*, 2014 for the rationale behind this approach), but also all males not captured in a given year but suspected of being present outside of our nest box system (i.e. captured on the same farm on both previous and following years). These assignations were based on a 90% confidence level, assuming a 2% mistyping error rate and

we used the percentage of social fathers captured as the percentage of candidate fathers known (variable among years, range: 64 – 88%). The mean probability of exclusion of a second parent with 6 loci was always larger than 0.99. Secondly, social fathers, when known, were tested for being genetic fathers of offspring in their nest using the likelihood-based approach of Lemons *et al.* (2015). Briefly, we re-ran the parental analysis with the social father as the unique candidate father for a given nestling, and we defined the proportion of sampled fathers as the proportion of nestlings without any locus mismatch with its social father (i.e. the probability of the social male being the true father). We then extracted, for each nestling, the critical LOD score associated with 95% confidence that its social father was not its true father and we compared these scores with those observed in regular parental analyses. Males significantly assigned to nestlings in the initial parental analysis (i.e. step 1) were considered as their genetic fathers. For the remaining nestlings (i.e. without a significant male assignment at step 1), if their social fathers could not be excluded (i.e. step 2), they were considered as their genetic fathers but otherwise no genetic father were assigned to them (see Figure S4.A1 for the exact number of fathers assigned to a nestling at each step).

Summary statistics for both pedigrees were obtained with the package PEDANTICS (Morrissey *et al.*, 2007; Morrissey and Wilson, 2010) in R v3.2.0 (R Core Team, 2015). These statistics were computed for complete pedigrees, but also for pedigrees pruned to contain only informative individuals based on the availability of phenotypic data for each trait (hereafter pruned pedigrees) and are presented for each trait category in Table 4.1 (see also Table S4.A2 for more information on each trait).

Table 4.1 Summary statistics for social and genetic pedigrees, in complete pedigrees (Total) or pruned pedigrees detailed for each trait category (means for all morphological, reproductive and nestling traits) based on data collected between 2004 and 2014 in our study system in southern Québec. Summary statistics for all traits are presented in Table S4.A2.

	Social pedigree				**Genetic pedigree**			
	Total	Morphological	Reproductive	Nestling	Total	Morphological	Reproductive	Nestling
Records	13446	2541	1531	7500	13446	2539	1523	7487
Maternities	10509	116	54	5797	10509	116	54	5797
Paternities	7325	81	36	5292	5656	64	25	4456
Full Sibs	18077	5	3	12315	6811	1	1	5341
Maternal Sibs	47124	9	5	23164	47124	9	5	23164
Paternal Sibs	35452	6	4	22979	29309	4	2	20160
Maternal Grandmothers	381	4	1	270	381	4	1	270
Maternal Grandfathers	277	2	1	221	207	2	1	159
Paternal Grandmothers	465	6	3	360	397	4	3	328
Paternal Grandfathers	315	3	1	260	242	0	0	204

Estimations of quantitative genetic parameters

To estimate additive genetic (co)variances of our focal traits, we used both univariate and multivariate animal models (Kruuk, 2004; Wilson *et al.*, 2010). Fixed effects (e.g. age, sex) were included for some traits based on mixed model analyses detailed in Supporting Information. For adult morphological and reproductive traits, full univariate animal models were constructed as follows:

$$V_P = V_A + V_{PE} + V_Y + V_R \quad (1)$$

and for nestling traits:

$$V_P = V_A + V_{BY} + V_B + V_R \quad (2)$$

where V_P is the phenotypic variance after accounting for fixed effects, V_A is the additive genetic variance, V_{PE} is the permanent environmental effect, V_Y and V_{BY} are the variance among years and among birth years respectively, V_B is the variance among broods and V_R the residual variance. A visual inspection revealed that all traits followed a Gaussian distribution and animal models were resolved with a restricted maximum likelihood method (REML), using both the social and the genetic pedigree. Final animal models were constructed by sequential model-building from a model with only residual variance to more complex ones, with a comparison of models at each step using a likelihood ratio test (LRT; see Table S4.C1-3 for the increasing levels of complexity). Only V_A from the incubation duration models did not significantly improve the model likelihood ($\chi^2 < 0.01$, $P > 0.99$).

We also constructed three multivariate animal models, one for each trait category. For each of them, we first included the same variance terms as for univariate models. However, due to convergence problems when including V_{PE} for morphological and reproductive traits, we decided to use a model without the V_{PE} term on a reduced dataset comprising only one observation per individual (randomly chosen). Moreover, since we observed no V_A for

incubation duration, this trait was not included in the multivariate analysis of reproductive traits. Covariances among traits for each variance components were estimated using unstructured variance models. Significance was tested by comparing a model including covariance estimation to a model where covariance was constrained to be equal to zero using LRT.

We estimated heritability ($h^2 = V_A/V_P$, Falconer and Mackay, 1996) and coefficient of genetic variation ($CV_A = \sqrt{V_A} / \bar{X}$, where $\bar{X}$ is the trait mean, Houle, 1992) for all traits within each analysis. For multivariate analyses we also estimated additive genetic correlations (r_A) between each pair of traits. All animal model analyses were conducted with ASRemL v3.0.5 (VSN International Ltd, Hemel Hempstead, UK). Standard errors (SEs) for variance components and h^2 estimates were computed directly by ASRemL.

Simulation analyses

We simulated three different datasets of phenotypic data within both the social and genetic pedigree structures with different levels of complexity. In all cases, simulated traits were normally distributed among all individuals, with $V_P=1$, $\bar{X}=0$ and h^2 of 0.1, 0.3 and 0.5 for the focal trait. In dataset 1, we simulated phenotypes using a unique observation by individual to reflect the simplest scenario possible. In dataset 2, in addition to h^2, different genetic and environmental correlations between traits were also implemented to simulations to assess the difference in power, precision and accuracy when using multivariate models. More specifically, we simulated phenotypes with h^2 similar as dataset 1 for the focal trait, while fixing h^2 of the two other traits at 0.3, for 3 different values of r_A, 0.1, 0.3 and 0.5, while fixing environmental correlation at 0.3. Finally, in dataset 3, we simulated phenotypic traits with a more complex underlying structure based on equations 1 and 2 to better reflect our empirical dataset. For nestling traits, we simulated phenotypes with $V_Y=0.1$, $V_B=0.3$, while for morphological and reproductive traits we simulated phenotypic values with $V_Y=0.1$, $V_{PE}=0.1$, thus implying multiple observations per individual. Simulations were performed in R, and

breeding values were simulated with the package PEDANTICS. While simulations were performed within total social and genetic pedigree structures, only individuals with complete information in the empirical dataset were kept in these three simulated datasets.

Different animal models were performed on simulated datasets to answer two different questions. First, to check if there was a bias when not accounting for EPP, datasets simulated with the genetic pedigree were resolved with animal models using both the genetic and the social pedigrees (GG and GS analyses, respectively) and were compared. Moreover, to look at inherent differences in reliability caused by pedigree structures, datasets simulated with the social pedigree were analysed using the social pedigree (SS analysis) and were compared to GG analysis. Animal models used for datasets 1 and 2 included only V_A and V_R as variance components (and covariance in dataset 2), while for dataset 3 they included all components described in equations 1 and 2. All these scenarios within each analysis were repeated 100 times for each trait, and animal model analyses were conducted with ASRemL.

Precision and accuracy for h^2 and r_A estimates were checked visually with boxplots (i.e. median for accuracy and distribution of estimates for precision) and also by computing the mean squared error (MSE) for each scenario. MSE is defined as $E\left[\left(\hat{d}-d\right)^2\right]$, where d is the true value (e.g. the simulated parameter of h^2 or r_A) and $\hat{d}$ is the estimated value; a small MSE indicates high precision and accuracy (Bolker, 2008). Root MSE (RMSE) was used to allow a comparison at the scale of the estimates (see also de Villemereuil *et al.*, 2013). For dataset 2 results, only h^2 of the focal trait and the two associated r_A were reported. Power to detect significant h^2 and r_A within each scenario were estimated by computing the proportion of estimates that were 2 times larger than their SE. While only LRTs can be used in formal hypothesis testing, this "rule of thumb" approach is a practical indicator of statistical testing that could be easily integrated to simulation analyses (see also Wilson *et al.*, 2011 for a similar approach). Finally, we also checked the identifiability of variance terms in dataset 3 using Spearman's rank correlation between estimates of phenotypic variance components. In this

case, a low correlation between two components would indicate a high identifiability whereas a high correlation would indicate a low identifiability.

Results

Pedigrees

Our total dataset comprised a total of 13,446 individuals, with 2,839 individuals observed only as adults, 10,472 observed only as nestlings and 135 observed at both states (i.e. recruits). From the field observations, we were able to determine the identity of the social father for 69.2% of nestlings (82.2% since 2006). Parental analyses allowed us to determine the genetic father for 53.3% of nestling (66.3% since 2006). Overall, 49.3% of nestlings successfully genotyped with a known social father were extra pair young (N_{total}=6382).

Pedigree size varied among the type of pedigree and traits (Table 4.1). As expected, the social pedigree always had a higher number of links related to father identities than its genetic counterpart, a difference mainly caused by unsampled males contributing to the genetic pool of nestlings. Nestling traits had a much higher number of links than morphological and reproductive traits, which were both of similar order of magnitude, but with slightly fewer links for the latter (Table 4.1). Pedigree sizes were similar for each trait within a given category (Table S4.A2).

Estimations of quantitative genetic parameters (empirical data)

Variance components, and resulting heritability values, estimated from empirical data varied among traits and also often importantly between pedigrees (social vs. genetic) and between models (univariate vs. multivariate) (Figure 4.1, Table 4.2). Biases in h^2 and CV_A resulting from using social instead of genetic pedigrees were moderate for morphological traits (h^2: -84−-7%; CV_A: -60−-3%), almost null for reproductive traits (all around 0%) and highly

variable for nestling traits (h^2: -19−608%; CV_A: -10−167%). Estimates of r_A obtained with the social and genetic pedigree were similar, except between nestling wing length and body mass where r_A was positive and significantly different from 0 with the social pedigree (r_A = 0.56±0.13, χ^2 = 9.92, P = 0.002), but not with the genetic pedigree (r_A = 0.25±0.19, χ^2 =1.32, P = 0.25; Figure S4.C1).

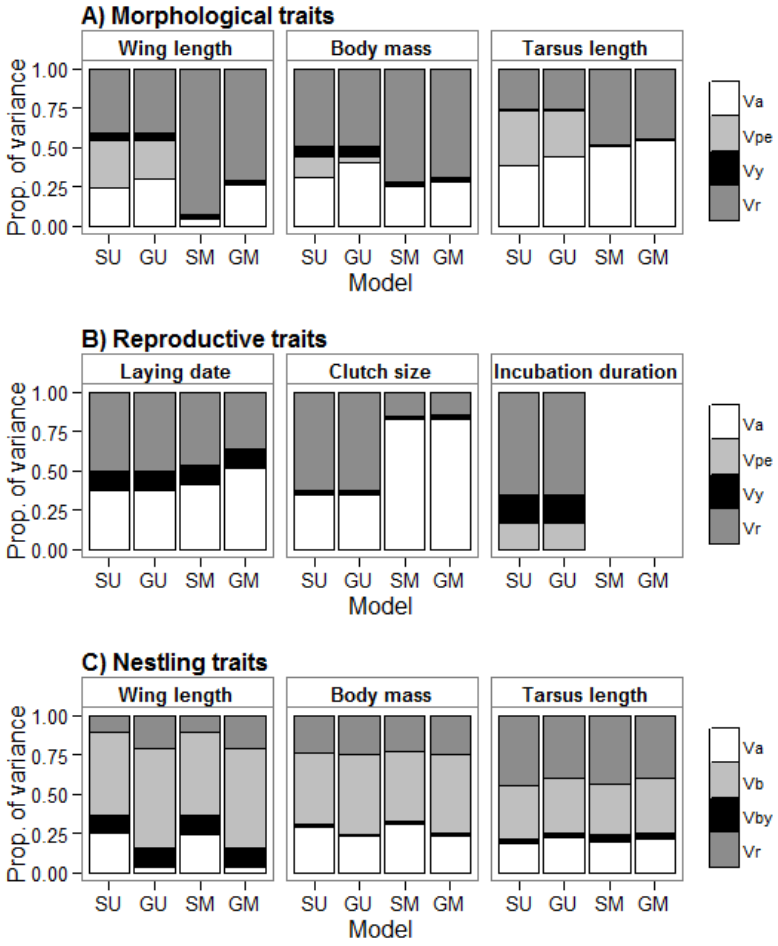

Figure 4.1 Proportion of phenotypic variance estimated from final animal models on empirical dataset, for A) morphological, B) reproductive and C) nestling traits. Different models were assessed for each trait: univariate models using social (SU) and genetic (GU) pedigree, and multivariate models using social (SM) and genetic (GM) pedigree.

Table 4.2 Summary of final A) univariate and B) multivariate animal models using empirical datasets and either the social pedigree or the genetic pedigree. Differences between estimates obtained from the social pedigree relative to the genetic one are presented, with the percentage of difference in parentheses. Standard errors are in parentheses for h^2 estimates. Note that CV_A could not be computed for laying date because of an arbitrary zero for this trait.

	Social pedigree		**Genetic pedigree**		**Difference (%)**	
	h^2	CV_A	h^2	CV_A	h^2	CV_A
A) Univariate models						
Morphological traits						
Wing length	0.24 (0.11)	0.013	0.30 (0.11)	0.015	-0.06 (-20%)	-0.002 (-11%)
Body mass	0.31 (0.10)	0.040	0.40 (0.10)	0.046	-0.09 (-24%)	-0.006 (-12%)
Tarsus length	0.38 (0.13)	0.021	0.44 (0.13)	0.023	-0.06 (-14%)	-0.002 (-7%)
Reproductive traits						
Laying date	0.38 (0.04)	-	0.38 (0.04)	-	0.00 (0%)	-
Clutch size	0.35 (0.03)	0.095	0.35 (0.03)	0.095	0.00 (0%)	0.000 (0%)
Incubation duration	-	-	-	-	-	-
Nestling traits						
Wing length	0.25 (0.06)	0.082	0.04 (0.02)	0.031	0.21 (608%)	0.051 (167%)
Body mass	0.29 (0.08)	0.053	0.23 (0.04)	0.047	0.06 (26%)	0.006 (14%)
Tarsus length	0.18 (0.06)	0.018	0.22 (0.04)	0.019	-0.04 (-19%)	-0.002 (-10%)
B) Multivariate models						
Morphological traits						
Wing length	0.04 (0.18)	0.006	0.26 (0.17)	0.014	-0.21 (-84%)	-0.008 (-60%)
Body mass	0.25 (0.15)	0.035	0.28 (0.16)	0.037	-0.02 (-8%)	-0.002 (-4%)
Tarsus length	0.51 (0.13)	0.025	0.54 (0.13)	0.025	-0.04 (-7%)	-0.001 (-3%)
Reproductive traits						
Laying date	0.41 (0.46)	-	0.52 (0.46)	-	-0.11 (-21%)	-
Clutch size	0.82 (0.48)	0.153	0.83 (0.48)	0.154	-0.01 (-1%)	0.001 (<1%)
Incubation duration	-	-	-	-	-	-
Nestling traits						
Wing length	0.24 (0.06)	0.081	0.04 (0.02)	0.031	0.21(587%)	0.051 (163%)
Body mass	0.31 (0.07)	0.055	0.23 (0.04)	0.047	0.07 (32%)	0.008 (16%)
Tarsus length	0.20 (0.06)	0.018	0.21 (0.04)	0.019	-0.01 (-6%)	-0.001 (-3%)

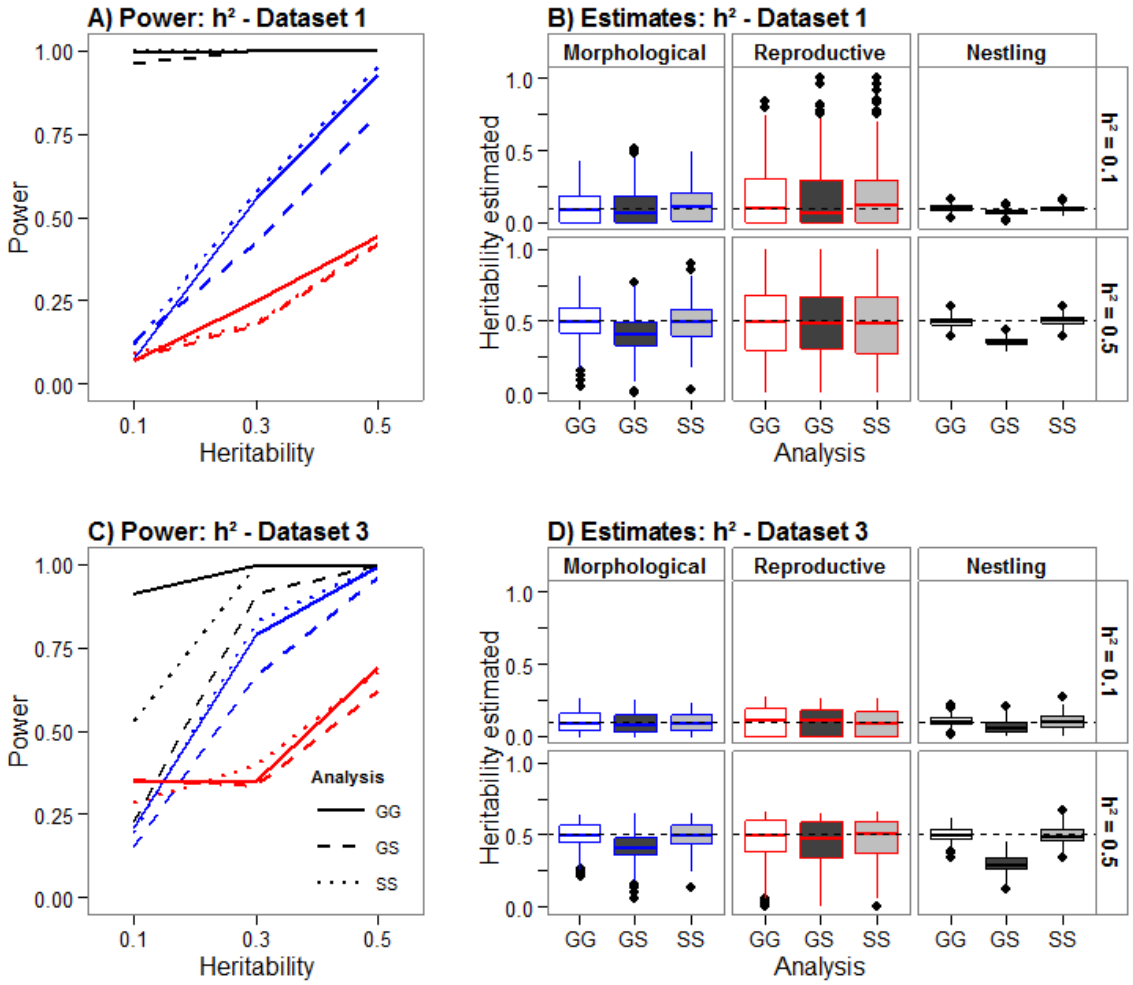

Figure 4.2 Power, precision and accuracy of heritability estimated from univariate animal models, for dataset 1 (panel A and B; simulation of h^2 only) and dataset 3 (panel C and D; simulation of h^2 and other source of resemblance among individual, see main text for details) simulated on the pedigree structure of morphological traits (blue lines), reproductive traits (red lines) and nestling traits (black lines). Power of detecting significant heritability over 300 simulated trait values (100 simulations per trait) are presented in panels A and C. Distribution of these estimates are represented by boxplots (1st quartile, median, 3rd quartile) for 2 levels of heritability (dotted lines represent the h^2 simulated value of 0.1 and 0.5) in panels B and D. Analysis types refer to datasets simulated with the genetic pedigree and analysed with both the genetic

and the social pedigree (GG and GS analyses, respectively), and datasets simulated with the social pedigree and analysed with the social pedigree (SS analysis).

Reliability of quantitative genetics estimates (simulated data)

Visual inspection of boxplots (Figure 4.2bd, 4.3bd) and comparison of RMSE values (Figure S4.D1-2) showed complementary information on precision and accuracy of h^2 and r_A estimates. Precision of h^2 and r_A estimates varied greatly depending on trait category (nestling > morphological > reproductive; mean±SD RMSE for h^2/r_A: $\overline{X}_{nestling}$ = 0.055±0.045/0.081 ±0.025, $\overline{X}_{morpho}$ = 0.122±0.026/0.342±0.061, $\overline{X}_{repro}$ = 0.200±0.045/0.430±0.025) but also among dataset used (dataset 3 > 2 > 1; mean±SD RMSE for h^2: $\overline{X}_{dataset1}$ = 0.144±0.086, $\overline{X}_{dataset2}$ = 0.127±0.072, $\overline{X}_{dataset3}$ = 0.100±0.047). Accuracy for h^2 estimates was high for all GG and SS analyses (except for reproductive traits in dataset 2), but downwardly biased in GS analyses for nestling and reproductive traits, an effect more important as simulated h^2 increased (mean±SD RMSE: $\overline{X}_{GG}$ = 0.117±0.076, $\overline{X}_{GS}$ = 0.143±0.061, $\overline{X}_{SS}$ = 0.115 ±0.075). Accuracy for r_A were similar for all types of analysis (mean±SD RMSE: $\overline{X}_{GG}$ = 0.283 ±0.160, $\overline{X}_{GS}$ = 0.294±0.151, $\overline{X}_{SS}$ = 0.275±0.158).

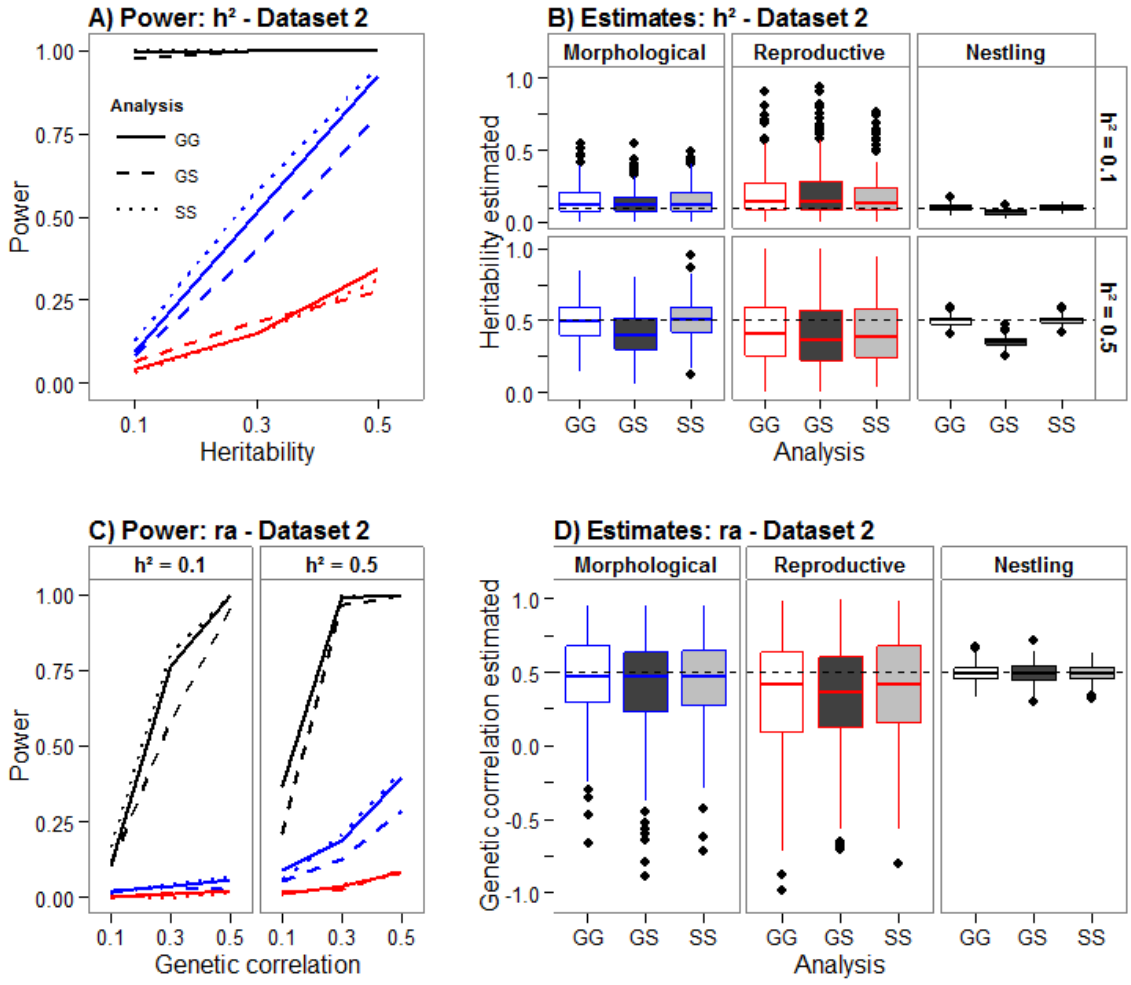

Figure 4.3 Power, precision and accuracy of heritability and genetic additive correlation estimated from multivariate animal models, for dataset 2 (simulation of h^2 and r_A) simulated on the pedigree structure of morphological traits (blue lines), reproductive traits (red lines) and nestling traits (black lines). Power of detecting significant heritability and genetic correlation, both over 300 trait values (100 simulations per trait) simulated, are presented in panels A and C, respectively. Distribution of these estimates are represented by boxplots (1st quartile, median, 3rd quartile), in panel B for heritability (for 2 levels of h^2 – dotted lines represent the h^2 true value of 0.1 and 0.5 simulated) and in panel D for genetic correlation (for 1 value of r_A – dotted lines represent the r_A true value of 0.5 simulated; 5 estimates > 1 are not presented). Analysis types refer

to datasets simulated with the genetic pedigree and analysed with both the genetic and the social pedigree (GG and GS analyses, respectively), and datasets simulated with the social pedigree and analysed with the social pedigree (SS analysis). For graphical representation, r_A was fixed at 0.3 in panels A and B and h^2 was fixed at 0.5 in panel D.

Power to detect significant h^2 or r_A was greater for traits with larger sample size (nestling > morphological > reproductive; Figure 4.2ac, 4.3ac), but we observed no difference among traits within each category (data not shown). For multivariate animal models from dataset 2, power to detect h^2 was similar for the different genetic correlations tested (data not shown), while power to detect genetic correlation increased with increasing heritability (Figure 4.3c). Within a trait category, power was similar between GG and SS analyses, except for nestling traits from dataset 3 were the simulated brood effect decreased power in SS analysis (Figure 4.2c). Finally, power comparison between GG and GS analyses revealed generally lower power in morphological and nestling traits for GS (Figure 4.2ac, 4.3ac).

For adult traits, V_A and V_{PE} estimates from dataset 3 were highly negatively correlated, suggesting that these terms were almost completely confounded (morphological traits: r_s = -0.95, $P < 0.001$; reproductive traits: r_s = 0.94, $P < 0.001$, results from GG analysis with h^2 = 0.3; Figure S4.D3-4). For nestling traits, V_A and V_B estimates were also correlated, but to a lesser extent (r_s = -0.42, $P < 0.001$; Figure S4.D5). All other variance terms showed correlations non-significantly different from 0 (all $|r_s| < 0.09$, $P > 0.12$).

Finally, given the large differences observed for some reproductive traits between standard errors (SE) of h^2 estimates from empirical and simulated datasets (Table 4.2, Figure S4.D6a), we further inspected the impact of model parameters on SEs. More specifically, we assessed the impact of not accounting for an important cause of resemblance among individuals by running dataset 3 animal models and omitting V_{PE} or V_B components. By doing so, we observed a power of 1 at all traits, and all h^2 estimates were very precise but showed a bias

equivalent to the variance component not accounted for (i.e. 0.1 for morphological and reproductive traits and 0.4 for nestling traits; Figure S4.D7). As suspected, SEs for these biased h^2 estimates were small and similar to those obtained in empirical analyses where V_{PE} was almost completely confounded with V_A (Figure S4.D6b).

Discussion

Despite the importance of obtaining precise and accurate genetic (co)variance estimates when assessing a population's adaptive potential, their reliability is rarely explicitly tested or reported in wild populations. Here, we formally assessed the reliability of genetic (co)variance estimates, in a species with a high rate of extra-pair reproduction and low recruitment, with a combination of empirical and simulated data. Altogether, our simulation analyses emphasized the limits of this particular dataset by revealing an important lack of precision in h^2 and r_A estimates for all adult traits, a lack of power to detect significant effects and identifiability problems between V_A and V_{PE}. Moreover, we observed a large bias in h^2 when using the social pedigree instead of the genetic one, and also when non-genetic causes of resemblance among individuals (i.e. repeated measurements or brood effects) were not accounted for in our analyses. We briefly discuss below i) the difficulties that make nearly impossible the generalization of estimate reliability from one study system to another, ii) the hidden problems related to model parameterization, iii) the impacts of high levels of EPP on genetic variance estimates, and iv) we finally conclude on the accessibility and utility of simulation analyses to address all these potential problems.

Estimate reliability and generalization among studies

At first glance, our sample size seemed large enough to be powerful, with more than 10 years of sampling and a number of records within our pruned pedigrees that was larger than the median number of records typically reported for similar studies in the literature. Indeed, a recent compilation of quantitative genetics estimates obtained from wild populations by

Postma (2014) showed that for estimates obtained with animal models, the median number of records for life history traits was 377 (range 6 − 4992; from 39 studies, covering 19 species) and 363 for morphological traits (range 50 − 38,024; from 47 studies, covering 22 species). This suggests that sample size alone is not sufficient to infer pedigree quality, as it may not reflect the underlying structure of pedigrees (see also Wilson *et al.*, 2010). In our study system, the low recruitment of nestlings (1.3%) results in very few grandparent links within our pedigree, which greatly reduces its power. However, this problem is far less important for nestling traits since the high EPP rate results in a genetic pedigree containing several half-sib families, which increase its power (see GG vs SS, Figure 4.2c) despite a smaller number of observations compared to the social pedigree for all categories (Table 4.1). Thus, given that pedigree structures are different from one study system to another, their impact on the reliability of quantitative genetic estimates is unlikely to be easily predicted without incorporating simulation analyses.

Hidden problems related to model parameterization

The choice of which variance terms to include in a model is a crucial step that can have substantial impacts on reliability of genetic variance estimates (Kruuk and Hadfield, 2007; Wilson, 2008). In general, the decision to include or not a particular variance term should rely on LRTs. However, in some cases even if the inclusion of a particular component is not improving the model likelihood, it may still have to be included (Wilson *et al.*, 2010). This is the case for components of variance attributable to repeated measurement of a given individual (V_{PE}). In our models, we had to account for multiple observations by fitting a V_{PE} term in our model, but this component was almost completely confounded with V_A. This situation probably occurred because of the low number of observations by individual (1.5 for morphological traits and 1.2 for reproductive traits), a situation that should be present in other short-lived species datasets. In such case, using a dataset with only one observation per individual may lead to more accurate estimates and easier model convergence.

Standard errors are often viewed as predictors of an estimate accuracy or significance, but this can be misleading (Krzywinski and Altman, 2013). Estimates from animal models have generally smaller standard errors than those obtained with parent-offspring regressions (Kruuk, 2004; Postma, 2014), partly due to their integration of multiple observations for a given individual (Åkesson *et al.*, 2008). As previously stated, failing to account for multiple observations can create unpredictable bias in phenotypic variance component estimates (Kruuk and Hadfield, 2007) and considerably reduce standard errors around these biased estimates (see Figure S4.D6). Moreover, it seems that problems of identifiability could also result in reduced standard errors. For example, in our empirical analyses, small standard errors were estimated around h^2 estimates for reproductive traits from univariate animal models (SE range: 0.03 – 0.04), which could have led us to misleadingly conclude that our dataset was powerful and our estimates were precise for these traits.

Impact of high level of EPP on estimates

In theory, EPPs, if not accounted for, could downwardly bias additive genetic variance and resulting heritability estimates (Charmantier and Réale, 2005). Yet, previous simulation studies showed that even if biases increased with the importance of EPPs, with increasing heritability and when focal traits were directly related to the number of extra-pair young produced, underestimations were generally smaller than 15% (Firth *et al.*, 2015). In our study, the rate of EPP was higher than those previously tested so far (up to 40% in Charmantier and Réale 2005, 12.5% in Firth et al. 2015), but its effect on quantitative genetics estimates was complex. First, our simulations analyses showed that the bias on h^2 estimates resulting from using the social instead of the genetic pedigree was increasing with sample size (i.e. higher for nestling traits). Also, our empirical analyses suggested that the impact of not accounting for EPP was noticeable for morphological and nestling traits, but sometimes resulted in higher h^2 and CV_A when using the social pedigree (i.e. for nestling wing length and body mass). A similar unexpected pattern was previously reported in blue tits (*Cyanistes caeruleus*), where h^2 estimates from a social pedigree were sometimes higher than those from a genetic pedigree (Charmantier and Réale, 2005). These positive biases observed in empirical data could be due

to social father influence (for example through parental care), which could be captured in V_A components (Griffith *et al.*, 1999; Charmantier and Réale, 2005). To assess this possible problem, we performed an additional animal model analysis including mother and social father identities as additional variance components. We found that new h^2/CV_A values obtained using the social pedigree were now smaller than values obtained with the genetic one (change in estimates for body mass h^2 = -0.06 (-26%), CV_A = -0.011 (-26%); wing length h^2 = -0.04 (-100%), CV_A = -0.41 (-100%); difference (%) calculated with univariate animal models). This further emphasizes the importance of model parameterization on reliability of variance component estimates.

Conclusion

Simulation analyses are now widely accessible to anyone with minimal programming skills in R, for instance by using the "*phensim"* function within the package PEDANTICS or the "*rbv"* function within the package MCMCglmm (Hadfield, 2010). Since each study system is unique, in terms of pedigree structure, number of repetitions by individual or potential causes of pedigree errors, it is difficult to predict the reliability of quantitative genetics estimates without testing it formally. With more simulation studies like this one on different types of study systems it could become possible to establish a threshold for each important parameter (e.g., recruitment rate, sample size, number of grandparent, connectedness). Without this information, detailed simulations should be routinely included when reporting quantitative genetics analyses of new wild populations to explicitly assess the precision and accuracy of genetic variance components (see also Morrissey *et al.*, 2007).

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Conflict of interest

The authors declare no conflict of interest.

Data archiving

Data will be deposited in the Dryad repository prior to publication.

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Chapitre 5

RÉPONSES ÉVOLUTIVES

Description de l'article et contribution

L'hétérogénéité environnementale est fréquemment mentionnée comme une possible cause des disparités entre les réponses évolutives prédites et les tendances phénotypiques observées, mais cette hypothèse est rarement testée. Les effets de l'hétérogénéité environnementale sur les composantes de variances phénotypiques étaient le point de départ de cet article, sujet sur lequel Dany Garant m'avait proposé de travailler dès le tout début de mon doctorat. Initialement, les analyses devaient s'effectuer sur les traits reproducteurs, mais en raison des résultats du chapitre précédent, l'orientation a changé vers des traits morphologiques d'oisillons. Se sont greffées à ce projet les idées d'intégrer en premier lieu des analyses de sélections, puis les prédictions des réponses évolutives à partir de l'équation du reproducteur et du second théorème de la sélection. Les résultats de cet article suggèrent entre autres un effet de l'environnement sur les patrons de sélection et des composantes de variance phénotypiques ainsi que de grandes disparités entre les approches utilisées pour prédire les réponses évolutives, mais aussi avec les tendances phénotypiques observées.

Pour cet article, j'ai élaboré les idées avec Dany Garant, j'ai effectué les analyses statistiques et j'ai rédigé une première version du manuscrit. J'ai également participé à la collecte de données sur le terrain durant trois saisons d'échantillonnage (2012-2014). Dany a supervisé le processus et corrigé quelques versions du manuscrit. Fanie Pelletier et Marc Bélisle ont contribué aux réflexions entourant l'interprétation des résultats et ont également révisé le manuscrit.

Evolutionary responses of morphological traits across different life-history stages and heterogeneous environments

en préparation pour *Journal of Evolutionary Biology*

Audrey Bourret, Marc Bélisle, Fanie Pelletier and Dany Garant

Abstract

Despite accumulating examples of selection acting on heritable traits in the wild, predicted evolutionary responses are often different from observed phenotypic trends. Various explanations have been suggested for these mismatches. These include environmental heterogeneity as well as within-individual changes across lifespan that can create important variation in genetic architecture of traits and selection acting on them, but also the methodological approach used to infer those responses. Here, we used a long-term dataset on Tree swallow (*Tachycineta bicolor*) to first assess the effects of environmental variation and differences among nestling life-history stages on the genetic (co)variances of morphological traits (body mass, wing and tarsus length) and the selection acting on them. Then we estimated their evolutionary responses using the breeder's equation and the secondary theorem of selection approaches and we compared the predictions obtained to the phenotypic trends observed in our system. Our results showed variation in selection across ages and stronger selection in harsher environmental conditions. Variance of traits was also different among contrasted environments, mainly because of changes in the brood identity component of variance. Evolutionary responses predicted with both approaches differed strikingly but were also different from the phenotypic trends observed.

Key words: body mass, Breeder's equation, phenotypic trends, quantitative genetics, secondary theorem of selection, tree swallow

Introduction

Predicting evolutionary responses to selective pressures in wild populations is a central goal in evolutionary biology and can help us understand population persistence under current global changes (Gienapp et al. 2008; Visser 2008; Gienapp and Brommer 2014). In order to respond to selection, a trait must display genetic variation within a given population, which is often quantified using its heritability (h^2, ratio of the additive genetic variance (V_A) over the phenotypic variance (V_P); Falconer and Mackay 1996). However, despite evidences of selection on heritable traits in wild populations (Kingsolver et al. 2001), predicted evolutionary responses are often different from observed phenotypic trends (Merilä et al. 2001b). In fact, there are now accumulating examples of populations showing evolutionary stasis over time or even phenotypic responses in opposite directions to those expected from selection patterns (Merilä et al. 2001b; Gienapp et al. 2008; Merilä 2012; Gotanda et al. 2015). Several explanations have been proposed to explain these mismatches, including a lack of consideration for factors that can bias estimations of selection and/or heritability and thus lead to erroneous predicted evolutionary responses (Merilä et al. 2001b).

Variation in environmental conditions can greatly influence evolutionary responses. For instance, there is evidence that selection frequently fluctuates both in strength and direction across time and space (Siepielski et al. 2009, 2013; Bell 2010; but see Morrissey and Hadfield 2012). Such changes in selection among years, for example, could explain why short term trends are not representative of those observed over longer periods (e.g. Grant and Grant 2002; Millet et al. 2015). Similarly, the genetic architecture of traits can vary depending on environmental conditions (reviewed in Hoffmann and Merilä 1999; Charmantier and Garant 2005). In wild populations, poorer environmental conditions are often associated with a reduction of V_A and/or an increase of environmental variance (V_E), which in both cases would result in lower h^2 (e.g. Charmantier et al. 2004; Robinson et al. 2009; Husby et al. 2011b). Fluctuating environments can also influence the strength and direction of genetic covariances among traits and the resulting G-matrix (Sgrò and Hoffmann 2004; Björklund and Gustafsson 2015; Wood and Brodie 2015), which can modify the constraints imposed on evolutionary

responses. Moreover, environmental heterogeneity can generate a negative covariance between selection and genetic variance, thus constraining evolutionary responses in all environments (Wilson et al. 2006). It is therefore critical to consider the heterogeneity in environmental conditions in our assessment of evolutionary responses.

Genetic architecture of traits and selection acting on them can also change throughout an individual's lifespan and thus traits measured at different life stages can be treated as separated characters for which different evolutionary responses could be predicted (i.e. age-specific traits; Lande 1982; Arnold and Wade 1984). Previous studies have shown that covariance between fitness and traits can vary across ages (e.g. McAdam and Boutin 2003; Le Galliard and Ferrière 2008). For example, weaker covariance between fitness and traits later in life can result from senescence in long-lived species (Hamilton 1966; McElligott et al. 2002; Kervinen et al. 2015). Heritability of traits (e.g. Gebhardt-Henrich and van Noordwijk 1994; Réale et al. 1999; Charmantier et al. 2006; Hadfield et al. 2013) and additive genetic covariances among traits (e.g. Atchley 1984; Irwin and Carter 2013) can also differ across ages, which can be attributed to changes in V_A due to variations in gene expression during ontogeny (Atchley 1984). Alternatively, it can result from changes in V_E, for example, following selection episodes targeting the environmental component of a trait (van Noordwijk 1988) or change in maternal effects with age (Wilson and Réale 2006). Investigating how heritability and selection change during different life-history stages can not only bring insights on the evolutionary potential of traits but also improve our assessment of the role of specific parts of the lifecycle in observed phenotypic trends.

Predictions of evolutionary responses have often been obtained using the so-called breeder's equation (e.g. in its univariate form: $R = h^2 * S$, where R is the expected change in mean phenotype between 2 generations and S is the selection differential; henceforth BE; Lush 1937). An important assumption underlying this equation is that the relationship between the trait and the fitness component used to assess S is causal (Morrissey et al. 2010). In wild populations, this assumption is, however, often violated since fitness-trait correlations can be induced by covariations with environmental variables (Rausher 1992; Kruuk et al. 2003). For

example, variation in resource availability across space can affect independently size of traits (e.g. adult body mass) and fitness proxies (e.g. young survival). Such non-causal relationships may lead to biases in predicted evolutionary responses (Price et al. 1988; Rausher 1992; Stinchcombe et al. 2002; Kruuk et al. 2003). In general, a more accurate assessment of evolutionary responses could be achieved by directly estimating the additive genetic covariances between traits and fitness (i.e. the so-called secondary theorem of selection; henceforth STS; Morrissey et al. 2010, 2012; Stinchcombe et al. 2014). However, contrary to the BE, the STS is uninformative on the causes underlying phenotypic changes, such as discriminate between direct and indirect selection. Thus comparing predictions obtained from both approaches is very valuable to provide a more rigorous understanding of evolutionary responses and their potential causes.

Here, we used a detailed dataset collected over 3 generations (8 years) from a population of tree swallows (*Tachycineta bicolor*) breeding in southern Québec (Canada) to 1) assess the effects of variation in environmental conditions and differences among life-history stages on genetic architecture of nestling morphological traits and selection acting on them, 2) estimate potential evolutionary responses of these traits based on the BE and the STS, and 3) compare these predicted responses to phenotypic trends observed in our study system. Tree swallows are small insectivorous migratory birds that breed in cavity holes and nest boxes across North America (Winkler et al. 2011). They produce one clutch per year, containing on average 5 eggs (Winkler et al. 2011). These socially monogamous birds display one of the highest rate of extra-pair paternity (EPP), with around 50% of nestlings resulting from extra-pair copulations (Dunn et al. 1994; Whittingham and Dunn 2001; Lessard et al. 2014). Here we focus on traits measured in nestlings during their development until fledging. Nestlings are sensitive to environmental conditions surrounding their natal sites (Ghilain and Bélisle 2008; Pigeon et al. 2013). Moreover, they can be easily captured to take measurements at different ages and, given the natural half-sib design created by the high EPP rate in this species, we can obtain precise and accurate quantitative genetic estimates for these traits (Bourret and Garant submitted).

More specifically, we first determined the impact of environmental heterogeneity on viability selection acting on three morphological nestling traits (body mass, wing length, tarsus length) measured at different stages from hatching to fledging. Based on the general trends observed in wild populations, we were expecting stronger selection in harsher environmental conditions (e.g. Wilson et al. 2006; reviewed in Siepielski et al. 2013) but also selection fluctuating in strength depending on life history stage (e.g. McAdam and Boutin 2004). We also estimated quantitative genetic components (additive genetic (co)variance, h^2 and coefficient of additive genetic variation (CV_A)) of these age-specific traits. We were anticipating to obtain reduced h^2 estimates in harsher environmental conditions because of either a decrease in V_A or an increase in V_R, or both (Charmantier and Garant 2005), as well as changes in h^2 across nestling development. Then, we predicted evolutionary responses using BE and STS approaches for our overall dataset and also for contrasted environmental conditions. Given our predictions for selection on, and h^2 of nestling traits in contrasted environments (e.g. stronger selection on lesser heritable traits in harsher environmental conditions), we expected that the predicted evolutionary response should be similar across environments (Wilson et al. 2006). Finally, we compared these predictions to phenotypic trends observed, and we expected that STS predictions would be more accurate than BE predictions (Morrissey et al. 2012).

Methods

Study system and data collection

The study system is located in southern Québec and is composed of 400 nest boxes equally distributed across 40 farms (i.e. 10 per farm) over an area covering 10,200 km² (detailed in Ghilain and Bélisle 2008). From 2007 to 2014, nest boxes were visited every 2 days to monitor tree swallow breeding activities (e.g. laying, hatching and fledging dates). Adults and 12-day-old nestlings were ringed with an aluminium band (US Fish and Wildlife Service) for individual identification. Prior to their banding, nestlings were individually marked using a nail clipping code. Blood samples (adults and 12-day-old nestlings; taken on P8 grade filter

papers (Fisher Scientific)) or muscular tissues (nestlings dead before 12 days old; preserved in 95% EtOH) were collected for molecular sexing and paternity assignments (see *Molecular analyses* section below).

Morphological measurements were taken on nestlings at different stages before fledging (which occurs around 18-22 days after hatching). Body mass (±0.01 g) was measured with a digital scale at 2, 6 12 and 16 days after hatching, primary feather length (hereafter wing length; ±0.02 mm) was measured with a caliper at 6, 12 and 16 days, while tarsus length (±0.02 mm) was measured only at 16 days. We defined nestling fitness as the survival from hatching to fledging (i.e. 0 or 1) and we computed a nestling's relative fitness as the ratio of its survival divided by the annual survival rate for a given age.

Environmental heterogeneity

We characterized environmental heterogeneity within our study system using five environmental variables. First, the studied farms are located across an agricultural gradient, from intensive monocultures in the West of the study area, to prairies and woodlands in the East. Several agricultural variables at local and regional scales were previously shown to influence traits and fitness of tree swallows in our system (see Ghilain and Bélisle 2008, Lessard et al. 2014). Thus, we used the proportion of intensive (e.g. corn, soy and cereal) and non-intensive (e.g. prairies) cultures at 2 spatial scales (500 m and 5 km) around each nest box. Landscape characterisation at 5 km was based on Landsat-7 satellite images captured between August 1999 and May 2003 (Canadian Wildlife Service 2004). Land use at 500 m was determined yearly in the field by visual inspection (see Porlier et al. 2009 for details). Proportions of intensive and non-intensive cultures were calculated with ArcView GIS Spatial Analyst v2.0a (ESRI 2005). As a fifth environmental variable, we used the proportion of the 10 nest boxes occupied each year by tree swallow for each farm. Since nest boxes are located in a similar fashion on each farm (i.e. spaced by 50 m along a single field margin, Ghilain and Bélisle 2008), the total area covered by nest boxes on each farm is very similar. This proportion of occupancy represents a measure of local density (hereafter density) and provides

a good proxy of environmental quality, where higher density farms represent better habitats (see Bourret et al. 2015).

Molecular analyses

DNA was extracted from blood samples and muscular tissues with a standard salt extraction method (Aljanabi and Martinez 1997) and its concentration for each sample was determined by electrophoresis on a 2% agarose gel with a molecular weight standard. Molecular sexing was performed following Lessard et al. (2014) to determine nestling sexes and confirm adult sex obtained from field observations. Each individual was also genotyped at 6 microsatellite loci following Lessard et al. (2014) to conduct parentage assignment; PCR products were visualised using an AB3130xl automated DNA sequencer and allele lengths were determined using GeneMapper v4.1 (Applied Biosystems).

Selection analyses

Selection analyses were conducted on both raw and standardized (zero mean, unit variance) values of traits for each age. We first obtained linear (i) and non-linear (j) selection differentials for each trait, which represents the combined effect of direct and indirect selection on the focal trait (Lande and Arnold 1983). For standardized traits, linear selection differentials were estimated from the coefficient of the regression between relative nestling fitness and the trait value (and twice its squared value for quadratic non-linear terms; Stinchcombe et al. 2008). For raw trait values, we multiplied these regression coefficients by the phenotypic variance of each trait. We then computed selection gradients to assess the direct effect of selection on each trait for a given age (Lande and Arnold 1983). For 6 and 12-day-old nestlings, linear selection gradients (β_i) for body mass (x_1) and wing length (x_2) were obtained following this linear model:

$$\omega = \alpha + \beta_1 x_1 + \beta_2 x_2 + \varepsilon \quad (1)$$

where ω is the relative fitness, α the intercept, and ε the residual term. Similarly, for the non-linear quadratic (γ_i) and correlational (γ_{ij}) terms, we used this linear model:

$$\omega = \alpha + \beta_1 x_1 + \beta_2 x_2 + (\gamma_1/2){x_1}^2 + (\gamma_2/2){x_2}^2 + \gamma_{12} x_1 x_2 + \varepsilon \quad (2)$$

For 16-day-old nestlings, equations 1 and 2 were expanded to include tarsus length (see equations S1−2 in Supporting Information). Hour of measurement, expressed as a proportion of 24 hours (e.g. midday = 0.5), was added as a predictor variable in models including body mass (all ages) and wing length (16 days old only). Statistical significance of selection differentials and gradients was assessed with generalized linear mixed models (logit link and binomial error structure) relating survival (0 or 1) to standardized values of traits and including year, farm and brood identity as random effects.

We assessed variation in selection related to environmental conditions using a two-step approach. First, since most environmental variables were moderately to highly correlated among each other (see Figure S5.A1), we selected the environmental variable showing the best fit by comparing different models based on the second-order Akaike information criterion (AICc). Each environmental variable was divided as a 2-level factor (low or high value) based on the median value obtained from all nestling observations to allow direct comparisons of selection, quantitative genetic parameters, and predicted evolutionary responses between contrasted environments (harsh or good). For each age, we built generalized linear mixed models (logit link and binomial error structure) relating survival (0 or 1) to standardized trait values, one environmental variable and their interactions. Year, farm and brood identity were included as random effects. Since model comparisons showed that only a model including density for nestlings at 12 days old had a lower AICc value than null models (Table S5.A1), we kept density as our environmental variable for subsequent analyses. As a second step, we used a sequential model building approach to assess if linear and non-linear terms of selection were fluctuating depending on density (as in Appendix A of Chenoweth and Blows 2005, see also Porlier et al. 2009, Millet et al. 2015 for similar approaches). Briefly, we compared a model including trait-density interactions (or squared values of traits for the non-linear term)

to a model without these interactions using a likelihood ratio test (LRT). When the full model was a significant improvement in likelihood over the basic model, we compared the full model with a model where one of the trait-environment interactions was removed successively.

All selection analyses were conducted in R v3.2.0 (R Core Team 2015). Mixed models were fitted with the *lme4* package (Bates et al. 2014) and AICc model comparisons performed with the *AICcmodavg* package (Mazerolle 2015). Statistical significance was based on an α-level of 0.05.

Pedigree construction and quantitative genetic analyses

The pedigree was built based on genetic information following Bourret and Garant (submitted). Briefly, dam identities were obtained from field observations (i.e. females captured during the incubation period) and confirmed genetically. Sire identities were obtained from paternal assignations performed with a likelihood approach set at 90% confidence level using CERVUS v3.0.7 (Kalinowski et al. 2007; Lemons et al. 2015). Candidate genetic fathers of a given nestling included all males captured, or suspected of being present outside of our nest box system within a given year (i.e. captured on the same farm on both previous and following years), within a 15-km radius of the nestling's nest box. When a male was significantly assigned to a nestling, it was considered as its genetic father. When this procedure failed to determine the genetic father, we attempted to exclude the nestling's social father (i.e. the male captured in the nestling's nest box during food provisioning) as being the genetic father at a 95% confidence level following Lemons et al. (2015). If we failed to exclude the social father, the latter was considered the genetic father, otherwise no genetic father was assigned to the nestling.

Quantitative genetic parameters were estimated with univariate and multivariate animal models (Kruuk 2004) for each age separately. Animal models were fitted with a restricted maximum likelihood method (REML) using ASRemL v3.0.5 (VSN International Ltd, Hemel Hempstead, UK). This approach is appropriated given the Gaussian distribution of studied

traits (de Villemereuil *et al.*, 2013). First, univariate animal models were constructed for each trait using the following model:

$$V_P = V_A + V_Y + V_B + V_R \quad (3)$$

where V_P is the phenotypic variance after accounting for fixed effects (only hour of measurement, as for selection analyses), V_A is the additive genetic variance, V_Y is the variance among birth years, V_B is the variance among broods and V_R is the residual variance. Multivariate animal models were used when more than one trait was sampled at a given age and covariances among traits were estimated for each variance components using unstructured variance models. For all models, we tested the statistical significance of additive genetic (co-)variances by comparing a model including the focal estimate to a model where this estimate was constrained to be equal to zero using a LRT. We estimated heritability ($h^2 = V_A/V_P$, Falconer and McKay 1996) and the coefficient of genetic variation ($CV_A = \sqrt{V_A}/\bar{X}$, where $\bar{X}$ is the trait mean, Houle 1992) for each trait in all models.

Finally, we estimated the above variance components at low and high densities by re-fitting univariate and multivariate animal models in both environments. To test for statistical difference in (co-)variance between environments, we compared a model where the focal variance component was constrained to be equal in both environments to an unconstrained model (i.e. variance estimated independently in both environments) using a LRT. Total phenotypic variances for each trait (raw data) observed in each environment were compared using *F* tests.

Evolutionary responses

We assessed the expected change in mean phenotype between 2 generations (*R*) with both the breeder's equation (BE) and the secondary theorem of selection (STS). In its univariate form, the breeder's equation (UVBE; Lush 1937) is defined as:

$$R = h^2 * S \quad (4)$$

where h^2 is the heritability and S the selection differential. We applied this equation on body mass for 2-day-old nestlings. When more than one trait was measured at a given age (6, 12 and 16 days), we applied a multivariate form of the breeder's equation (MVBE, also known as the Lande equation, Lande 1979):

$$R = G * \beta \quad (5)$$

where G is the variance-covariance additive genetic matrix and β the vector of selection gradients. All of the components necessary to apply BEs were obtained as described in previous sections.

According to the STS (Robertson 1966, 1967; Price 1970), evolutionary change is estimated from:

$$R = cov_A(\omega, x) \quad (6)$$

where $cov_A(w,x)$ is the genetic covariance between relative fitness (w) and a trait (x). We obtained this estimate from additive genetic variance-covariance matrices of multivariate animal models that included, in addition to all traits sampled at a given age, the relative fitness (i.e. the G_{ZW} matrix of Stinchcombe et al. 2014). Within these models, the V_Y component of relative fitness was fixed to zero (see also Morrissey et al. 2012).

BE and STS equations were applied to the overall dataset and to both density subsets. Standard errors (SEs) for R estimated with the STS were computed directly by ASRemL. With the UVBE, R SEs were approximated assuming that squared relative standard errors were additive (see equation A1 in Morrissey et al. 2012). Finally, for R estimated with the MVBE, associated SEs were estimated following Kingsolver et al. (2015; as described in their

Appendix B). Briefly, we randomly generated 10,000 *G* matrices (following Meyer and Houle 2013 and using the *mvtnorm* package, Genz et al. 2015) and 10,000 bootstrapped *S* (with the *boot* package, Canty and Ripley 2015) that we multiplied accordingly to equation 5. MVBE *R* SEs were then defined as the standard deviation of these 10,000 newly obtained *R* estimates.

Finally, we computed the observed changes in mean phenotype for all trait-age combinations and compared them to the predicted changes. Observed changes were assessed by regressions between the observed annual mean phenotypes and years. The obtained coefficients were then multiplied by the generation time (i.e. 2.38 years (SD = 1.19), computed as the mean age at reproduction for females with known age (n=97 observations on 63 females); Charlesworth 1980) to obtain a phenotype change per generation (i.e. same unit as *R* predicted with equation 4−6). Predicted and observed *R* were compared for each trait-age combinations using *t*-tests.

Results

Between 2007 and 2014, we sampled 7104 nestlings (see Table S5.A2 for sample sizes by year and age). During this period, the overall nestling survival from hatching to fledging was 0.77 (5598 fledglings out of 7253 hatchlings); the proportion of nestling surviving until fledgling increased with age: annual mean survival (range): at 2 days: 0.79 (0.65−0.88); 6 days: 0.83 (0.71−0.91); 12 days: 0.91 (0.81−0.95); 16 days: 0.97 (0.94−>0.99). We assigned a genetic father to 5052 nestlings (71.1% of all nestlings or 77.2% of nestlings with successful DNA amplification).

Selection analyses

Body mass, wing length and tarsus length were all under direct and/or indirect viability selection in nestlings. Selection differentials varied in strength among traits and among ages within a trait (Table 5.1, S5.A3). Linear terms were all positive and significant, while small negative non-linear components, representing an asymptote for highest trait values (Figure

S5.A2), were significant for 6 out of the 8 trait-age combinations (Table 5.1). Selection gradients showed significant direct positive linear effects of selection on body mass and wing length, with the exception of wing length at 6 days old which was negative (Table 5.2, S5.A4). Non-linear components were small but significant for body mass at all ages (negative) and wing length at 16 days (positive). Only a small positive correlational selection between body mass and tarsus length at 16 days old significantly differed from zero (Table 5.2).

Table 5.1 Standardized linear (*i*) and non-linear (*j*) selection differentials (±SE) for all traits measured for nestlings at 2, 6, 12 and 16 days old. Significant values are in bold (see Table S5.A3 for selection differentials on raw data).

Trait	Age (days)	*i* ± se	*j* ± se
Body mass	2	**0.068 ± 0.006**	**-0.086 ± 0.010**
	6	**0.127 ± 0.005**	**-0.095 ± 0.007**
	12	**0.136 ± 0.004**	**-0.064 ± 0.004**
	16	**0.055 ± 0.002**	**-0.048 ± 0.002**
Wing length	6	**0.060 ± 0.006**	**-0.039 ± 0.008**
	12	**0.102 ± 0.004**	**-0.089 ± 0.005**
	16	**0.049 ± 0.002**	-0.030 ± 0.002
Tarsus length	16	**0.017 ± 0.002**	-0.010 ± 0.003

We observed environmental fluctuations in the strength of selection for body mass at 2 and 12 days old only (Figure 5.1, Table S5.A5-6). At 2 days old, the negative non-linear term of selection differential for body mass was stronger in low density environment (Figure 5.1a, Table S5.A6). At 12 days old, the linear component of selection gradient for body mass was stronger in farms with lower densities, while the non-linear component of selection was more stabilizing (negative) in these farms (Figure 5.1b, Table S5.A6).

Table 5.2 Standardized linear (β_i), non-linear (γ_i) and correlational (γ_{ij}) selection gradients (±SE) for all traits (body mass, wing and tarsus length) measured for nestlings at 6, 12 and 16 days old. Significant values are in bold (see Table S5.A4 for selection gradients on raw data).

	6 days	12 days	16 days
β_{MASS}	**0.184 ± 0.008**	**0.115 ± 0.004**	**0.046 ± 0.002**
β_{WING}	**-0.077 ± 0.008**	**0.038 ± 0.004**	**0.037 ± 0.002**
β_{TARSUS}			0.002 ± 0.078
γ_{MASS}	**-0.050 ± 0.007**	**-0.067 ± 0.007**	**-0.035 ± 0.003**
γ_{WING}	0.015 ± 0.007	-0.021 ± 0.007	**0.004 ± 0.003**
γ_{TARSUS}			0.001 ± 0.003
$\gamma_{MASS\text{-}WING}$	0.018 ± 0.015	0.010 ± 0.006	-0.017 ± 0.002
$\gamma_{MASS\text{-}TARSUS}$			**0.011 ± 0.002**
$\gamma_{WL\text{-}TARSUS}$			0.001 ± 0.002

Quantitative genetics analyses

Univariate and multivariate animal models led to very similar results, we thus only present results from multivariate analyses whenever possible (see Table S5.A7 for complete results). We found important variation in V_A, h^2 and CV_A between and within nestling morphological traits (Figure 5.2a-c). For body mass, h^2 increased throughout nestling development while CV_A slightly decreased during the same period. For wing length, h^2 and CV_A were more stable across ages except for the higher CV_A observed at 6 days old. Most trait-age combinations revealed V_A components that were significantly different from 0 ($P \leq 0.012$). Only wing length showed nonsignificant V_A at 6 days ($\chi^2 = 3.20$, df = 2, $P = 0.20$) and 16 days ($\chi^2 = 3.98$, df = 3, $P = 0.26$). We also obtained positive additive genetic covariances between all pairs of traits showing significant V_A (Figure S5.A3a).

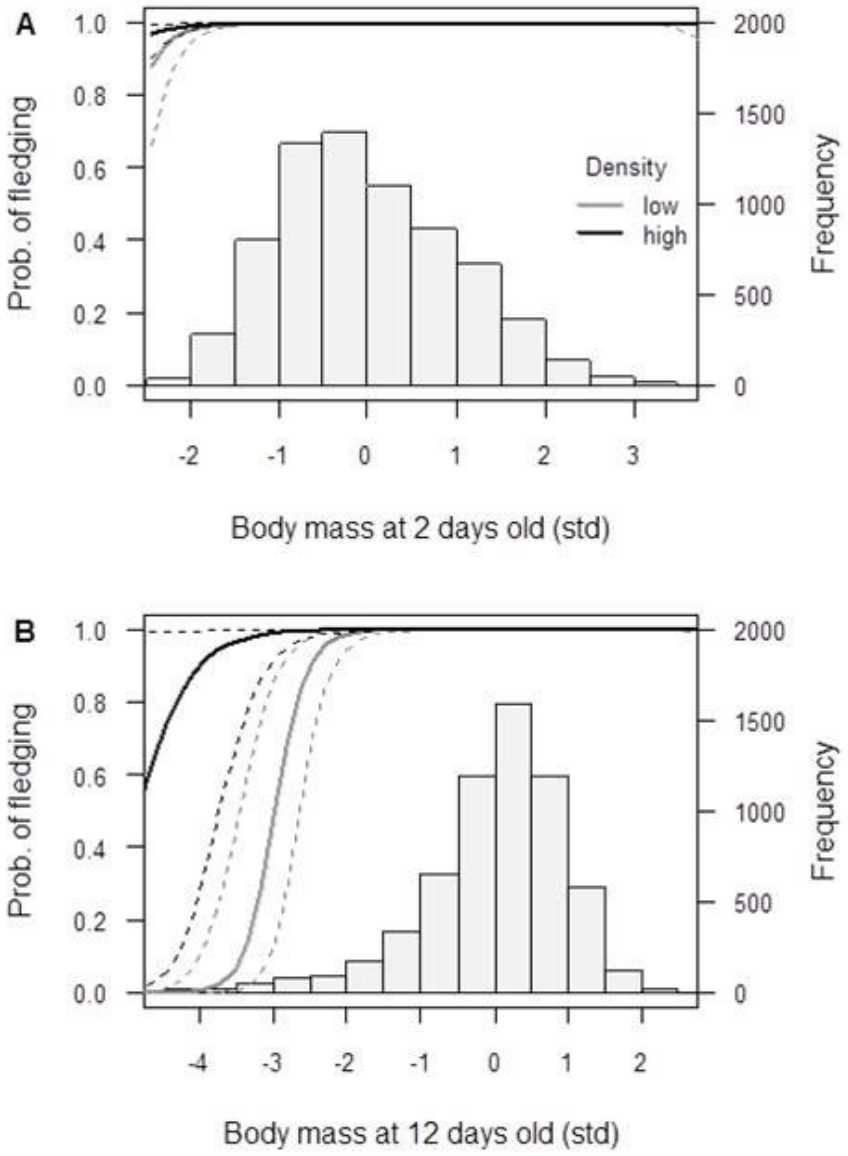

Figure 5.1 Predicted probability of fledging (solid lines) in relationship to body mass for A) 2-day-old and B) 12-day-old nestlings, in low (gray) and high (black) density environments. Confidence intervals at 95% (dashed lines) and histograms of observed mass values (gray bars) are presented.

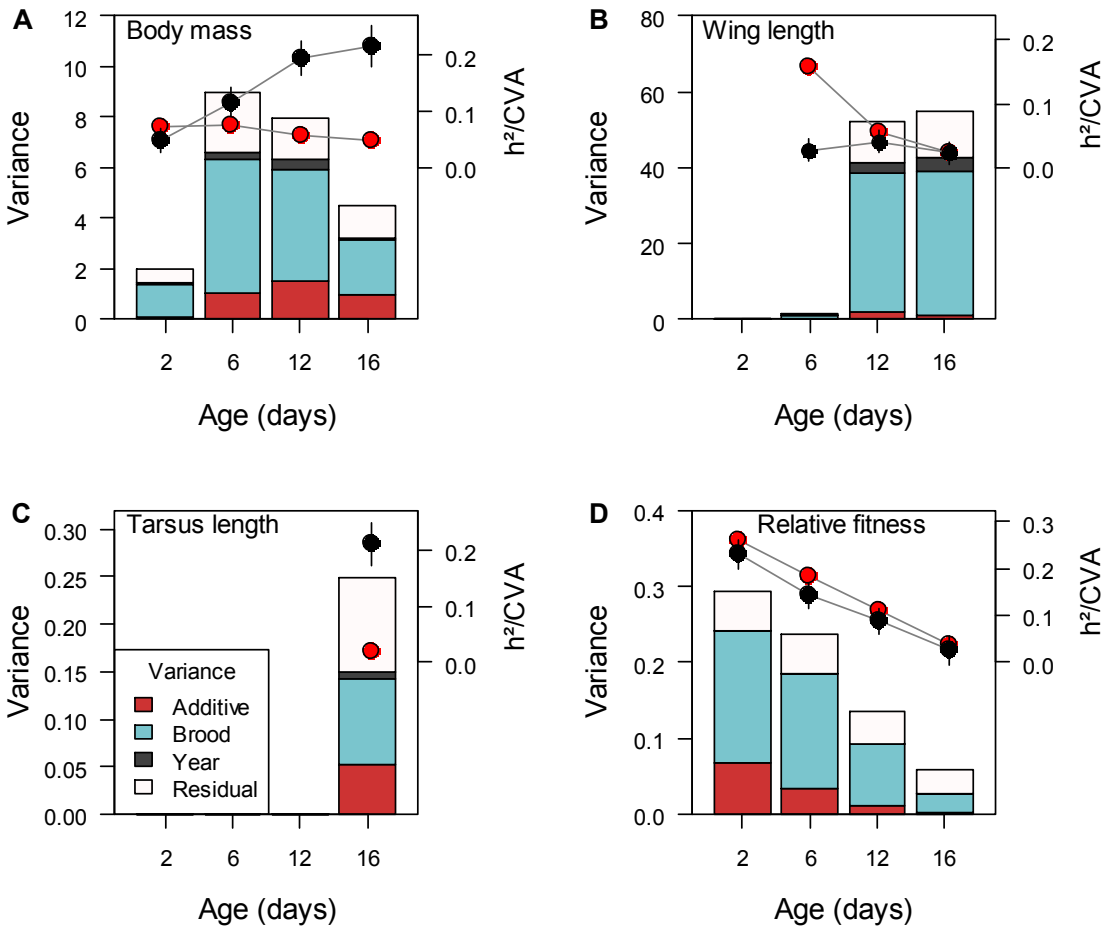

Figure 5.2 Phenotypic variance components (stacked bars), heritability (h^2, black dots) and CV_A (red dots) for nestling A) body mass, B) wing length, C) tarsus length and D) relative fitness at different ages (2, 6, 12 and 16 days old). Errors bars on h^2 estimates represent standard errors. Phenotypic variance components for morphological traits (A-C) were obtained from animal models including all measured morphological traits at a given age while those for relative fitness (D) included all morphological traits and relative fitness.

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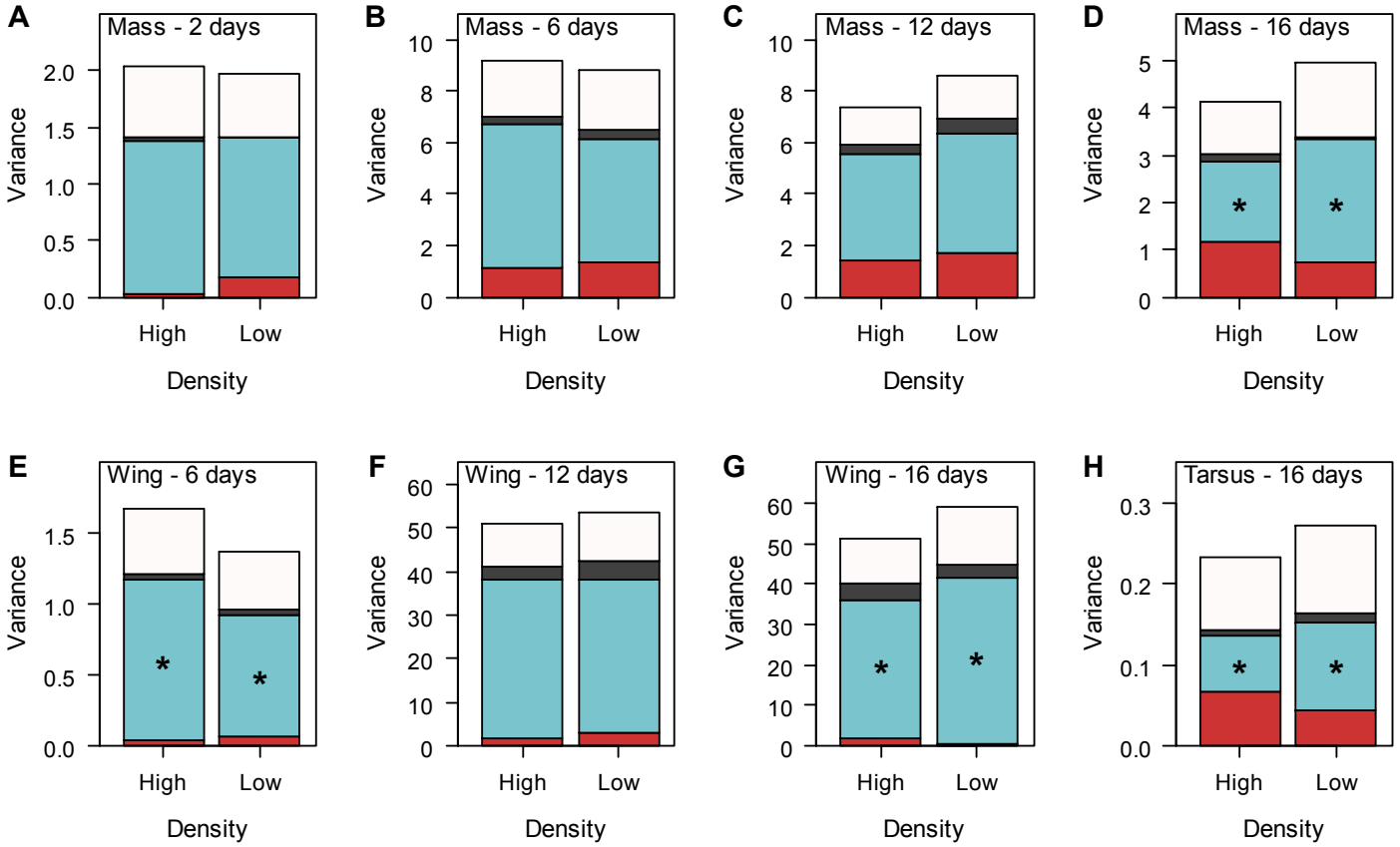

Figure 5.3 Phenotypic variance components of all 8 trait-ages in high and low density environments. Variance components are additive genetic (V_A, red), brood (V_B, blue), year (V_Y, dark gray) and residual (V_R, white). Asterisks (*) indicate significant differences in variance components between environments.

Phenotypic variance components differed between low and high density environments (Figure 5.3). Overall phenotypic variance was significantly larger in low density environments for body mass at 12 days ($F_{3334,2804} = 0.83$, $P < 0.001$) and for all traits at 16 days (body mass: $F_{3074,2575} = 0.83$, $P < 0.001$; wing length: $F_{3061,2559} = 0.84$, $P < 0.001$; tarsus length: $F_{3061,2575} = 0.86$, $P < 0.001$). On the contrary, overall phenotypic variance was larger in high density environments for wing length at 6 days ($F_{3619,3087} = 1.20$, $P < 0.001$; all other trait-age: $P > 0.08$). For specific components of phenotypic variance, only V_B showed significant difference between the two environments, being larger in high density environments for wing length at 6 days ($\chi^2 = 10.24$, df = 1, $P = 0.001$) and larger in low density environments for all traits at 16 days (body mass: $\chi^2 = 11.16$, df = 1, $P < 0.001$; wing length: $\chi^2 = 4.02$, df = 1, $P = 0.045$; tarsus length: $\chi^2 = 9.60$, df = 1, $P = 0.002$; all other trait-age variance components: $P > 0.08$). Similarly, only the covariance component related to brood identity showed variation between environments, being larger in high density environments at 6 days between body mass and wing length ($\chi^2 = 4.62$, df = 1, $P = 0.032$) and larger in low density environments at 16 days between body mass and wing length ($\chi^2 = 15.74$, df = 1, $P < 0.001$) as well as between wing length and tarsus length ($\chi^2 = 4.36$, df = 1, $P < 0.037$; Figure S5.A3b; all other covariances for trait-age combinations : $P > 0.05$).

Evolutionary responses

Observed phenotypic trends suggested temporal decreases in trait values, which were significant for body mass at 6 and 12 days old and for tarsus length at 16 days old (Figure 5.4; all other *P*-values > 0.05). However, we observed no differences in mean trait values or in the slope of these trends between low and high density environments (linear models including an interaction between year and environment, all *P*-values > 0.11).

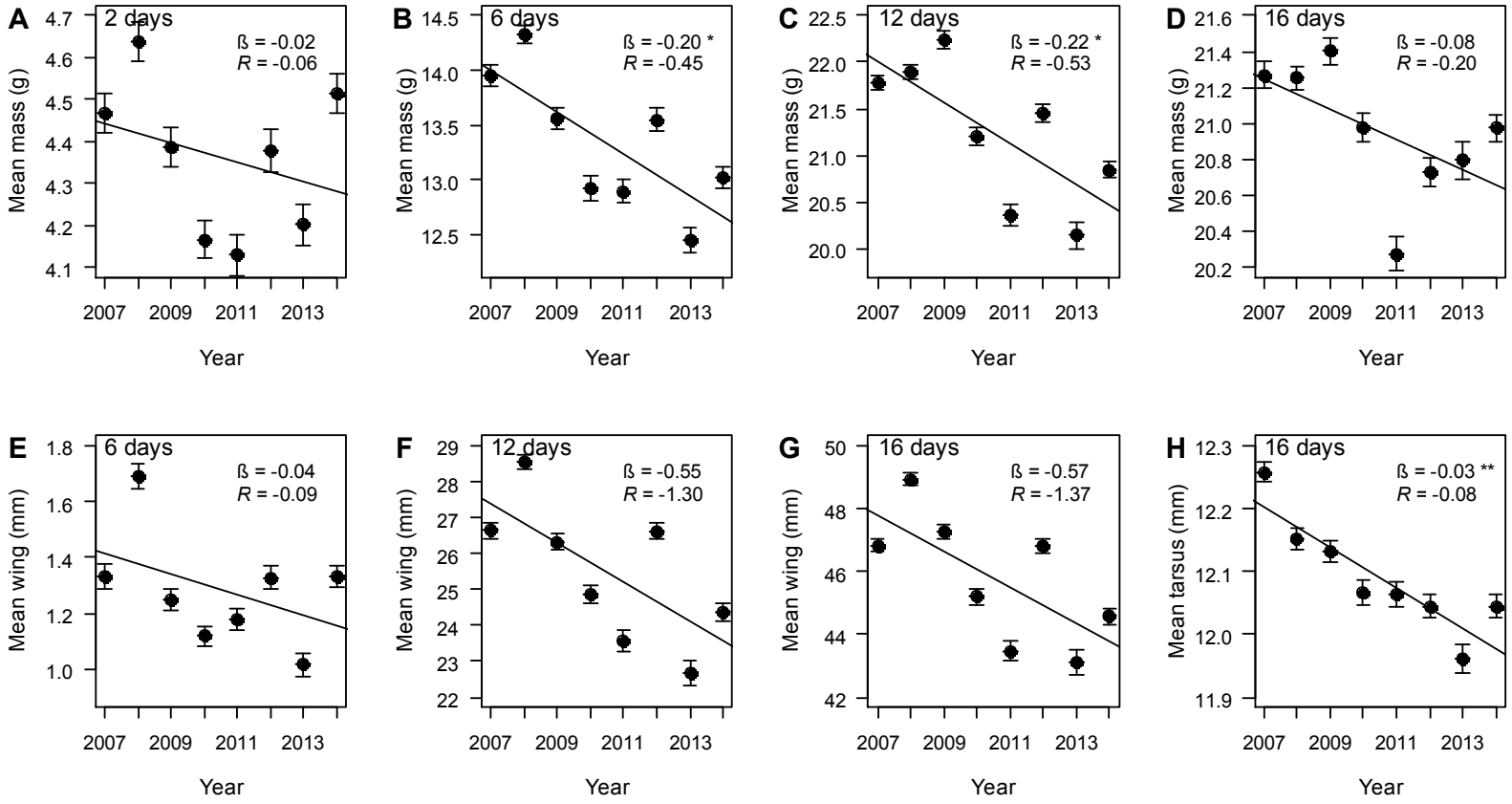

Figure 5.4 Observed phenotypic trends between 2007 and 2014 for 8 trait-age combinations (A-H). Annual trait means (black dots), model predictions (black line, β = model coefficient) and observed phenotypic changes between 2 generations (*R*) are presented. Asterisks indicate different significance levels, $P < 0.05$ (*) and $P < 0.01$ (**) and error bars represent standard errors**.**

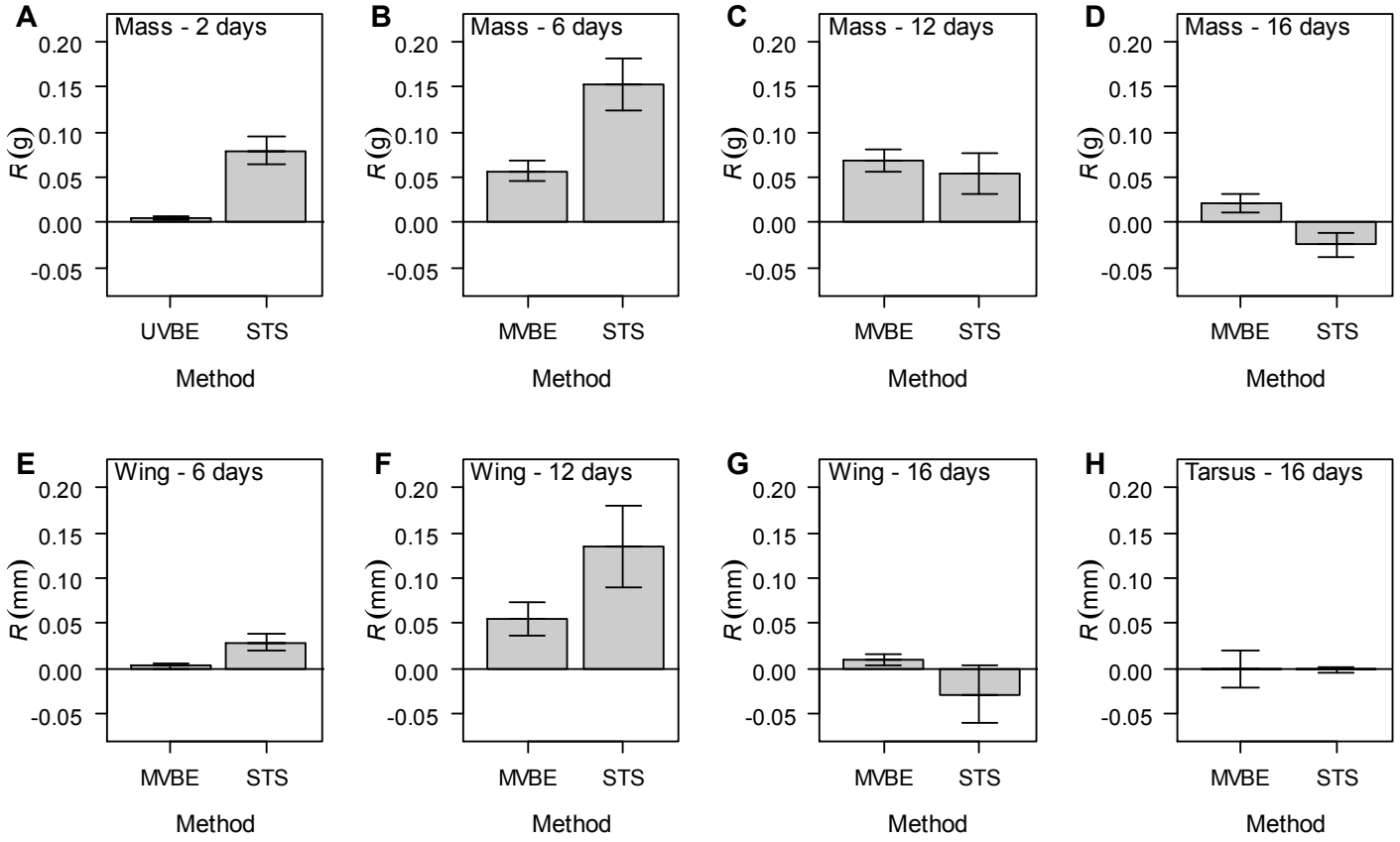

Figure 5.5 Evolutionary change (*R*) predicted with the breeder's equation (univariate (UVBE) and multivariate (MVBE) versions) and the secondary theorem of selection (STS) for 8 trait-age combinations (A-H). *R* represents the change in mean trait between generations and is expressed in the trait unit measurement. Errors bars represent standard errors.

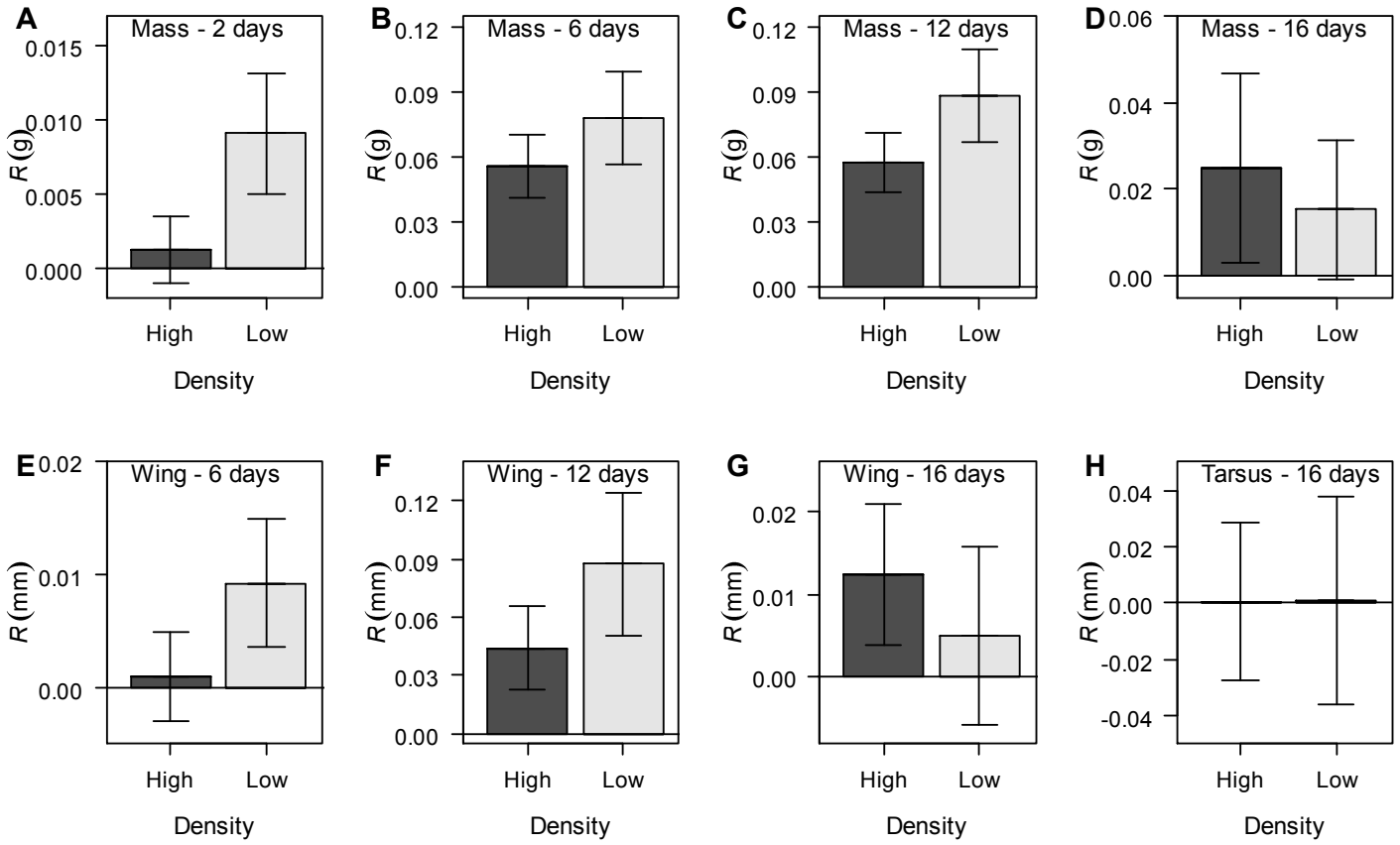

Figure 5.6 Evolutionary changes (R) predicted with the breeder's equation for 8 trait-age combinations (A-H) in low and high density environments. R represents the change in mean trait between 2 generations and is expressed in the trait measurement unit. Errors bars represent standard errors.

As a prerequisite for the use of the second theorem of selection (STS), we observed significant V_A for relative fitness at most ages, but which were declining with age (Figure 5.2d, only V_A for relative fitness of 16-day-old nestlings was not different than zero, χ^2 =7.20, df = 4, P = 0.13). The application of the breeder's equation (BE) and of the STS to our data led to very different predicted evolutionary changes for some age-trait combinations (Figure 5.5). For example, although positive, STS predictions were larger than those obtained from BE for body mass at 2 and 6 days as well as for wing length at 6 and 12 days (Figure 5.5a,b,e,f). Moreover, STS and BE predictions were in opposed direction for body mass and wing length in 16-day-old nestlings (Figure 5.5d,g). Importantly, however, predicted R obtained with both the BE and the STS approach differed significantly from observed phenotypic trends (see Figure 5.4) for body mass at 6 and 12 days, wing length at 12 and 16 days and tarsus length at 16 days (Table S5.A8; all other P-values > 0.05).

Finally, we observed no significant additive genetic variance for relative fitness in high density environments for 12 and 16-day-old nestlings, and thus STS predictions showed convergence problems when including nestling morphological traits under these particular conditions. We thus present results for BE predictions only (STS predictions for 2 and 6-day-old nestlings can be found in Supporting Information, Figure S5.A4). Our results suggested differences between low and high density environments and this, despite large standard errors around evolutionary response predictions (Figure 5.6). We observed a general trend for larger responses in low density environments for all nestling traits at 2, 6 and 12 days. For 16-day-old nestlings, the difference seemed either null or larger in high density environments.

Discussion

Environmental heterogeneity and changes during lifespan can both affect evolutionary responses through their effects on selective pressures and genetic architecture of traits. In this study, we observed important fluctuations in viability selection acting on nestling tree swallows as well as changes in heritability and additive genetic variation of traits from

hatching to fledging. For instance, selection on body mass was stronger in low density environments for 2 and 12-day-old nestlings, and the differences in phenotypic variances between contrasted environments were mainly caused by changes in the brood identity component of variance. Evolutionary responses predicted according to the breeder's equation (BE) and the secondary theorem of selection (STS) were mostly positive but differed strikingly for most trait-age combinations. We also observed a trend for larger evolutionary responses to be predicted in low density environments. However, predicted evolutionary responses, using either the BE or STS, did not match the negative phenotypic trends observed during the same period.

Changes throughout nestling development

Assessing the strength of selection acting on age-specific traits can reveal critical periods during the lifespan of individuals. For tree swallow nestlings, selection on body mass was stronger at intermediate ages (6 and 12 days old), which correspond to the period of highest mass gain (Zach and Mayoh 1982; McCarty 2001). As for most passerines, tree swallow body mass follows a sigmoid growth curve during the nestling phase and adult body mass is generally reached around 12 days old (Zach and Mayoh 1982; McCarty 2001). For wing length, selection varied both in strength and direction during nestling development. Despite the positive selection differential at 6 days old, direct selection acting on wing length (estimated with selection gradients) was negative. At this age, 24 % of nestlings still showed no growth of primary feathers, and this trait could thus also be considered as a presence-absence trait, or early versus late primary feather growth phenotypes. When considered as such, the observed fitness-wing relationship was still negative and significant (β_{WING} = -0.009±0.007, $P < 0.001$), suggesting that it is advantageous for nestlings to delay their wing growth. At this stage, tree swallow nestlings still have poor thermoregulation capacity (Marsh 1980) and therefore energy allocation dedicated to growth may favour body mass increase over wing growth given the tight relationship between body mass increase and surface-area-to-volume ratio decrease which limits heat loss (e.g. Pereyra and Morton 2001). However, given the positive phenotypic correlation between wing length and body mass ($r_s = 0.81$, $P < 0.001$)

and the strong direct selection for higher body mass, the negative direct selection acting on wing length was masked, resulting in an overall positive selection for this trait. Later in development (12 and 16 days old), direct selection on wing length turns positive, resulting this time in a higher overall selection strength for both body mass and wing length compared to the direct selection acting on them.

Changes in genetic architecture across life-history stages can be observed through changes in h^2 and CV_A. While h^2 represents the proportion of the total phenotypic variance explained by additive genetic variance and may be indicative of past selection pressures eroding standing genetic variation (given the negative correlation between selection acting on a trait and its h^2, e.g. Kruuk et al. 2000; McCleery et al. 2004; Wheelwright et al. 2014), CV_A is rather viewed as an indicator of a trait potential to respond to selective pressures, i.e. trait evolvability (Houle 1992; Hansen et al. 2011). Relationships between h^2 and CV_A are however equivocal – being sometimes positive (e.g. Teplitsky et al. 2009), negative (e.g. McCleery et al. 2004) or absent (e.g. Wheelwright et al. 2014). Here, we observed a gradual increase in h^2 for body mass across ages, caused by concurrent variation in V_A and overall V_P. On one side, change in V_A during development could be explained in part by changes in gene expression (Atchley 1984). On the other side, selection episodes between life-history stages could reduce overall V_P, but also specific components such as V_B and V_R when selection is targeting environmental deviations (van Noordwijk 1988; see also Hadfield et al. 2013), thus increasing h^2. On the contrary, the small decrease in body mass CV_A over the same period would suggest that for a similar selective pressure applied on this trait, the relative evolutionary response would be reduced as the nestling development progresses. For wing length, the very low h^2 estimates obtained were consistent with the absence of h^2 observed for the same trait in savannah sparrow nestlings (*Passerculus sandwichensis*, Wheelwright et al. 2014). Yet, adult wing length is generally highly heritable (mean h^2±SD = 0.47±0.19, calculated from 17 estimates from 8 species, Postma 2014) and a recent study suggested that adult feather growth is also heritable (Siberian jay, *Perisoreus infaustus*, h^2 = 0.10, Gienapp and Merilä 2010). It should be noted that the high CV_A observed for wing length at 6 days old was caused by the very small trait mean ($\bar{X}$±SD = 1.30±1.24 mm).

Variation between environments

Stronger selective pressures could be expected under harsher environmental conditions (Siepielski et al. 2013), a pattern we observed here with higher linear and/or non-linear components of selection gradients in lower density environments for body mass at 2 and 12 days old. The chosen environmental variable − the percentage of nest boxes occupied by tree swallows − is a proxy of environmental quality in tree swallow populations because individuals tend to aggregate in higher quality sites (Dunn and Winkler 1999; Bourret et al. 2015). This environmental variable probably encompasses agricultural characteristics as well as other underlying components reflecting habitat quality (e.g. insect abundance, predation risk, etc.). Our results suggest that for a given body mass, nestlings hatched in a low density environment had a lower probability of fledging than those in a high density environment. However, we observed no difference in mean body mass between environments (for all ages $P > 0.26$, linear mixed models including year, farm and brood identity as random effects), only larger overall V_P at 12 and 16 days old in low density environments (see *Quantitative genetics* results). A similar pattern was also previously observed in experimental manipulations where deprived tree swallow nestlings reached the same body mass as control individuals (Wiggins 1990). This suggests compensation through parental feeding effort or a trade-off in resource allocations with other traits, including those expressed later in their life.

Change in genetic architecture between environments can be caused by variations in V_A and/or V_E (Charmantier and Garant 2005). Our results suggest that the changes we observed were due the environmental component. The observed decrease in V_B in high density environments for all 16-day-old traits is in line with previous observations in a blue tit (*Cyanistes caeruleus*) population where experimentally deparasitized broods (mimicking a higher environmental quality) showed a drastic reduction in V_B and V_R for tarsus length compared to control broods (Charmantier et al. 2004). This decrease could be explained by lower variance in parental care quality in higher quality environments since differences in individual quality could not be exacerbated. Interestingly, covariances in brood components among traits in low density environments were positive and strong, which could suggest little evidences of trade-offs in

resource allocation among traits (Figure S5.A3b). For wing length at 6 days old, V_B and overall V_P were reduced in low density environments but there was no difference in the proportion of nestlings with non-zero wing length (P=0.68, generalized linear mixed models (logit link and binomial error structure) including year, farm and brood identity as random effects), a pattern suggesting that feather growth were more similar across broods under more restrictive environmental conditions. Finally, it should be noted that changes in h^2 and CV_A between environments were very small for all trait-age combinations (Figure S5.A5).

Evolutionary responses

The most striking pattern we observed was that the differences between predicted evolutionary responses obtained with the BE and STS approach were generally larger than the differences observed between environmental conditions for a given method. The most probable reason for this discrepancy is that the BE approach can lead to biased estimations when fitness-trait relationships are induced by a covariation with an environmental variable (see Morrissey et al. 2010), a situation highly likely for developmental traits. In tree swallow nestlings, a high food availability (i.e. insects) has a direct positive influence on body mass and feather growth (McCarty and Winkler 1999), and spatial variation in this resource may generate an environmental covariance with fitness. However, while the STS approach may lead to more accurate predictions (Morrissey et al. 2012), its use is still restricted to few studies probably because of limited V_A for fitness components. Additive genetic variance in fitness is theoretically expected to be very low given its tight relationship with selection that would have led its depletion to occur rapidly (Fisher 1930). This is consistent with low V_A observed for fitness components in wild populations (e.g. relative fitness: Morrissey et al. 2012; lifetime reproductive success: Merilä and Sheldon 2000; McCleery et al. 2004; Teplitsky et al. 2009; McFarlane et al. 2014). Also, even when present, very small V_A components may lead to large sampling errors around fitness-trait covariance estimates, impeding the use of the STS approach (Morrissey et al. 2010; see also Shaw and Shaw 2014). Here, we observed between 2 and 12 days old significant V_A for relative fitness in survival, which can be considered as an exceptional finding. Interestingly, the reduction in overall relative fitness variance (i.e. V_P)

across life-history stages we observed represents a reduction in the opportunity for selection through lifespan as predicted by the theory (Arnold and Wade 1984). The similar trend observed for V_A corresponds to the expectations following previous selection episodes. Because of this fitness reduction through time, restricting analysis to nestlings sampled just before fledging (i.e. at 16 days old) would have led to conclude in no significant V_A for fitness, which further emphasizes the importance of considering the differences that may occur among life-history stages.

Phenotypic trends observed during this 8-year-study were negative for all traits, significantly for body mass at 6 and 12 days old and tarsus length at 16 days old, revealing a decrease in nestling body size. Patterns detected in our study are similar to recent observations of body mass decline in other bird populations (reviewed in Yom-Tov et al. 2006; Gardner et al. 2014), including adult females from this population (Rioux Paquette et al. 2014). Two general explanations are usually proposed to explain the patterns of size decrease: evolutionary responses to climate change under the general idea of Bergmann's rule (i.e. size latitudinal cline) or phenotypic plastic responses caused by degradation of environmental conditions (Teplitsky et al. 2008; Husby et al. 2011a). In this study we have assessed evolutionary responses and observed phenotypic trends that differed largely and significantly for 5 out of the 8 trait-age combinations under both the BE or the STS approach (Table S5.A8). Similar discrepancies were observed in other study systems when predictions were obtained with BE (e.g. Kruuk et al. 2001; Garant et al. 2004; Morrissey et al. 2012), potentially related to the drawbacks of BE discussed above. However, to our knowledge, the few predictions obtained using STS were mostly similar to observed phenotypic trends (Morrissey et al. 2012; Stinchcombe et al. 2014; Pigeon et al. 2016). It is thus less clear if the discrepancies observed between STS predictions and observed phenotypic trends in our system were caused by the degradation of environmental conditions over time, were methodological in nature or both. Degradation of environmental conditions could induce plastic changes and also potentially mask evolutionary changes (Cooch et al. 1991; Garant et al. 2004). Such changes in conditions are suspected in this study system given a temporal decline in nest box occupancy across years (Rioux Paquette et al. 2014; Bourret et al. 2015), a pattern also observed at larger spatial

scales (Nebel et al. 2010; Shutler et al. 2012; Michel et al. 2016). Also, methodological issues such as the choice of fitness proxy may have a large influence on predicted evolutionary responses. Here we were limited to the use of survival from hatching to fledging because of the very low nestling recruitment rate (~1%) that prevents to follow most of nestlings up to adulthood. However, it would have been interesting to assess the consequences of using another fitness component (e.g. survival to recruitment, lifetime reproductive success) on STS predictions.

Conclusion

Mismatch between observed and predicted evolutionary have been puzzling evolutionary ecologists (e.g. Merilä et al. 2001a; Garant et al. 2004; Husby et al. 2011a; Gienapp and Merilä 2014). Our results add to the evidences that predicted evolutionary responses of traits rarely match the phenotypic trends observed in wild populations. Our analyses suggest that changes in environmental conditions and across life-history stages in terms of genetic architecture of traits and selective pressure on them can affect predictions from evolutionary models. Their relative impacts on predicted evolutionary responses were, however, relatively small compared to methodological differences in approaches used for their inference. Further analyses and data gathered in this system and in other study systems are thus needed to disentangle the potential causes for the persistent discrepancies between predicted and observed phenotypic trends.

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Chapitre 6

DISCUSSION GÉNÉRALE ET CONCLUSION

Discussion générale

Résumé

Le potentiel évolutif est un élément clé de la persistance à long terme d'une population, lui permettant ou non de répondre aux modifications dans les pressions de sélection. Dans mes travaux de thèse, j'ai étudié les rôles de la plasticité phénotypique et de la variabilité génétique sur le potentiel évolutif d'une population d'Hirondelle bicolore retrouvée dans un environnement hétérogène. Une première série d'analyses a montré que la date de ponte dans ce système d'étude est liée à plusieurs facteurs environnementaux, à des niveaux individuels et populationnels (chapitre 2). Plus particulièrement, les températures printanières semblent induire des réponses plastiques individuelles suivant le patron populationnel observé. En revanche, ces réponses plastiques individuelles pourraient être contraintes dans les fermes peu fréquentées par les hirondelles, c'est-à-dire celles de faibles densités. Le rôle que peut jouer la plasticité adaptative comme réponse aux changements environnementaux semble donc inégal entre les populations d'une même espèce puisque potentiellement limité dans les conditions environnementales les plus contraignantes. Ensuite, l'étude de la variation observée à des gènes candidats liés à la phénologie a montré leurs relations avec la variation observée à deux traits reproducteurs, soit la date de ponte et le temps d'incubation (chapitre 3). De plus, plusieurs de ces relations présentaient des interactions statistiques avec des variables environnementales, dont la densité locale, suggérant l'importance des interactions génotype-environnement sur l'expression des phénotypes en nature. Les deux chapitres suivants de ma thèse se sont plutôt tournés vers une approche en génétique quantitative. Comme étude préliminaire dans ce système, la fiabilité des estimations d'héritabilité de différents traits

morphologiques (adultes et oisillons) et reproducteurs ainsi que des corrélations génétiques entre eux a été déterminée (chapitre 4). Les différentes analyses ont montré les limites actuelles du jeu de données pour l'analyse des traits adultes, autant morphologiques que reproducteurs, ainsi que l'utilité des simulations pour vérifier la précision et l'exactitude des estimations en génétique quantitative. Finalement, les effets de l'hétérogénéité environnementale, des changements au cours de l'ontogénie et de deux approches méthodologiques sur les prédictions des réponses évolutives en milieu naturel ont été évalués simultanément dans une dernière étude qui se voulait intégratrice (chapitre 5). Autant la sélection que l'architecture génétique des traits changeaient selon la qualité de l'environnement (densité faible ou élevée), et des variations étaient aussi présentes au cours du développement des oisillons. L'équation du reproducteur et le second théorème de la sélection ont prédit des réponses évolutives différentes, mais aucune des approches ne semblait plus adéquate vu la grande disparité des prédictions avec les changements phénotypiques observés. Dans les sections qui suivent, je discuterai de ce que ma thèse a permis d'apprendre sur la variation génétique et les différents rôles que peut jouer l'environnement sur le potentiel évolutif en population naturelle, et je soulèverai au passage quelques limites rencontrées.

La variation génétique

La quantité de variation génétique observée à un trait donné est souvent considérée comme un premier indice du potentiel évolutif de ce dernier (Falconer et Mackay, 1996). La première approche utilisée dans ma thèse pour quantifier la variation génétique présente pour des traits d'intérêt était par des gènes candidats ciblés en raison de leur implication dans la régulation du rythme circadien. Les 4 gènes candidats étudiés (CLOCK, NPAS2, ADCYAP1 et CREB1) montraient de la variation entre les individus dans le nombre de répétitions de leur patron de courtes répétitions en tandem (c.-à-d., *short tandem repeats*). Cette variation était associée à la variation phénotypique pour la date de ponte et/ou le temps d'incubation. Si, tel que supposé, les relations établies sont causales, alors ces traits présentent un certain potentiel évolutif. De ces quatre gènes, seul CREB1 montrait une variabilité génétique réduite par rapport à ce qui est observé chez d'autres espèces aviaires (3 allèles, dont un avec une fréquence de 96,7 %;

voir aussi le Tableau S3.E1). Il aurait aussi été intéressant d'aller vérifier s'il existe des interactions entre les différents gènes candidats étudiés, particulièrement CLOCK et NPAS2. Finalement, il faut noter que la variation observée à ces gènes n'explique qu'une faible proportion de la variance phénotypique, plus précisément autour de 2,0 % pour la date de ponte et de 2,5 % pour la durée d'incubation (R^2 marginal d'un modèle mixte ayant comme effets fixes seulement les 4 gènes candidats; Nakagawa et Schielzeth 2013). Cette observation n'est pas surprenante puisque des études effectuées au niveau génomique suggèrent que de la variation phénotypique des traits est expliquée par de nombreux locus à petite taille d'effet (Flint et Mackay, 2009; Manolio *et al.*, 2009; Visscher, 2008). Par exemple, sur les 50 000 SNPs testés pour expliquer la variation dans la taille de couvée chez le Gobemouche à collier (*Ficedula albicollis*), le locus expliquant le plus de variation n'en expliquait que 3,9 % (Husby *et al.*, 2015). Les études ciblant des gènes précis, même s'ils expliquent très peu de la variation phénotypique observée, peuvent permettre de suivre et prédire les changements génétiques au fils du temps (Harrisson *et al.*, 2014).

La génétique quantitative propose une approche différente permettant d'estimer la variance génétique d'un trait à partir d'information sur l'apparentement entre les individus d'une population (Falconer et Mackay, 1996). J'ai obtenu à travers les chapitres 4 et 5 de telles estimations pour une douzaine de traits, reproducteurs ou morphologiques (Tableau 4.2, S5.A7). Or, en raison des limites du jeu de donnée dans l'obtention d'estimations précises et exactes (voir chapitre 4), peu d'informations peuvent être tirées des estimations de variance génétique additive (V_A), d'héritabilité (h^2) et des coefficients de variance génétique (CV_A) obtenues pour tous les traits adultes (morphologiques et reproducteurs), si ce n'est que la présence d'une composante de variance génétique pour la majorité d'entre eux. Néanmoins, l'analyse de traits morphologiques chez les oisillons a permis d'en apprendre davantage sur leur potentiel évolutif. Premièrement, la quantité de V_A semble variable au cours de leur développement et donc le potentiel évolutif également. Ensuite, lorsque comparés entre eux, les traits ne présentaient pas des niveaux similaires de variance génétique. Plus particulièrement, la longueur d'aile présentait une faible proportion de V_A par rapport à V_P, et tel que discuté précédemment, malgré une présence plus importante une fois rendue au stade

adulte (Postma, 2014). Il serait intéressant de vérifier, au lieu de la variance génétique pour un âge précis, celle des caractéristiques des courbes de croissance (p. ex., Irwin et Carter, 2013). Ces caractéristiques pourraient présenter différents niveaux d'h^2 et de CV_A, et possiblement mieux refléter la biologie sous-jacente à la croissance de l'aile (Atchley, 1984). Au final, le potentiel évolutif semblait différent entre les différentes combinaisons trait-âge.

L'héritabilité est la mesure traditionnellement utilisée pour comparer le potentiel évolutif des traits (Falconer et Mackay, 1996). Cette utilisation est par contre vivement critiquée par certains chercheurs puisque l'héritabilité d'un trait ne serait pas liée à son évolvabilité, c'est-à-dire à sa capacité de réponse évolutive à la sélection par unité de force de sélection (Hansen *et al.*, 2011; Houle, 1992). Il est souvent suggéré que les traits fortement associés à l'aptitude phénotypique montrent une plus faible valeur d'h^2 non pas en raison d'une plus faible V_A mais plutôt d'une plus forte V_R (Houle, 1992; Price et Schluter, 1991). Le CV_A représenterait donc une meilleure mesure du potentiel évolutif d'un trait (Hansen *et al.*, 2011; Houle, 1992). Ici, les valeurs d'h^2, de CV_A, de coefficient de variation résiduel (CV_R) et de différentiel de sélection standardisé (*S*, représentant la force s'association entre un trait et l'aptitude phénotypique) peuvent être comparées pour les diverses combinaisons trait-âge étudiées chez les oisillons au chapitre 5 (Figure 6.1). D'une part, la relation entre l'h^2 et la sélection *S* agissant sur eux est très faible, mais reste tout de même dans la direction attendue (c.-à-d., négative; Figure 6.1a). De l'autre, la relation entre le CV_A et la sélection *S* était plutôt positive, comme ce que l'on retrouve dans la littérature (Figure 6.1b; Houle, 1992; Postma, 2014, mais voir Teplitsky *et al.* 2009 pour un patron contraire). Par contre, il ne semble pas y avoir une relation claire entre CV_R et *S* (Figure 6.1c). Finalement, les valeurs d'h^2 et de CV_A semblent corrélées négativement, tel qu'observé généralement dans la littérature (Figure 6.1d; Hansen *et al.*, 2011; Postma, 2014). Ces différentes relations présentées sont bâties sur un nombre très restreint d'observations (N=8), appartenant à une seule catégorie de trait (morphologique) et aucune n'est significative. Néanmoins, elles révèlent la complexité de définir efficacement le potentiel évolutif d'un trait.

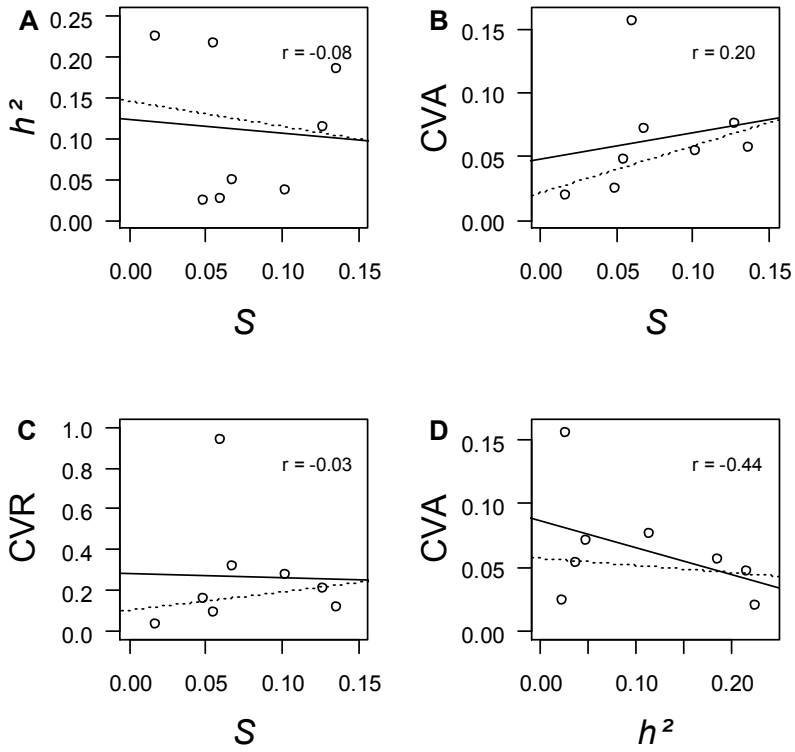

Figure 6.1 Relations entre l'héritabilité (h^2), le coefficient de variation génétique (CV_A), le coefficient de variation résiduel (CV_R) et le différentiel de sélection standardisé (S) des 8 combinaisons trait-âge pour les oisillons (cercle). La ligne pleine représente la prédiction d'un modèle linéaire simple alors que la ligne pointillée montre plutôt la prédiction du même modèle en enlevant les observations pour la longueur de l'aile à 6 jours. Le coefficient de corrélation (r) est aussi présenté. Les données sont tirées des résultats du chapitre 5.

Les traits ne sont pas indépendants les uns des autres et, lorsque présentes, les corrélations génétiques entre eux peuvent contraindre ou faciliter les réponses à la sélection (Tableau 1.1; Falconer et Mackay, 1996; Teplitsky *et al.*, 2014a). Chez les oisillons, des covariances génétiques positives et significatives ont été observées entre la masse et la longueur d'aile des

oisillons de 12 jours et la masse et la longueur du tarse de ceux de 16 jours (Figure S4.C1, S5.A3). Dans tous les cas, vu les gradients de sélection positifs pour les traits impliqués, il n'y aurait pas de contrainte à leur évolution, mais plutôt une facilitation (Tableau 1.1). Toutes les autres covariances testées n'étaient pas significativement différentes de zéro, possiblement en raison du manque de puissance des données dans certains cas, particulièrement pour les traits d'adultes (voir la Figure S4.C1). Par contre, d'autres types de covariance génétique qui n'ont pas été pris en compte pourraient influencer le potentiel évolutif des traits chez l'Hirondelle bicolore, comme celles entre les différents âges (p. ex., Badyaev et Martin, 2000; Hadfield *et al.*, 2013) ou celles entre les sexes (p. ex., Forstmeier *et al.*, 2011; Mainguy *et al.*, 2009; Poissant *et al.*, 2010). Il serait pertinent d'aller étudier ces deux types d'interactions chez les oisillons pour compléter le portrait de leur potentiel évolutif.

Une limitation importante aux questions pouvant être répondues par une approche en génétique quantitative dans ma thèse était la qualité du pédigrée disponible. Plus particulièrement, le plus gros du problème se situait au niveau du très faible taux de recrutement des oisillons en tant qu'adultes (1,3 %), limitant de ce fait notre connaissance de l'apparentement entre les adultes. La qualité du pédigrée va assurément augmenter d'elle-même par l'ajout de données année après année. Dans un monde idéal (c.-à-d., avec des ressources financières illimitées), il serait également possible d'établir les coefficients d'apparentement entre tous les individus capturés dans le système d'étude à l'aide de marqueurs génétiques à haute densité (Bérénos *et al.*, 2014). Il y a fort à parier que les individus bagués la première fois comme adultes dans le système d'étude soient apparentés entre eux à un certain degré. En effet, les oisillons montrent une tendance à recruter comme adultes près de leur lieu de naissance puisque la médiane des distances des recaptures entre le stade oisillon et adulte est de 9.1 km (Figure 6.2). Cette observation est aussi similaire à ce qui a été fait à plus large échelle (médiane = 2,3 km, N = 592, Winkler *et al.*, 2005). Conséquemment, les adultes nichant le plus près géographiquement devraient également être génétiquement plus semblables (mais voir les résultats de Porlier *et al.*, 2009, qui suggèrent l'absence d'un tel patron). En plus d'ajouter de l'information sur l'apparentement entre les adultes arrivant dans le système (c.-à-d., 95 % des adultes), ce type d'analyse pourrait mieux

connecter les oisillons entre eux dans le pédigrée, particulièrement les 26 % pour lesquels aucun père génétique n'a été déterminé avec les assignations parentales. Finalement, ces informations pourraient permettre de vérifier directement l'apparentement génétique entre les partenaires. En effet, bien qu'il y ait peu de raisons de suspecter un fort apparentement entre les partenaires, ignorer de tels effets s'ils sont présents pourraient entre autres biaiser nos estimations d'héritabilité (Abney *et al.*, 2000). Étant donné que le coût associé aux technologies de séquençage est en baisse, il reste permis de penser qu'un jour elle sera plus accessible pour les espèces non modèles comme l'Hirondelle bicolore (van Dijk *et al.*, 2014).

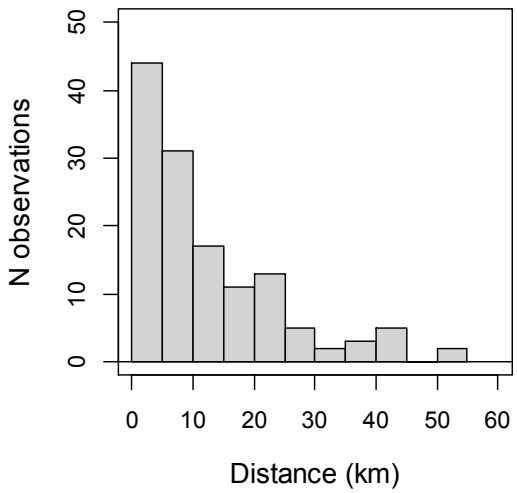

Figure 6.2 Histogramme des distances euclidiennes (km) observées entre la ferme où un individu est né et celle où il a été observé pour la première fois en tant qu'adulte (N=133 individus). La distance moyenne (± écart type) entre les fermes est de 42,2±21,1 km (étendue : 1,9 – 103,1 km).

Les multiples facettes de l'environnement

L'hétérogénéité environnementale a pris différentes formes tout au long de ma thèse. Premièrement, elle a pris la forme de variation dans l'espace, en latitude et en longitude, à laquelle ont été associées des variations phénotypiques – dates de ponte plus hâtive plus au nord (chapitre 2) – et génotypiques – cline longitudinale dans les allèles du gène NPAS2 chez les femelles (chapitre 3). Ensuite, l'environnement variait aussi dans le temps, ce qui a pu être quantifié au niveau des déterminants environnementaux de la ponte – augmentation des températures et déclin dans le pourcentage d'occupation des nichoirs (chapitre 2) – mais aussi extrapolé pour expliquer les discordances entre les réponses évolutives prédites et les patrons phénotypiques observés (chapitre 5). La variation environnementale au niveau des températures printanières a aussi été interprétée comme un indice permettant la synchronisation des évènements de reproduction dans un contexte de plasticité phénotypique anticipatoire (chapitre 2). Bien que nos analyses ne testaient pas la causalité de la relation entre température et date de ponte, de tels liens ont été largement documentés chez les oiseaux (Caro *et al.*, 2013; Visser *et al.*, 2009). Au niveau des gènes candidats, des interactions avec diverses variables environnementales (températures printanières, densité locale et latitude) ont également été mises de l'avant. Finalement, la qualité de l'environnement a été étudiée au moyen des variations dans densité locale, et elle semblait contraindre les réponses plastiques – réponses individuelles à la température moins prononcées à faible densité (chapitre 2) – et évolutives – augmentation des pressions de sélections et de la composante génétique liée à l'identité de la couvée (V_B) à faible densité (chapitre 5). L'environnement semble donc affecter le potentiel évolutif des populations simultanément dans plus d'une dimension, et ce potentiellement par différents mécanismes.

La densité locale, définie tout au long de ma thèse comme le pourcentage de nichoirs occupés par un couple d'hirondelles en première couvée, est le facteur environnemental qui est ressorti le plus fréquemment comme étant important (chapitre 2, 3, 5). Les déterminants de l'occupation des nichoirs par l'Hirondelle bicolore ont été étudiés précédemment par Robillard *et al.* (2013); la probabilité d'occupation des nichoirs était négativement corrélée avec la

présence de Moineaux domestiques (*Passer domesticus*) et positivement corrélée avec le succès reproducteur de l'année précédente et avec la distance entre les nichoirs et les bâtiments de fermes. Fait intéressant, le pourcentage de cultures intensives à 500 mètres ne semblait pas avoir d'impacts sur l'occupation des nichoirs par les hirondelles, seulement sur l'occupation des nichoirs par les moineaux (plus forte occupation dans les milieux plus intensifs). D'autres facteurs n'ayant pas été formellement testés pourraient aussi affecter la densité locale, comme l'abondance de ressources alimentaires (c.-à-d., d'insectes; Hussell, 2012; voir aussi Rioux Paquette *et al.*, 2013) ou encore le taux de prédation (Etterson *et al.*, 2007; McIntyre *et al.*, 2014). Jusqu'à présent, les caractéristiques liées à l'utilisation des terres agricoles (p. ex., proportion d'agriculture intensive ou extensive dans un rayon de 500 m ou 5 km) représentaient les indices de choix de la qualité de l'environnement dans ce système d'étude particulier; alors que certaines études ont établi des liens entre des traits d'histoire de vie de l'Hirondelle bicolore et ces caractéristiques agricoles (p. ex., Baeta *et al.*, 2012; Ghilain et Bélisle, 2008; Lessard *et al.*, 2014; Pigeon *et al.*, 2013), d'autres n'ont pas observé les relations attendues (p. ex., Rioux Paquette *et al.*, 2014; cette étude, voir le Tableau S5.A1). Il serait intéressant d'inclure la densité plus systématiquement dans les études futures sur ce système afin de déterminer dans quels cas la densité locale explique mieux les différences entre individus que les caractéristiques plutôt liées à l'agriculture.

Finalement, puisque toute variation phénotypique induite par l'environnement peut être englobée dans le concept de plasticité phénotypique (Bateson, 2015; DeWitt et Scheiner, 2004; Stearns, 1989), on peut considérer que la plasticité phénotypique a été présente tout au long de ma thèse sans nécessairement être explicitement nommée ainsi (chapitre 2, 3, 5). Bien que le rôle, adaptatif ou non, des réponses plastiques observées dans ma thèse n'ait pas été formellement testé (voir Ghalambor *et al.*, 2007), il est tout de même possible de penser que les réponses plastiques liées à la température et à la latitude étaient adaptatives, comme la plasticité individuelle et populationnelle de la date de ponte (chapitre 2) et les interactions GxE avec les gènes candidats (chapitre 3). Au contraire, les réponses plastiques liées aux variations dans la densité locale semblaient plutôt non adaptatives, telles que la plasticité populationnelle de la date de ponte (chapitre 3), les interactions GxE avec les gènes candidats

(chapitre 3) et variations dans l'architecture génétique des traits (chapitre 4). Pour en revenir au rôle encore peu documenté de la plasticité phénotypique dans le potentiel évolutif des populations naturelles, il serait important de décortiquer les différentes réponses plastiques qui arrivent simultanément afin de mieux comprendre l'importance relative de la plasticité qui est adaptative et de celle qui ne l'est pas (Forsman, 2015). Éventuellement, la meilleure approche pour prédire la persistance ou non d'une population face aux changements dans les conditions environnementales devrait inclure des informations portant à la fois sur des aspects génétiques, biotiques et démographiques (Chevin *et al.*, 2010; Lavergne *et al.*, 2010). En outre, des études comme celles présentées dans ma thèse constituent les fondations d'une compréhension plus globale des différents phénomènes qui peuvent toucher simultanément les populations.

Conclusion

Cette thèse se voulait une étude du potentiel évolutif en population naturelle, plus particulièrement des aspects de plasticité phénotypique et de variation génétique. À travers les différents chapitres qui la composent, j'ai pu montrer leur importance et leurs limites dans les réponses possibles aux changements dans les pressions de sélection, de même que la contribution considérable de l'hétérogénéité environnementale dans les patrons observés. Les études du potentiel évolutif en nature restent rarissimes, et les résultats de mes travaux de recherche améliorent les connaissances sur quelques-uns des facteurs qui peuvent le moduler. De plus, ces travaux représentent une première évaluation de l'architecture génétique de traits chez l'Hirondelle bicolore depuis ceux de Wiggins (1989, 1990) il y a 25 ans. Au final, mes résultats pourront servir de points de départ pour continuer l'investigation du potentiel évolutif chez cette espèce et de pistes de réflexion pour les études dans d'autres systèmes. Plusieurs questions restent sans réponses quant aux futurs des populations faisant face à des changements environnementaux importants, et la clé d'une meilleure compréhension reste encore l'acquisition de connaissances à travers différents systèmes biologiques, incluant celui de l'Hirondelle bicolore dans le Sud du Québec.

ANNEXES

Annexes Chapitre 2

Appendix A: Supplementary information on methods and results

Table S2.A1 Sample sizes of clutches and female tree swallows (for first breeding attempts only) between 2004 and 2013.

	2004	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**Total**
Clutches	216	292	295	256	244	226	217	214	200	216	2376
Females	180	257	250	217	212	186	198	181	180	172	2033
SY	-	46	17	19	30	28	35	36	40	34	285
ASY	-	211	233	198	182	158	163	145	140	138	1568

Table S2.A2 Information on the meteorological stations used in the analysis. Station name and ID refer to Environment Canada unique identification (http://meteo.qc.ca/), and underlined stations were used in the sliding windows analysis. Latitude and longitude are in decimal degrees, and the number of farms closest to each meteorological station is also reported.

Station name	Station ID	Latitude	Longitude	N closest farms
Brome	7020840	45.18	-72.57	2
Bromptonville	7020860	45.48	-71.95	3
Farnham	7022320	45.30	-72.90	3
Granby	7022800	45.38	-72.72	6
Marieville	7024627	45.40	-73.13	3
Richmond	7026464	45.63	-72.13	5
St-Guillaume	7027302	45.88	-72.77	5
Ste-Madeleine	7027517	45.62	-73.13	3
Ste-Hyacinthe	7027361	45.57	-72.92	3
St-Nazaire	7027588	45.73	-72.62	7

Details on treatment of meteorological data: From the raw data of Environment Canada, daily value with indicator "E" (i.e., estimated) and "C" (i.e., precipitation occurred, amount uncertain) were excluded. When more than five consecutive daily values were missing, they were replaced with those from the closest meteorological station (7 replacement periods over 10 years of data from 5 meteorological stations).

Table S2.A3 Descriptive statistics of environmental variables included in the statistical analyses prior to standardization, for A) the environmental determinant analysis (see table 2.1), B) the change in laying date analysis (see table 2.2) and C) the random regression model (see table 2.3).

Analysis	**Environmental variable**	**Range**	**Mean**	**Standard deviation**
A) Environmental determinants	Density	0.1 – 1.0	0.6	0.3
	Latitude	45.26 – 45.99	45.56	0.18
	Elevation	-20.7 – 259.0	71.5	83.2
	Temperature	4.93 – 10.57	8.01	1.15
	Precipitation	0.00 – 9.00	1.97	2.07
B) Change in laying date				
SY dataset	ΔTemperature	-2.31 – 3.04	0.50	1.35
	ΔDensity	-0.50 – 0.50	0.00	0.20
ASY dataset	ΔTemperature	-3.13 – 4.04	0.32	1.62
	ΔDensity	-0.70 – 0.40	0.00	0.16
TOTAL dataset	ΔTemperature	-3.13 – 4.04	0.42	1.56
	ΔDensity	-0.70 – 0.50	0.00	0.17
C) Random regression model	Latitude	45.26 – 45.99	45.56	0.19
	Temperature	4.93 – 10.57	8.03	1.15
	Density	0.10 – 1.00	0.75	0.22

Table S2.A4 Linear mixed effects model used to assess if slopes of within-individual (β_W) and between-individual environmental components (β_B) are similar or not (following van de Pol and Wright 2009, equation 3; see also table 2.3). The random effect structure was identical to model 5 in the random regression analyses.

Estimates of fixed effects	**Estimate**	**S.E.**	**t-value**	**P-value**
Intercept (β_0)	138.605	0.752	184.41	<0.001
Age	7.274	0.646	11.25	<0.001
Latitude	0.638	0.303	2.11	0.048
$Temperature_{within}$ (β_W)	-1.408	0.468	3.01	0.004
$Temperature_{between}$ - $Temperature_{within}$ (β_B- β_W)	0.414	0.472	0.88	0.38
$Density_{within}$ (β_W)	-0.347	0.421	0.82	0.41
$Density_{between}$ - $Density_{within}$ (β_B- β_W)	-1.039	0.500	2.80	0.039

Reference

van de Pol M. and J. Wright. 2009. A simple method for distinguishing within- versus between-subject effects using mixed models. Animal Behaviour 77:753–758.

Appendix B: Additional individual plasticity analyses

Results from the random regression model and the environmental determinant analyses suggest that habitat with lower density could constraint laying date plasticity in response to spring temperature (see Results section in the main text). To further explore this hypothesis, we conducted additional individual plasticity analyses using our datasets subdivided into high and low breeder densities. These additional analyses were conducted for both plasticity analyses (change in laying date and random regression model) and subsets were created based on the median of mean individual values of observed densities (table S2.B1-3) or for the quarter of lower/higher mean individual values of observed densities (table S2.B2, change in laying date analysis only).

Table S2.B1 Analyses of change in laying date between two consecutive years by female tree swallows in relationship to change in spring temperature and breeder density for a subset of A) low density of breeders (158 females) and B) high density of breeders (173 females). The subsets were created based on the median of mean individual values of observed densities (18 females with the median were excluded).

Model	Variable	Estimate	S.E.	t-value	P-value
Low density of breeders	Intercept	-2.037	0.694	2.94	0.004
	Age	-6.566	1.545	4.25	<0.001
	ΔTemperature	-1.738	0.656	2.63	0.009
High density of breeders	Intercept	-2.697	0.608	4.43	<0.001
	Age	-6.673	1.540	4.33	<0.001
	ΔTemperature	-2.644	0.539	4.91	<0.001

Table S2.B2 Analyses of change in laying date between two consecutive years by female tree swallows in relationship to change in spring temperature and breeder density for a subset of A) low density of breeders (87 females) and B) high density of breeders (80 females). The subsets were divided based on the quartiles of higher/lower of mean individual values of observed densities.

Model	Variable	Estimate	S.E.	t-value	P-value
Low density of breeders	Intercept	-3.263	0.973	3.35	0.001
	Age	-5.292	2.074	2.55	0.013
	ΔTemperature	-2.010	0.976	2.06	0.043
High density of breeders	Intercept	-1.781	0.466	3.83	<0.001
	Age	-3.440	1.195	2.88	0.005
	ΔTemperature	-3.665	0.461	7.96	<0.001

Table S2.B3 Random regression analyses of the effect within-individual (β_W) and between-individual (β_B) components of spring temperature on female tree swallow laying dates for a subset of A) low density of breeders (434 observations on 176 females) and B) high density of breeders (456 observations on 175 females). The subsets are divided based on the median of mean values of observed densities (19 females with the median were excluded). Estimates of fixed effects and variance components of random effects (in bold) are presented.

A) Low density of breeders							
Models			Log-L	Test	d.f.	LRT	P-value
1**. Year + Farm + Female**			-1359.6		9		
2. Year + Farm + Female X $Temperature_{within}$			-1359.9	1 vs. 2	11	0.03	0.99
Estimates of fixed effects	**Estimate**	**S.E.**	**t-value**	**P-value**	**Random effects**		**Var**
Intercept (β_0)	139.500	0.789	176.79	<0.001	Female (intercept)		7.765
Age	7.301	0.892	8.18	<0.001	Year (intercept)		3.404
Latitude	0.835	0.522	1.60	0.12	Farm (intercept)		3.923
$Temperature_{within}$ (β_W)	-1.548	0.605	2.56	0.016	Residual		21.982
$Temperature_{between}$ (β_B)	-0.785	0.658	1.19	0.24			
B) High density of breeders							
Models			Log-L	Test	d.f.	LRT	P-value
1. **Year + Farm + Female**			-1410.2		9		
2. Year + Farm + Female X $Temperature_{within}$			-1410.0	1 vs. 2	11	0.30	0.86
Estimates of fixed effects	**Estimate**	**S.E.**	**t-value**	**P-value**	**Random effects**		**Var**
Intercept (β_0)	137.866	0.739	186.58	<0.001	Female (intercept)		6.527
Age	7.571	1.060	7.14	<0.001	Year (intercept)		3.207
Latitude	0.415	0.409	1.02	0.33	Farm (intercept)		1.439
$Temperature_{within}$ (β_W)	-1.904	0.572	3.33	0.003	Residual		21.726
$Temperature_{between}$ (β_B)	-1.566	0.644	2.43	0.019			

Annexes Chapitre 3

Appendix A: Environmental determinants of incubation duration

The method used to define incubation duration in this study (i.e. [hatching date - incubation initiation date]) differed slightly from the traditional way of calculating incubation duration (i.e. hatching date – [laying date + clutch size – 1]). In fact, the method used here gives a better fit with incubation duration obtained from thermocrons placed within a subset of nest boxes in 2013 (N=34, r=0.88, P<0.001) compared to the traditional method (N=34, r=0.19, P=0.27). These thermocrons collected temperature every 2 minutes from a few days after laying date till hatchling date and days with abrupt temperature increase were considered as incubation initiation date.

For incubation analysis, daily mean temperature (°C) and daily rainfall (mm) were obtained from sliding windows analyses following the methodology described in Bourret *et al.* (submitted) with slight modifications. We tested windows varying from 5 to 121 days, from Julian days 60 to 181 (respectively March 1 and June 31 in non-leap years) for a total of 6903 windows. The strongest correlation of incubation duration and mean temperature was between Julian days 138 and 159 (May 18 – June 8; r=-0.999, P<0.001, while for rainfall, this window was between Julian days 105 and 117 (April 15 – 27; r=-0.990, P=0.010). We then used these periods as our references for computing both annual mean temperatures and annual rainfalls of ten meteorological stations near our farms.

We investigated the relative importance of different environmental variables on annual mean incubation duration observed on a farm (N=148, 12 farm-year with no observations) with a linear model (farm identity was tested as random effect using a Likelihood Ratio Test (LRTs) but was not significant and thus we used a simple linear model). The full model included annual mean temperature and rainfall (from sliding window analysis), breeding density (% of nest boxes occupied on a farm), longitude and latitude (decimal degree) and two-way interactions with breeding density. We did not include year and elevation as they were both

highly correlated with temperature (r=0.74) and elevation (r=0.92), respectively. All explanatory variables were standardized (zero mean, unit variance, Table S3.B3). We determined the final model by sequentially removing the least significant term from the model based on its *P*-value until all remaining variables were significant (α=0.05) (Crawley 2007). This model suggests a negative relationship between incubation duration and both longitude and temperature (Table S3.A1).

Table S3.A1 Final linear model of the environmental determinants of mean incubation duration (N=148) in tree swallows. Explanatory variables were standardized and adjusted R^2 for fixed effects was 0.195.

Variables	**Estimates**	**S.E.**	**t-value**	***P*-value**
Intercept	11.340	0.076	149.87	<0.001
Longitude	-0.233	0.084	2.79	0.006
Temperature	-0.513	0.084	6.14	<0.001

Appendix B: Supplementary information on methods

Table S3.B1 Sample size of breeders (males and females) and nests (for first breeding attempts only) used in this study for laying date/incubation duration of tree swallows breeding between 2010 and 2013 in the southern Quebec study system.

	2010	**2011**	**2012**	**2013**	**Total**
N clutches	217/165	214/157	200/154	216/124	847/600
with 2 adults known	142/133	121/118	119/117	103/101	485/469
with 2 adults known and genotyped	142/133	119/116	119/117	102/100	470/466

Table S3.B2 Details of PCR products and amplification conditions for candidate gene amplifications, following Jonhsen *et al.* (2008; CLOCK) and Steinmeyer *et al.* (2009; ADCYAP1, CREB1, NPAS2).

	CLOCK	**NPAS2**	**ADCYAP1**	**CREB1**
PCR products				
Buffer GOLD (1X)	1	1	1	1
$MgCl_2$ (mM)	2.5	2	1	2
dNTPs (µM)	0.20	0.20	0.20	0.20
BSA (mg/ml)	0.40	-	-	-
Reverse primer (mM)	0.50	0.50	0.50	0.50
Forward primer (mM)	0.25	0.50	0.50	0.50
Taq – Amplitaq Gold – Life technologies (U)	1	1	1	1
DNA (ng)	10	10	10	10
PCR amplification conditions				
Initial denaturation	92 °C / 3 min	95 °C / 3 min	95 °C / 3 min	95 °C / 3 min
Denaturation	92 °C / 30 s	95 °C / 30 s	95 °C / 30 s	95 °C / 30 s
Annealing	53 °C / 30 s	53 °C / 30 s	51 °C / 30 s	55 °C / 30 s
Elongation	72 °C / 30 s	72 °C / 60 s	72 °C / 60 s	72 °C / 60 s
Final elongation	72 °C / 30 s	72 °C / 7 min	72 °C / 7 min	72 °C / 7 min
N cycles	35	35	35	35

Table S3.B3 Descriptive statistics of variables included in the statistical analyses prior to standardization (zero mean and unit variance), for A) the environmental effects on genetic variation distribution analysis (see Table 3.2) and B) the genotypic effects on reproductive parameters analysis (see Table 3.3).

Analysis	**Environmental Variable**	**Range**	**Mean**	**Standard deviation**
A) Environmental effects on genetic variation distribution	Breeding density	0.1 – 1.0	0.7	0.2
	Latitude	45.26 – 45.99	45.56	0.18
	Longitude	-73.24 – -71.98	-72.65	0.33
	Mean temperature	10.95 – 14.19	12.52	0.75
B) Genotypic effects on reproductive parameters	Breeding density	0.1 – 1.0	0.7	0.2
	Latitude	45.26 – 45.99	45.55	0.18
	Longitude	-73.24 – -71.98	-72.64	0.33
	April temperature	6.21 – 10.57	8.38	1.11
	May temperature	13.75 – 18.86	16.69	1.15
	CLOCK female	358 – 370	365.6	2.7
	CLOCK male	358 – 370	365.6	2.6
	NPAS2 female	333 – 351	345.7	2.9
	NPAS2 male	333 – 360	345.8	3.1
	ADCYAP1 female	336 – 368	350.1	5.1
	ADCYAP1 male	338 – 370	350.4	5.3
	CREB1 female	524 – 528	526.0	0.6
	CREB1 male	524 – 528	525.9	0.5

Appendix C: Adjusted repeatability for laying date and incubation duration

We computed adjusted repeatability (R_{adj}) for laying date and incubation duration to assess the individual identity influence on these traits. To disentangle the relative impact of female and social male identity, we calculated R_{adj} from a linear mixed model where both identities were included as random effects (Liedvogel *et al.* 2009; Chakarov *et al.* 2013). We also included in this model female age class and relevant environmental variables described in the main text as fixed effects and year as random effect to account for possible confounding factors (year could not be included as a fixed effect because it was highly correlated with May temperature; r=-0.72, P<0.001, VIF=3.53) (Nakagawa & Schielzeth 2010).

For laying date, the adjusted repeatability for females and males were 0.320 and 0.181, respectively, while for incubation duration, it was 0.195 for females and 0.070 for male.

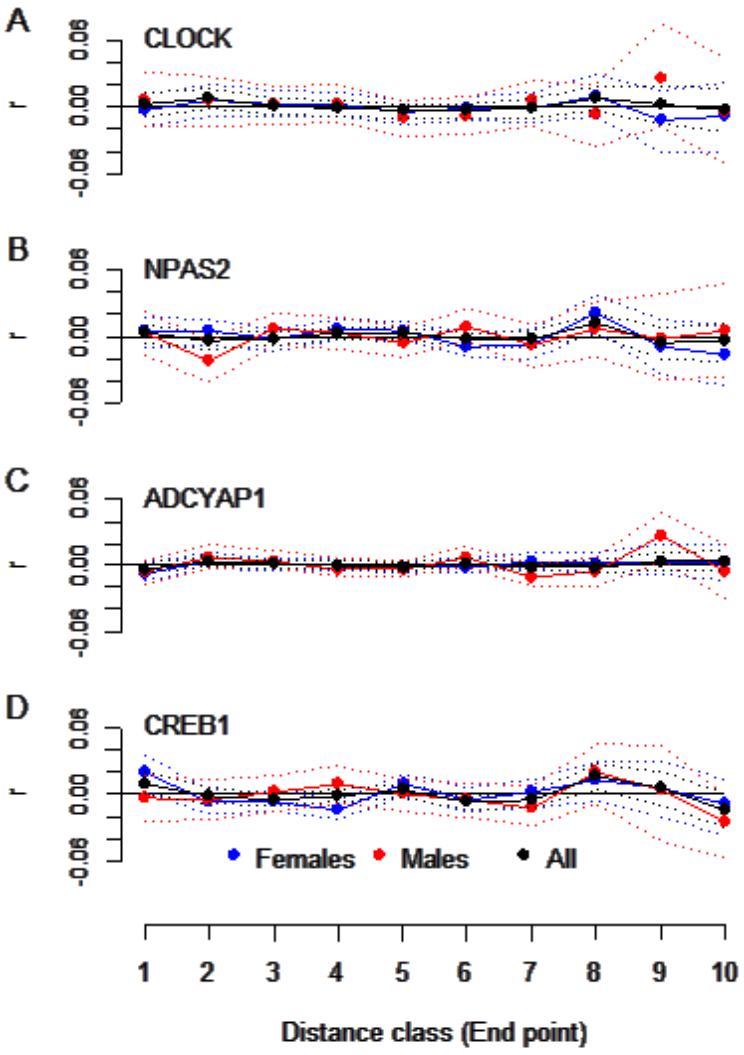

Figure S3.D1 Spatial autocorrelation analysis for A) CLOCK, B) NPAS2, C) ADCYAP1 and D) CREB1 alleles of tree swallows breeding between 2010 and 2013 in this study. Females (blue), males (red) and all individuals (black) were tested for 10 distance classes (circle). 95% confidence intervals are presented for both sexes and overall (dotted lines).

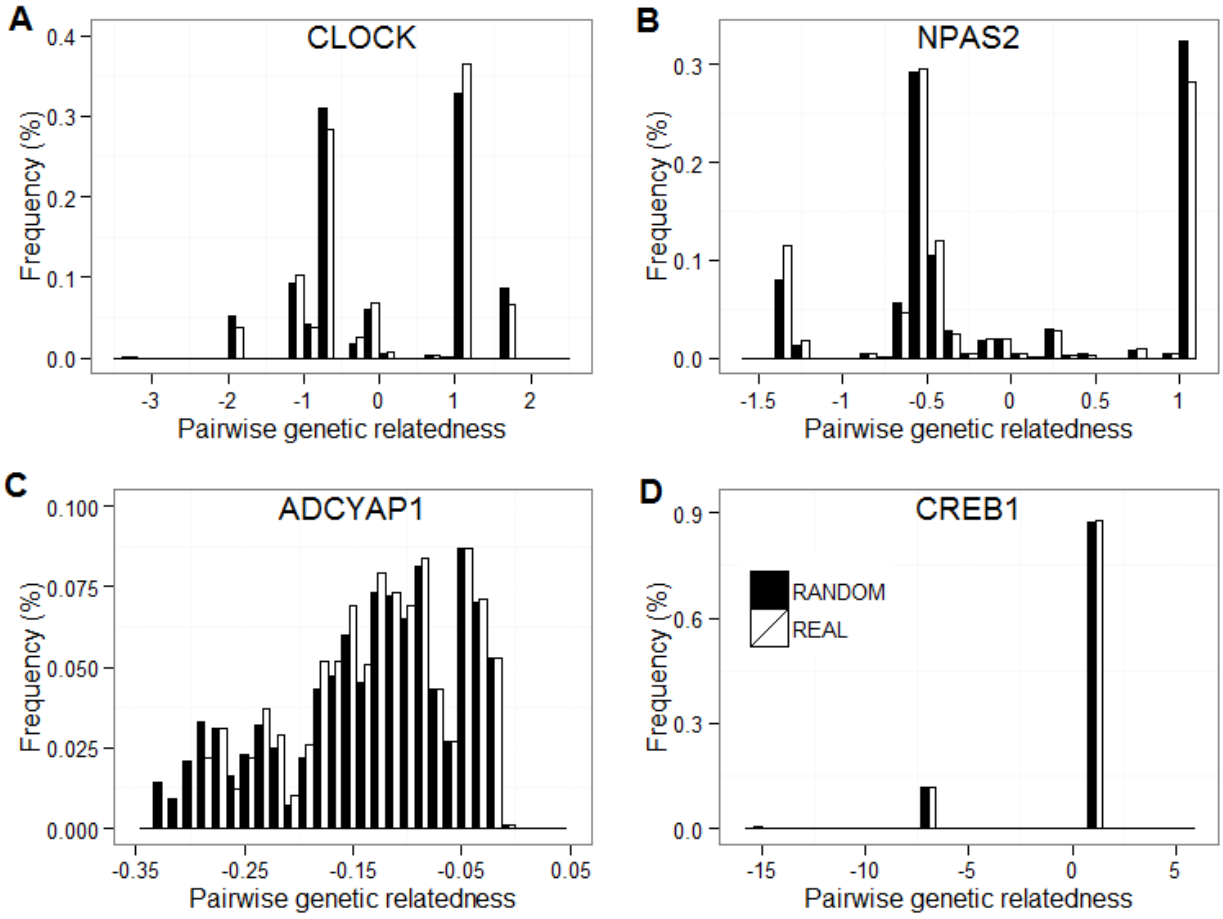

Figure S3.D2 Distribution of estimates of pairwise genetic relatedness (*r*) of observed mating pairs (white bar) and random mating pairs (black bar) of tree swallows breeding between 2010 and 2013 in this study for A) CLOCK, B) NPAS2, C) ADCYAP1 and D) CREB1.

Table S3.D1 Full linear mixed model of laying date for CLOCK male and female genotypes. Female age class and environmental variables were included as fixed effects and tested for interactions with breeder genotypes. Female identity, male identity and year were included as random effects and all explanatory variables were standardized. Bold variables were kept in the final model.

Variables	Estimates	S.E.	t-value	*P*-value
Intercept	138.919	0.877	158.39	<0.001
Age	5.440	0.705	7.72	<0.001
Density	-1.252	0.297	4.22	<0.001
April temperature	-1.058	0.479	2.21	0.033
CLOCK female	0.556	0.296	1.88	0.061
CLOCK female X Density	0.659	0.295	2.24	0.026
CLOCK male	0.496	0.286	1.73	0.084
Latitude	0.294	0.303	0.97	0.33
Longitude	-0.121	0.395	0.31	0.76
CLOCK male X April temperature	0.326	0.274	1.19	0.23
CLOCK female X Age	-0.719	0.702	1.02	0.31
CLOCK female X Latitude	-0.105	0.320	0.33	0.74
CLOCK male X Longitude	-0.360	0.331	1.09	0.28
CLOCK male X Density	-0.264	0.293	0.90	0.37
CLOCK female X April temperature	-0.239	0.269	0.89	0.38
CLOCK male X Longitude	0.212	0.361	0.59	0.55
CLOCK female X Latitude	-0.123	0.319	0.39	0.70
CLOCK female X CLOCK male	-0.072	0.256	0.28	0.78
CLOCK male X Age	0.143	0.612	0.23	0.82

Table S3.D2 Full linear mixed model of laying date for NPAS2 male and female genotypes. Female age class and environmental variables were included as fixed effects and tested for interactions with breeder genotypes. Female identity, male identity and year were included as random effects and all explanatory variables were standardized. Bold variables were kept in the final model.

Variables	Estimates	S.E.	t-value	*P*-value
Intercept	138.957	1.216	114.29	<0.001
Age	5.327	0.707	7.53	<0.001
Density	-1.136	0.295	3.86	<0.001
NPAS2 female	0.309	0.290	1.07	0.29
NPAS2 female X Density	-0.700	0.280	2.50	0.013
April temperature	-0.917	0.482	1.90	0.064
NPAS2 male	-0.405	0.284	1.43	0.15
Latitude	0.331	0.303	1.09	0.28
Longitude	-0.057	0.397	0.14	0.89
NPAS2 male X Density	-0.355	0.288	1.23	0.22
NPAS2 female X NPAS2 male	-0.353	0.316	1.12	0.27
NPAS2 female X April temperature	0.296	0.266	1.11	0.27
NPAS2 female X Age	0.731	0.704	1.04	0.30
NPAS2 male X Longitude	0.261	0.337	0.78	0.44
NPAS2 male X April temperature	0.282	0.296	0.96	0.34
NPAS2 male X Age	0.607	0.714	0.85	0.40
NPAS2 female X Latitude	0.119	0.318	0.37	0.71
NPAS2 male X Latitude	0.065	0.371	0.23	0.82
NPAS2 female X Longitude	0.021	0.357	0.06	0.95

Table S3.D3 Full linear mixed model of laying date for ADCYAP1 male and female genotypes. Female age class and environmental variables were included as fixed effects and tested for interactions with breeder genotypes. Female identity, male identity and year were included as random effects and all explanatory variables were standardized. Bold variables were kept in the final model.

Variables	Estimates	S.E.	t-value	*P*-value
Intercept	138.935	0.873	159.173	<0.001
Age	5.326	0.710	7.50	<0.001
Density	-1.238	0.298	4.16	<0.001
April temperature	-0.978	0.480	2.04	0.048
Latitude	0.372	0.306	1.22	0.23
ADCYAP1 female	0.103	0.293	0.35	0.73
ADCYAP1 female X Latitude	0.697	0.316	2.21	0.028
ADCYAP1 male	-0.066	0.288	0.23	0.82
Longitude	0.025	0.397	0.06	0.95
ADCYAP1 male X Age	-1.134	0.703	1.62	0.11
ADCYAP1 female X April temperature	-0.396	0.266	1.49	0.14
ADCYAP1 female X Age	-0.756	0.718	1.05	0.29
ADCYAP1 female X Density	-0.393	0.316	1.25	0.21
ADCYAP1 male X Latitude	0.306	0.294	1.04	0.30
ADCYAP1 female X ADCYAP1 male	-0.302	0.312	0.97	0.33
ADCYAP1 male X Longitude	-0.224	0.299	0.75	0.45
ADCYAP1 male X April temperature	-0.176	0.277	0.63	0.53
ADCYAP1 male X Density	-0.135	0.292	0.46	0.64
ADCYAP1 female X Longitude	0.113	0.350	0.32	0.75

Table S3.D4 Full linear mixed model of laying date for CREB1 male and female genotypes. Environmental variables were included as fixed effects and tested for interactions with breeder genotypes. Female age class was also included as fixed effect and female identity, male identity and year were included as random effects and all explanatory variables were standardized. Bold variables were kept in the final model.

Variables	Estimates	S.E.	t-value	*P*-value
Intercept	138.946	0.865	160.64	<0.001
Age	5.383	0.710	7.58	<0.001
Density	-1.231	0.298	4.13	<0.001
April temperature	-1.001	0.478	2.10	0.043
CREB1 male	-0.385	0.284	1.36	0.18
CREB1 female	-0.346	0.304	1.14	0.26
Latitude	0.282	0.304	0.93	0.35
Longitude	-0.044	0.397	0.11	0.91
CREB1 male X Density	0.360	0.271	1.33	0.18
CREB1 female X April temperature	-0.343	0.291	1.18	0.24
CREB1 female X Longitude	-0.253	0.286	0.88	0.38
CREB1 female X CREB1 male	0.193	0.213	0.91	0.37
CREB1 male X Longitude	0.272	0.306	0.89	0.38
CREB1 female X Density	-0.128	0.363	0.35	0.72
CREB1 male X Latitude	-0.104	0.356	0.29	0.77
CREB1 male X April temperature	-0.018	0.290	0.06	0.95
CREB1 female X Latitude	-0.009	0.366	0.03	0.98

Table S3.D5 Full linear mixed model of incubation duration for CLOCK male and female genotypes. Female age class and environmental variables were included as fixed effects and tested for interactions with breeder genotypes. Female identity, male identity and year were included as random effects and all explanatory variables were standardized. Bold variables were kept in the final model.

Variables	Estimates	S.E.	t-value	*P*-value
Intercept	11.326	0.363	31.21	<0.001
Age	0.318	0.161	1.97	0.049
Longitude	0.064	0.067	0.97	0.33
CLOCK female	-0.047	0.066	0.71	0.48
CLOCK male	-0.032	0.064	0.49	0.62
May temperature	-4.7^{-04}	0.132	0.00	1.00
CLOCK male X May temperature	0.115	0.065	1.77	0.078
CLOCK male X Longitude	0.142	0.075	1.91	0.057
CLOCK female X Age	-0.196	0.159	1.23	0.22
CLOCK female X Longitude	-0.050	0.075	0.68	0.50
CLOCK female X May temperature	-0.058	0.072	0.81	0.42
CLOCK female X CLOCK male	-0.025	0.059	0.42	0.67
CLOCK male X Age	-0.023	0.140	0.17	0.88

Table S3.D6 Full linear mixed models of incubation duration for NPAS1 male and female genotypes. Female age class and environmental variables were included as fixed effects and tested for interactions with breeder genotypes. Female identity, male identity and year were included as random effects and all explanatory variables were standardized. Bold variables were kept in the final model.

Variables	Estimates	S.E.	t-value	*P*-value
Intercept	11.323	0.360	31.43	<0.001
Age	0.332	0.160	2.08	0.039
NPAS2 male	0.144	0.063	2.28	0.023
Longitude	0.073	0.067	1.09	0.28
NPAS2 female	0.021	0.066	0.32	0.75
May temperature	0.009	0.131	0.07	0.95
NPAS2 male X May temperature	0.113	0.064	1.78	0.076
NPAS2 female X Age	-0.249	0.159	1.56	0.12
NPAS2 male X Longitude	-0.040	0.073	0.55	0.58
NPAS2 male X Age	0.076	0.163	0.49	0.64
NPAS2 female X NPAS2 male	0.016	0.074	0.22	0.83
NPAS2 female X May temperature	-0.006	0.063	0.09	0.93
NPAS2 female X Longitude	-0.009	0.078	0.11	0.91

Table S3.D7 Full linear mixed model of incubation duration for ADCYAP1 male and female genotypes. Female age class and environmental variables were included as fixed effects and tested for interactions with breeder genotypes. Female identity, male identity and year were included as random effects and all explanatory variables were standardized. Bold variables were kept in the final model.

Variables	Estimates	S.E.	t-value	*P*-value
Intercept	11.326	0.363	31.21	<0.001
Age	0.318	0.161	1.97	0.049
ADCYAP1 female	0.071	0.065	1.10	0.27
Longitude	0.067	0.064	1.00	0.32
ADCYAP1 male	0.032	0.064	0.51	0.61
May temperature	-0.021	0.131	0.16	0.88
ADCYAP1 female X Longitude	-0.081	0.071	1.13	0.26
ADCYAP1 male X Age	-0.147	0.161	0.91	0.36
ADCYAP1 female X Age	-0.107	0.162	0.55	0.51
ADCYAP1 female X ADCYAP1 male	0.038	0.071	0.54	0.59
ADCYAP1 male X May temperature	-0.019	0.059	0.32	0.75
ADCYAP1 male X Longitude	-0.030	0.074	0.40	0.69
ADCYAP1 female X May temperature	-0.003	0.068	0.04	0.97

Table S3.D8 Full linear mixed model of incubation duration for CREB1 male and female genotypes. Environmental variables were included as fixed effects and tested for interactions with breeder genotypes. Female age class was also included as fixed effect. Female identity, male identity and year were included as random effects and all explanatory variables were standardized. Bold variables were kept in the final model.

Variables	Estimates	S.E.	t-value	*P*-value
Intercept	11.302	0.315	35.89	<0.001
Age	0.322	0.159	2.02	0.044
May temperature	-0.097	0.094	1.03	0.30
CREB1 male	-0.108	0.062	1.73	0.088
CREB1 male X May temperature	0.217	0.058	3.73	<0.001
Longitude	0.044	0.088	0.50	0.62
CREB1 female	-0.004	0.066	0.06	0.95
CREB1 female X Longitude	0.065	0.057	1.14	0.26
CREB1 female X May temperature	0.119	0.072	1.66	0.097
CREB1 male X Longitude	0.051	0.074	0.69	0.49
CREB1 female X CREB1 male	0.017	0.049	0.34	0.73

Appendix E: Supplementary information on allelic diversity

Table S3.E1 Summary of studies assessing allelic diversity for CLOCK, NPAS2, ADCYAP1 and CREB1 in various bird species. Number of observed alleles (N alleles) and observed heterozygosity (*Ho*) are reported.

Species	**Localization**	**Candidate gene**	**N alleles**	***Ho***	**Reference**
Barn swallow (*Hirundo rustica*)	Milano, Italy	CLOCK	4	0.066	Caprioli *et al.* 2012
Barn swallow (*Hirundo rustica*)	Worldwild (5 sites)	CLOCK	3	0.030	Dor *et al.* 2011
Barn swallow (*Hirundo rustica*)	Boje, Nigeria	CLOCK	3	-	Saino *et al.* 2013
Blackcaps (*Sylvia atricapilla*)	Worldwild (14 sites)	CLOCK	8	-	Mueller *et al.* 2011
Blue tit (*Cyanistes caeruleus*)	Worldwild (14 sites)	CLOCK	9	0.489	Johnsen *et al.* 2007
Blue tit (*Cyanistes caeruleus*)	Wytham Woods, Oxfordshire, UK	CLOCK	6	0.565	Liedvogel *et al.* 2009
Blue tit (*Cyanistes caeruleus*)	Wytham Woods, Oxfordshire, UK	CLOCK	5	0.561	Olano-Marin *et al.* 2011
Blue tit (*Cyanistes caeruleus*)	Wytham Woods, Oxfordshire, UK	CLOCK	5	0.57	Steinmeyer *et al.* 2009
Blue tit (*Cyanistes caeruleus*)	Wytham Woods, Oxfordshire, UK	CLOCK	4	0.60	Steinmeyer *et al.* 2012
Bluethroat (*Luscinia svecica*)	Worldwild (12 sites)	CLOCK	7	0.213	Johnsen *et al.* 2007
Common buzzard (*Buteo buteo*)	Eastern Westphalia, Germany	CLOCK	1	-	Chakarov *et al.* 2013
Great tit (*Parus major*)	Wytham Woods, Oxfordshire, UK	CLOCK	5	0.077	Liedvodel & Sheldon 2010
Junco spp. (2 species)	Americas (15 sites)	CLOCK	8	0.294	Peterson *et al.* 2013
Pied flycatcher (*Ficedula hypoleuca*)	La Hiruela, Spain	CLOCK	5	0.722	Kuhn *et al.* 2013
Raptors (10 species)	Adlerwarte Berlebeck, Germany	CLOCK	2	-	Chakarov *et al.* 2013
Tachycineta spp. (5 species)	Americas (5 sites)	CLOCK	5	0.332	Dor *et al.* 2012

Species	**Localization**	**Candidate gene**	**N alleles**	***Ho***	**Reference**
Blackcaps (*Sylvia atricapilla*)	Worldwild (14 sites)	NPAS2	2	-	Mueller *et al.* 2011
Blue tit (*Cyanistes caeruleus*)	Wytham Woods, Oxfordshire, UK	NPAS2	8	0.742	Olano-Marin *et al.* 2011
Blue tit (*Cyanistes caeruleus*)	Wytham Woods, Oxfordshire, UK	NPAS2	5	0.75	Steinmeyer *et al.* 2009
Blue tit (*Cyanistes caeruleus*)	Wytham Woods, Oxfordshire, UK	NPAS2	6	0.75	Steinmeyer *et al.* 2012
Common buzzard (*Buteo buteo*)	Eastern Westphalia, Germany	NPAS2	2	0.014	Chakarov *et al.* 2013
Raptors (10 species)	Adlerwarte Berlebeck, Germany	NPAS2	2	-	Chakarov *et al.* 2013
Blackcaps (*Sylvia atricapilla*)	Worldwild (14 sites)	ADCYAP1	13	-	Mueller *et al.* 2011
Blue tit (*Cyanistes caeruleus*)	Wytham Woods, Oxfordshire, UK	ADCYAP1	14	0.637	Olano-Marin *et al.* 2011
Blue tit (*Cyanistes caeruleus*)	Wytham Woods, Oxfordshire, UK	ADCYAP1	7	0.68	Steinmeyer *et al.* 2009
Blue tit (*Cyanistes caeruleus*)	Wytham Woods, Oxfordshire, UK	ADCYAP1	9	0.64	Steinmeyer *et al.* 2012
Common buzzard (*Buteo buteo*)	Eastern Westphalia, Germany	ADCYAP1	3	0.312	Chakarov *et al.* 2013
Junco spp. (2 species)	Americas (15 sites)	ADCYAP1	16	0.772	Peterson *et al.* 2013
Raptors (10 species)	Adlerwarte Berlebeck, Germany	ADCYAP1	6	-	Chakarov *et al.* 2013
Blackcaps (*Sylvia atricapilla*)	Worldwild (14 sites)	CREB1	10	-	Mueller *et al.* 2011
Blue tit (*Cyanistes caeruleus*)	Wytham Woods, Oxfordshire, UK	CREB1	9	0.267	Olano-Marin *et al.* 2011
Blue tit (*Cyanistes caeruleus*)	Wytham Woods, Oxfordshire, UK	CREB1	7	0.27	Steinmeyer *et al.* 2009
Blue tit (*Cyanistes caeruleus*)	Wytham Woods, Oxfordshire, UK	CREB1	6	0.30	Steinmeyer *et al.* 2012
Common buzzard (*Buteo buteo*)	Eastern Westphalia, Germany	CREB1	3	0.093	Chakarov *et al.* 2013
Raptors (10 species)	Adlerwarte Berlebeck, Germany	CREB1	2	-	Chakarov *et al.* 2013

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Annexes Chapitre 4

Appendix A: Sample size

Table S4.A1 Number of tree swallows sampled for each trait within the three trait categories, A) morphological, B) reproductive and C) nestling traits, between 2004 and 2014 in our study system in southern Québec. Numbers are presented for individuals with known sex, except for reproductive traits, for which only females were included.

	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	Total
A) Morphological traits												
Wing length	188	353	529	443	415	381	390	337	338	368	429	4171
Females	188	279	354	245	235	217	225	202	205	222	240	2612
Males		74	175	198	180	164	165	135	133	146	189	1559
Body mass	175	341	443	428	404	367	365	323	322	342	410	3920
Females	175	270	288	235	228	209	211	193	191	209	227	2436
Males		71	155	193	176	158	154	130	131	133	183	1484
Tarsus length				436	405	378	378	329	326	351	417	3020
Females				242	229	214	214	197	197	210	232	1735
Males				194	176	164	164	132	129	141	185	1285
B) Reproductive traits												
Laying date	181	257	250	218	212	186	197	181	181	182	217	2262

	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	Total
Clutch size	181	257	250	218	212	186	197	181	181	182	217	2262
Incubation duration	160	248	207	196	187	159	168	158	155	131	196	1965
C) Nestling traits												
Wing length			200	783	867	775	655	538	643	536	861	5858
Females			75	398	462	391	381	289	370	263	441	3070
Males			82	336	386	364	273	239	261	259	414	2614
Body mass		9	208	786	869	775	654	538	665	543	860	5907
Females			80	401	463	391	381	289	387	268	440	3100
Males			85	336	387	364	272	239	265	261	414	2623
Tarsus length				776	867	775	651	538	667	543	861	5678
Females				395	461	391	379	289	388	268	441	3012
Males				334	387	364	271	239	266	261	414	2536

Table S4.A2 Summary statistics for A) social pedigree and B) genetic pedigree, for all individuals (total) and all traits within each category (morphological, reproductive, nestling).

	Total	Morphological			Reproductive			Nestling		
		Wing length	Body mass	Tarsus length	Laying date	Clutch size	Incubation duration	Wing length	Body mass	Tarsus length
Social pedigree										
Sample Size	13446	2844	2671	2108	1603	1610	1381	7560	7621	7318
Maternities	10509	130	115	102	56	56	49	5840	5887	5665
Paternities	7325	88	78	78	37	37	33	5321	5358	5196
Full Sibs	18077	6	4	4	3	3	2	12325	12430	12189
Maternal Sibs	47124	12	9	7	5	5	4	23275	23431	22786
Paternal Sibs	35452	7	5	5	4	4	3	23012	23319	22605
Maternal Grandmothers	381	4	4	4	1	1	1	271	271	269
Maternal Grandfathers	277	2	2	2	1	1	1	221	220	221
Paternal Grandmothers	465	6	6	6	3	3	3	360	364	356
Paternal Grandfathers	315	3	3	3	1	1	1	257	261	261
Genetic pedigree										
Sample Size	13446	2843	2672	2102	1594	1601	1373	7552	7610	7298
Maternities	10509	130	115	102	56	56	49	5842	5889	5669
Paternities	5656	69	60	62	26	26	23	4478	4505	4385
Full Sibs	6811	2	1	1	1	1	0	5346	5372	5304
Maternal Sibs	47124	12	9	7	5	5	4	23294	23450	22816
Paternal Sibs	29309	5	4	4	2	2	1	20139	20378	19964
Maternal Grandmothers	381	4	4	4	1	1	1	271	271	269
Maternal Grandfathers	207	2	2	2	1	1	1	159	158	159
Paternal Grandmothers	397	5	4	4	3	3	3	326	330	329
Paternal Grandfathers	242	1	0	0	0	0	0	204	204	204

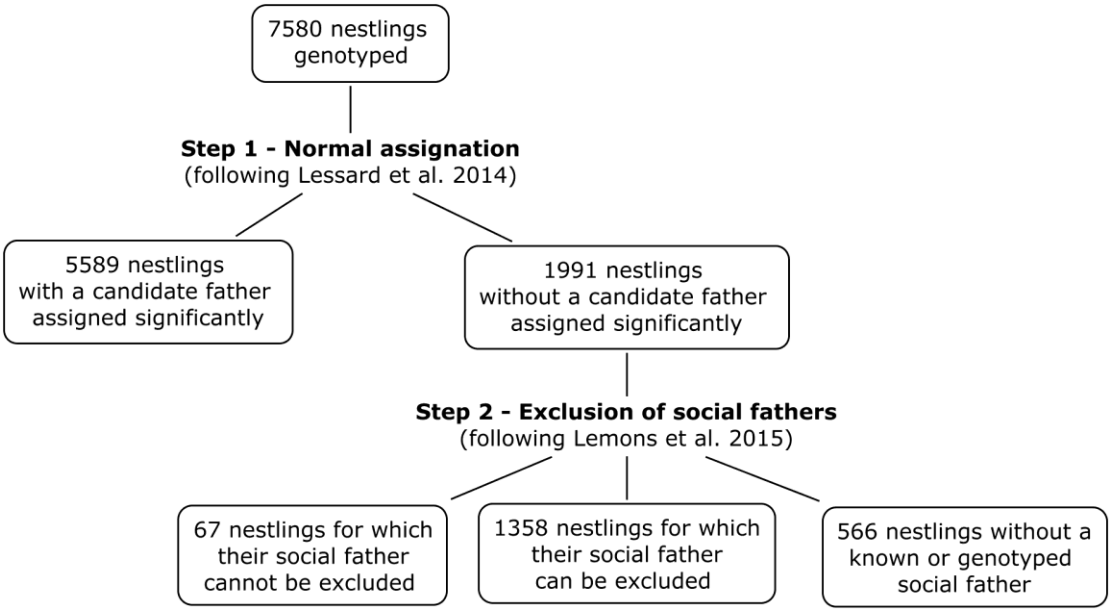

Figure S4.A1 Schematic representation of the number of nestlings assigned or not to a candidate father at each of the 2 steps. Males significantly assigned to nestlings in the initial parental analysis (i.e. step 1) were considered as their genetic fathers. For the remaining nestlings (i.e. without a significant male assignment at step 1), if their social fathers could not be excluded (i.e. step 2), they were considered as their genetic fathers but otherwise no genetic father were assigned to them.

Appendix B: Fixed effects included in animal models

Fixed effects included in animal models came from preliminary mixed model analyses and are summarized in Table S4.B1. Similar parameters were tested for each trait category and are detailed below (Table S4.B2-4). All statistical analyses were made with lme4 package (Bates et al. 2014) in R v3.2.0 (R core team 2015), and degree of freedom and associated *P*-values were determined with lmerTest package (Kuznetsova et al. 2013). Final models were determined by removing the less significant parameters based on *P*-value (α=0.05).

Table S4.B1 Fixed effects fitted in the univariate and multivariate animal models, for each trait within the three trait categories (morphological, reproductive, nestling). Female age classes are second-year (SY) or after-second-year (ASY).

Trait category	**Trait**	**Fixed effects**
Morphological	Wing length	Sex, Age (covariate), Julian day, Sex X Julian day
	Body mass	Sex, Age (covariate), Clutch number, Days, Day^2, Hour, Sex X Days, Sex X $Days^2$, Sex X Hour
	Tarsus length	None
Reproductive	Laying date	Age (SY/ASY)
	Clutch size	Age (SY/ASY)
	Incubation duration	Age (SY/ASY)
Nestling	Wing length	Sex, Hour
	Body mass	Sex, Hour
	Tarsus length	None

Fixed effects tested for morphological traits

For all morphological traits: sex and age (covariate). For wing length: day of measurement (in Julian day). For body mass: day since laying date and its squared value (in Julian day), hour of measurement (expressed as a proportion of 24 hours, e.g. midday = 0.5) and number of clutch (first vs others, factor). Finally, we also tested all two-way interactions including sex.

Table S4.B2 Linear mixed models analyses of adult morphological traits: a) wing length, b) body mass and c) tarsus length. Year and individual identity were included as random effects. Variables in bold are those retained at the end of model selection.

Models	Variables	Estimates	S.E.	d.f.	t-value	*P*-value
Wing length	**Intercept**	**119.7**	**0.898**	**1648**	**133.24**	**<0.001**
	Sex (male)	**-0.533**	**1.472**	**3789**	**0.36**	**<0.001**
	Age (covariate)	**0.523**	**0.043**	**2943**	**12.09**	**<0.001**
	Julian day	**-0.039**	**0.055**	**3999**	**7.13**	**<0.001**
	Sex X Julian day	**0.033**	**0.009**	**3783**	**3.64**	**<0.001**
	Sex X Age	-0.029	0.084	2357	0.34	0.73
Body mass	**Intercept**	**20.60**	**0.027**	**227.0**	**76.93**	**<0.001**
	Sex (male)	**1.046**	**0.444**	**3773**	**2.35**	**0.019**
	Age (covariate)	**0.203**	**0.011**	**3119**	**9.11**	**<0.001**
	Clutch number	**-0.611**	**0.071**	**3570**	**8.65**	**<0.001**
	Day since laying	**0.091**	**0.021**	**3679**	**4.34**	**<0.001**
	Day since laying²	**-0.006**	**0.001**	**3641**	**10.70**	**<0.001**
	Hour	**2.311**	**0.251**	**3704**	**9.21**	**<0.001**
	Sex X Day since laying	**-0.157**	**0.036**	**3781**	**4.31**	**<0.001**
	Sex X Day since laying²	**0.006**	**0.001**	**3686**	**7.27**	**<0.001**
	Sex X Hour	**-0.257**	**0.371**	**3642**	**3.39**	**<0.001**
	Sex X Age	-0.047	0.044	2709	1.07	0.29
	Sex X Clutch number	0.056	0.143	3496	0.39	0.70
Tarsus length	**Intercept**	**12.165**	**0.012**	**14.65**	**1001**	**<0.001**
	Age (covariate)	0.004	0.005	1110	0.68	0.49
	Sex (male)	0.005	0.018	2121	0.28	0.78
	Sex X Age	0.012	0.010	1496	1.30	0.19

Fixed effects tested for reproductive traits

For reproductive traits: age = second-year (SY) or after-second-year (ASY).

Table S4.B3 Linear mixed models analyses for female reproductive traits: a) laying date, b) clutch size and c) incubation duration. Year and female identity were included as random effects. Variables in bold are those retained at the end of model selection. Female age classes are second-year (SY) or after-second-year (ASY).

Models	**Variables**	**Estimates**	**S.E.**	**d.f.**	**t-value**	***P*-value**
Laying date	**Intercept**	**139.322**	**0.738**	**9.4**	**188.81**	**<0.001**
	Age (SY)	**7.129**	**0.382**	**1727.9**	**18.65**	**<0.001**
Clutch size	**Intercept**	**5.663**	**0.051**	**10.3**	**111.22**	**<0.001**
	Age (SY)	**-0.586**	**0.052**	**1743.4**	**11.16**	**<0.001**
Incubation duration	**Intercept**	**15.354**	**0.226**	**9.1**	**68.08**	**<0.001**
	Age (SY)	**-0.370**	**0.099**	**1744.8**	**3.73**	**<0.001**

Fixed effects tested for nestling traits

For all nestling traits: sex and hour of the measurement (expressed as a proportion of 24 hours, e.g. midday = 0.5).

Table S4.B4 Linear mixed models analyses for nestling traits: a) wing length, b) body mass and c) tarsus length. Brood identity and year were included as random effects. Variables in bold are those retained at the end of model selection.

Models	**Variables**	**Estimates**	**S.E.**	**d.f.**	**t-value**	***P*-value**
Wing length	**Intercept**	**43.516**	**1.120**	**45.0**	**38.84**	**<0.001**
	Sex (male)	**0.293**	**0.113**	**4414.0**	**2.60**	**0.009**
	Hour	**3.685**	**1.686**	**1240.0**	**2.19**	**0.029**
Body mass	**Intercept**	**19.05**	**0.265**	**160.0**	**71.81**	**<0.001**
	Sex (male)	**1.033**	**0.040**	**4581**	**26.08**	**<0.001**
	Hour	**2.797**	**0.460**	**1247**	**6.08**	**<0.001**
Tarsus length	**Intercept**	**12.087**	**0.023**	**7.0**	**374.3**	**<0.001**
	Sex (male)	0.016	0.011	4821	1.45	0.15
	Hour	0.133	0.099	1239	1.34	0.18

Table S4.C1 Animal model comparisons using LRT, for morphological traits, using A) the social pedigree and B) the genetic pedigree. SE of variance components are in parentheses.

Traits	Model	Variance components				Test				
		V_Y	V_{PE}	V_A	V_R	LogL	Models	Chisq	d.f.	*P*-value
A) Social pedigree										
Wing length	1				9.83 (0.22)	-6865.85				
	2	0.49 (0.23)			9.38 (0.21)	-6783.17	1 vs 2	165.36	1	<0.001
	3	0.50 (0.24)	5.42 (0.26)		4.08 (0.16)	-6504.63	2 vs 3	557.08	1	<0.001
	4	0.50 (0.23)	3.05 (1.10)	2.37 (1.10)	4.08 (0.16)	-6502.69	3 vs 4	3.88	1	0.049
Body mass	1				2.27 (0.05)	-3583.14				
	2	0.15 (0.07)			2.15 (0.05)	-3488.34	1 vs 2	189.60	1	<0.001
	3	0.16 (0.07)	1.01 (0.06)		1.15 (0.05)	-3317.40	2 vs 3	341.88	1	<0.001
	4	0.16 (0.07)	0.30 (0.23)	0.72 (0.23)	1.15 (0.05)	-3314.22	3 vs 4	8.30	1	0.004
Tarsus length	1				0.171 (0.004)	1154.93				
	2	0.001 (<0.001)			0.171 (0.004)	1159.24	1 vs 2	8.62	1	0.003
	3	0.001 (<0.001)	0.127 (0.005)		0.045 (0.002)	1553.10	2 vs 3	787.72	1	<0.001
	4	0.001 (<0.001)	0.061 (0.022)	0.066 (0.023)	0.045 (0.002)	1556.93	3 vs 4	7.66	1	0.006
B) Genetic pedigree										
Wing length	1				9.83(0.22)	-3583.14				
	2	0.49 (0.23)			9.38(0.21)	-6783.17	1 vs 2	165.36	1	<0.001
	3	0.50 (0.24)	5.42 (0.26)		4.08 (0.16)	-6504.63	2 vs 3	557.08	1	<0.001
	4	0.50 (0.23)	2.45 (1.11)	2.96 (1.12)	4.08 (0.16)	-6501.79	3 vs 4	5.68	1	0.017
Body mass	1				2.27 (0.05)	-3488.34				
	2	0.15 (0.07)			2.15 (0.05)	-3488.34	1 vs 2	189.60	1	<0.001
	3	0.16 (0.07)	1.01 (0.06)		1.15 (0.05)	-3317.40	2 vs 3	341.88	1	<0.001
	4	0.16 (0.07)	0.08 (0.22)	0.94 (0.23)	1.15 (0.05)	-331145	3 vs 4	11.90	1	<0.001
Tarsus length	1				0.171 (0.004)	1154.93				
	2	0.001 (<0.001)			0.171 (0.004)	1159.24	1 vs 2	8.62	1	0.003
	3	0.001 (<0.001)	0.127 (0.005)		0.045 (0.002)	1553.10	2 vs 3	787.72	1	<0.001
	4	0.001 (<0.001)	0.051 (0.022)	0.077 (0.022)	0.045 (0.002)	1558.41	3 vs 4	10.62	1	0.001

Table S4.C2 Animal model comparisons using LRT, for reproductive traits, using A) the social pedigree and B) the genetic pedigree. SE of variance components are in parentheses.

Traits	Model	Variance components				Test				
		V_Y	V_{PE}	V_A	V_R	LogL	Models	Chisq	d.f.	*P*-value
A) Social pedigree										
Laying date	1				40.92 (1.27)	-4880.83				
	2	4.71 (2.30)			36.62 1.14)	-4780.70	1 vs 2	200.26	1	<0.001
	3	5.20 (2.53)	16.04 (1.58)		21.66 (1.23)	-4734.91	2 vs 3	91.58	1	<0.001
	4	5.17 (2.51)	<0.01 (<0.01)	16.19 (1.58)	21.57 (1.22)	-4733.49	3 vs 4	2.84	1	0.09
Clutch size	1				0.793 (0.025)	-805.15				
	2	0.021 (0.012)			0.775 (0.024)	-789.33	1 vs 2	31.63	1	<0.001
	3	0.021 (0.012)	0.274 (0.031)		0.503 (0.027)	-740.57	2 vs 3	97.54	1	<0.001
	4	0.020 (0.011)	<0.001 (<0.01)	0.275 (0.031)	0.502 (0.027)	-739.06	3 vs 4	3.00	1	0.08
Incubation	1				2.560 (0.086)	-1715.49				
Duration	2	0.474 (0.230)			2.182 (0.074)	-1591.36	1 vs 2	248.26	1	<0.001
	3	0.486 (0.235)	0.438 (0.089)		1.742 (0.095)	-1576.55	2 vs 3	29.62	1	<0.001
	4	0.486 (0.235)	0.438 (0.089)	<0.001 (<0.001)	1.742 (0.095)	-1576.55	3 vs 4	0.00	1	1.00
B) Genetic pedigree										
Laying date	1				40.92 (1.27)	-4880.83				
	2	4.71 (2.30)			36.62 (1.14)	-4780.70	1 vs 2	200.26	1	<0.001
	3	5.20 (2.53)	16.04 (1.58)		21.66 (1.23)	-4734.91	2 vs 3	91.58	1	<0.001
	4	5.17 (2.51)	<0.01 (<0.01)	16.22 (1.58)	21.54 (1.22)	-4733.16	3 vs 4	3.50	1	0.06
Clutch size	1				0.793 (0.025)	-805.15				
	2	0.021 (0.012)			0.775 (0.024)	-789.33	1 vs 2	31.63	1	<0.001
	3	0.021 (0.012)	0.274 (0.031)		0.503 (0.027)	-740.57	2 vs 3	97.54	1	<0.001
	4	0.020 (0.011)	<0.001 (<0.01)	0.275 (0.031)	0.502 (0.027)	-739.08	3 vs 4	3.01	1	0.06
Incubation	1				2.560 (0.086)	-1715.49				
Duration	2	0.474 (0.230)			2.182 (0.074)	-1591.36	1 vs 2	248.26	1	<0.001
	3	0.486 (0.235)	0.438 (0.089)		1.742 (0.095)	-1576.55	2 vs 3	29.62	1	<0.001
	4	0.486 (0.235)	0.438 (0.089)	<0.001 (<0.001)	1.742 (0.095)	-1576.55	3 vs 4	0.00	1	1.00

Table S4.C3 Animal model comparisons using LRT, for nestling traits, using A) the social pedigree and B) the genetic pedigree. SE of variance components are in parentheses.

Traits	Model	Variance components				Test				
		V_{BY}	V_B	V_A	V_R	LogL	Models	Chisq	d.f.	*P*-value
A) Social pedigree										
Wing length	1				49.61 (0.93)	-13905.77				
	2	7.04 (3.58)			45.36 (0.85)	-13670.00	1 vs 2	471.54	1	<0.001
	3	6.69 (3.55)	36.49 (1.58)		13.25 (0.29)	-11826.34	2 vs 3	3687.32	1	<0.001
	4	6.75 (3.57)	30.13 (2.00)	14.14 (3.28)	5.82 (1.71)	-11815.62	3 vs 4	21.44	1	<0.001
Body mass	1				4.13 (0.08)	-6905.53				
	2	0.10 (0.05)			4.04 (0.08)	-6855.31	1 vs 2	100.44	1	<0.001
	3	0.08 (0.05)	2.57 (0.12)		1.67 (0.04)	-5640.47	2 vs 3	2429.68	1	<0.001
	4	0.08 (0.05)	1.97 (0.16)	1.25 (0.30)	1.01 (0.16)	-5630.79	3 vs 4	19.36	1	<0.001
Tarsus length	1				0.239 (0.005)	1216.77				
	2	0.007 (0.004)			0.233 (0.005)	1280.04	1 vs 2	126.54	1	<0.001
	3	0.008 (0.005)	0.104 (0.006)		0.134 (0.003)	1911.72	2 vs 3	1263.36	1	<0.001
	4	0.008 (0.005)	0.084 (0.008)	0.045 (0.015)	0.111 (0.008)	1916.39	3 vs 4	9.34	1	0.002
B) Genetic pedigree										
Wing length	1				49.61 (0.93)	-13905.77				
	2	7.04 (3.58)			45.36 (0.85)	-13670.00	1 vs 2	471.54	1	<0.001
	3	6.69 (3.55)	36.49 (1.58)		13.25 (0.29)	-11826.34	2 vs 3	3687.32	1	<0.001
	4	6.68 (3.54)	35.74 (1.61)	1.98 (1.02)	11.96 (0.70)	-11824.40	3 vs 4	3.88	1	0.049
Body mass	1				4.13 (0.08)	-6905.53				
	2	0.10 (0.05)			4.04 (0.08)	-6855.31	1 vs 2	100.44	1	<0.001
	3	0.08 (0.05)	2.57 (0.12)		1.67 (0.04)	-5640.47	2 vs 3	2429.68	1	<0.001
	4	0.07 (0.04)	2.14 (0.12)	0.97 (0.15)	1.05 (0.10)	-5610.73	3 vs 4	59.48	1	<0.001
Tarsus length	1				0.239 (0.005)	1216.77				
	2	0.007 (0.004)			0.233 (0.005)	1280.04	1 vs 2	126.54	1	<0.001
	3	0.008 (0.005)	0.104 (0.006)		0.134 (0.003)	1911.72	2 vs 3	1263.36	1	<0.001
	4	0.008 (0.005)	0.086 (0.006)	0.055 (0.010)	0.098 (0.007)	1931.63	3 vs 4	39.82	1	<0.001

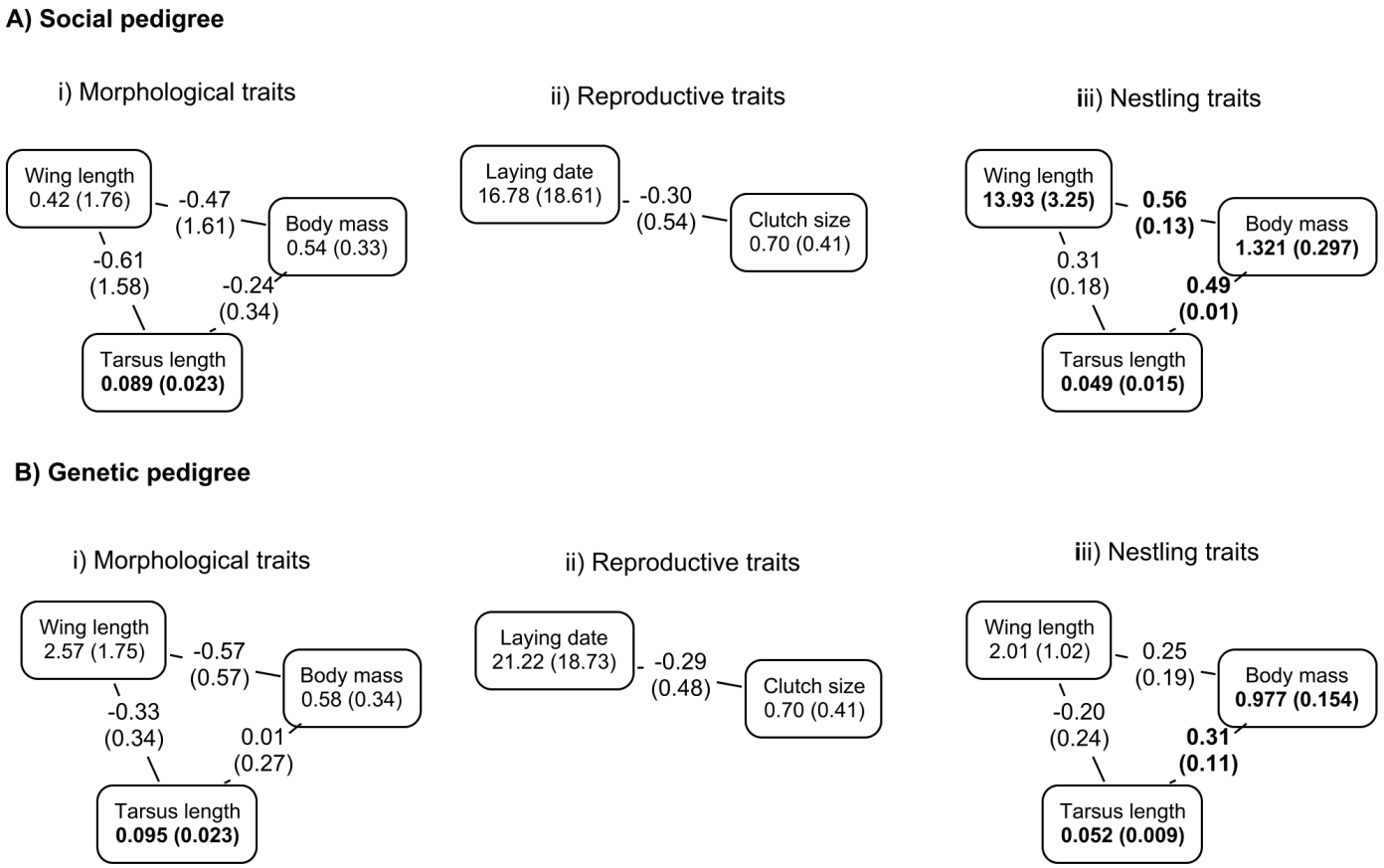

Figure S4.C1 Schematic representation of additive genetic correlations (r_A) obtained from multivariate animal models with A) social and B) genetic pedigrees, from the empirical dataset, on i) morphological, ii) reproductive and iii) nestling traits. Within each box are reported the additive genetic variance of a trait and r_A for the two related traits are presented on lines between boxes. SE are in parentheses, and values significantly different from zero are in bold.

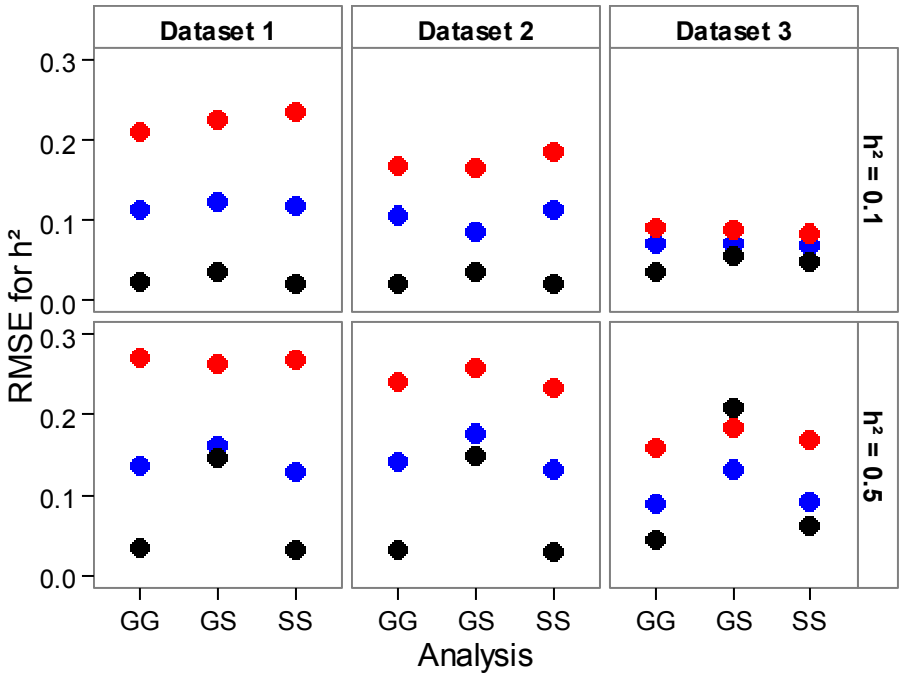

Figure S4.C1 RMSE values obtained for h^2 estimations for morphological traits (blue dots), reproductive traits (red dots) and nestling traits (black dots) simulated in the three datasets, for 2 levels of h^2. Analysis types refer to datasets simulated with the genetic pedigree and analysed with both the genetic and the social pedigree (GG and GS analyses, respectively), and datasets simulated with the social pedigree and analysed with the social pedigree (SS analysis).

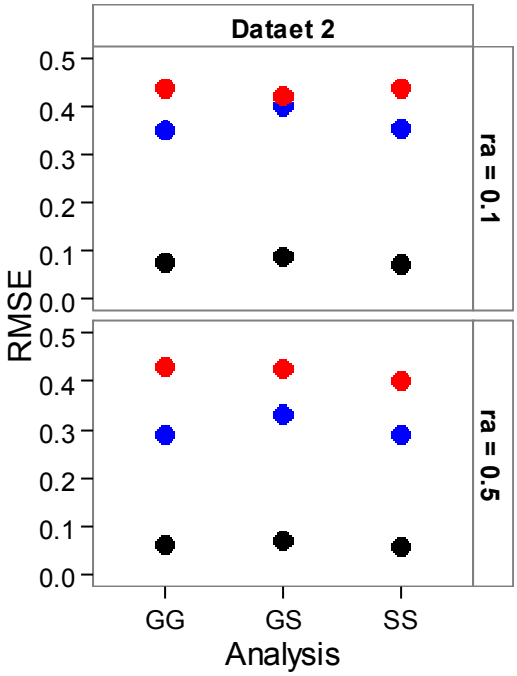

Figure S4.D2 RMSE values obtained for r_A estimations for morphological traits (blue dots), reproductive traits (red dots) and nestling traits (black dots) simulated in dataset 2, for 2 levels of r_A. Analysis types refer to datasets simulated with the genetic pedigree and analysed with both the genetic and the social pedigree (GG and GS analyses, respectively), and datasets simulated with the social pedigree and analysed with the social pedigree (SS analysis).

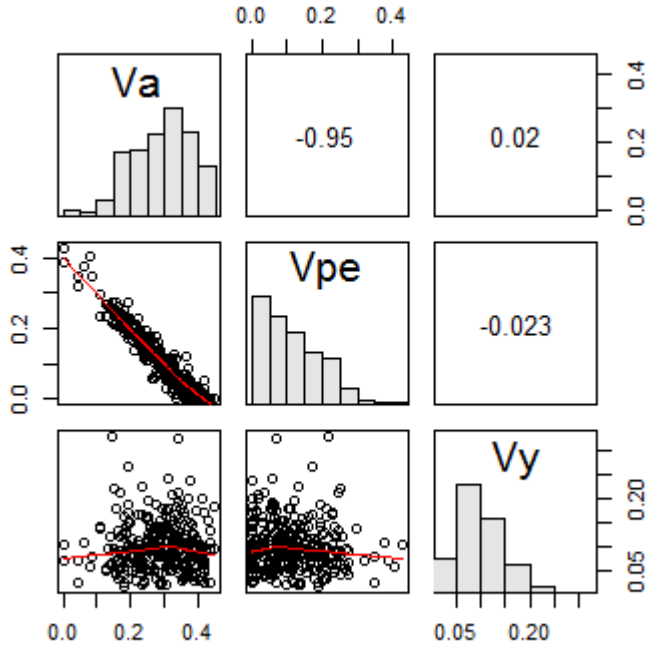

Figure S4.D3 Relationships between V_A, V_{PE} and V_Y estimated for morphological traits (dataset 3, GG analysis, $h^2 = 0.3$). Distributions of these estimates are presented on the diagonal (gray bars) while they are plotted together on the lower diagonal (circles). Spearman's rank correlation coefficients are presented on the upper diagonal.

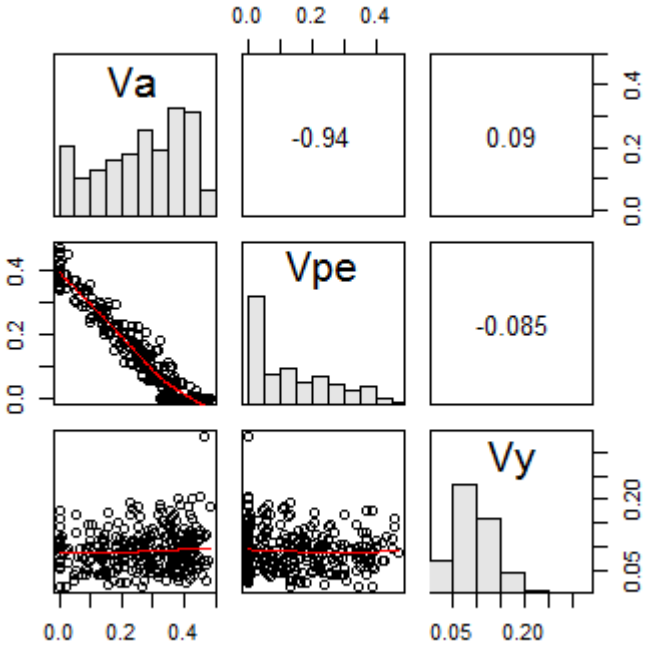

Figure S4.D4 Relationships between V_A, V_{PE} and V_Y estimated for reproductive traits (dataset 3, GG analysis, $h^2 = 0.3$). Distributions of these estimates are presented on the diagonal (gray bars) while they are plotted together on the lower diagonal (circles). Spearman's rank correlation coefficients are presented on the upper diagonal.

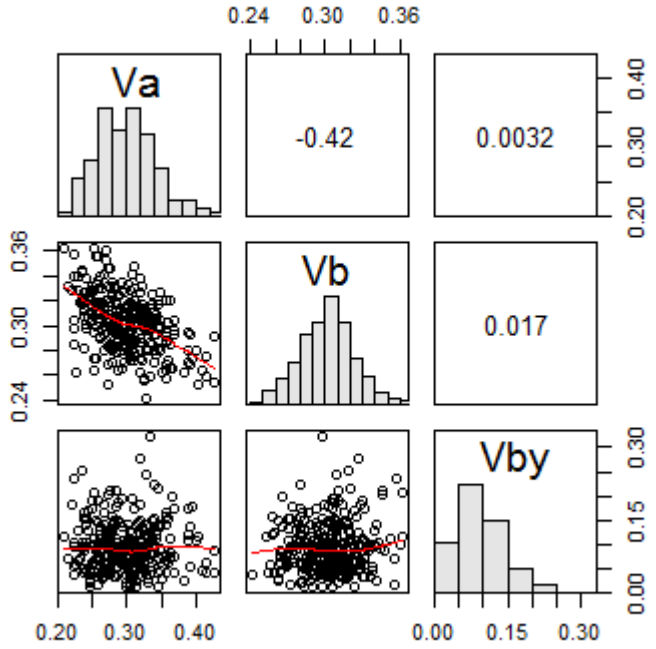

Figure S4.D5 Relationships between V_A, V_B and V_{BY} estimated for nestling traits (dataset 3, GG analysis, $h^2 = 0.3$). Distributions of these estimates are presented on the diagonal (gray bars) while they are plotted together on the lower diagonal (circles). Spearman's rank correlation coefficients are presented on the upper diagonal.

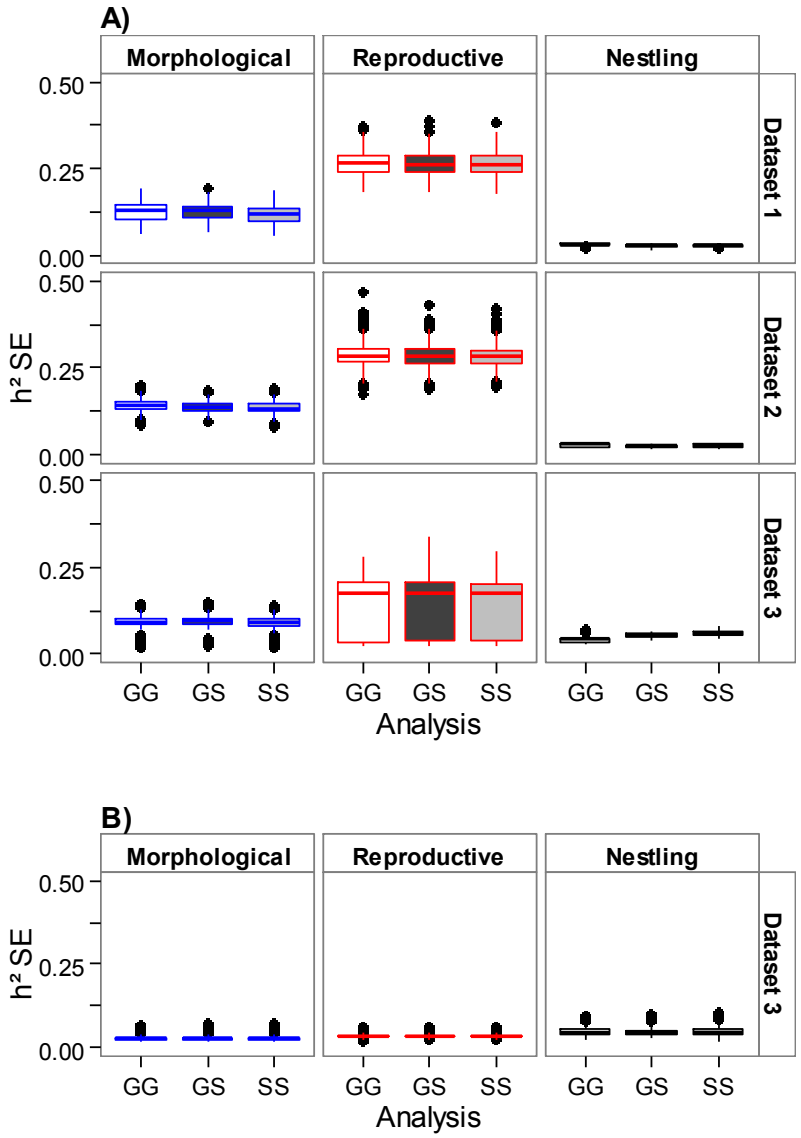

Figure S4.D6 Heritability standard errors (SE) observed for the 3 datasets of simulated morphological (blue), reproductive (red) and nestling (black) traits analysed with A) full animal models and B) animal models omitting an important cause of resemblance among individuals (V_{PE} or V_B). SE distributions are represented by boxplots (1st quartile, median, 3rd quartile). Analysis types refer to datasets simulated with the genetic pedigree and analysed with both the genetic and the social pedigree (GG and GS analyses, respectively), and datasets simulated with the social pedigree and analysed with the social pedigree (SS analysis).

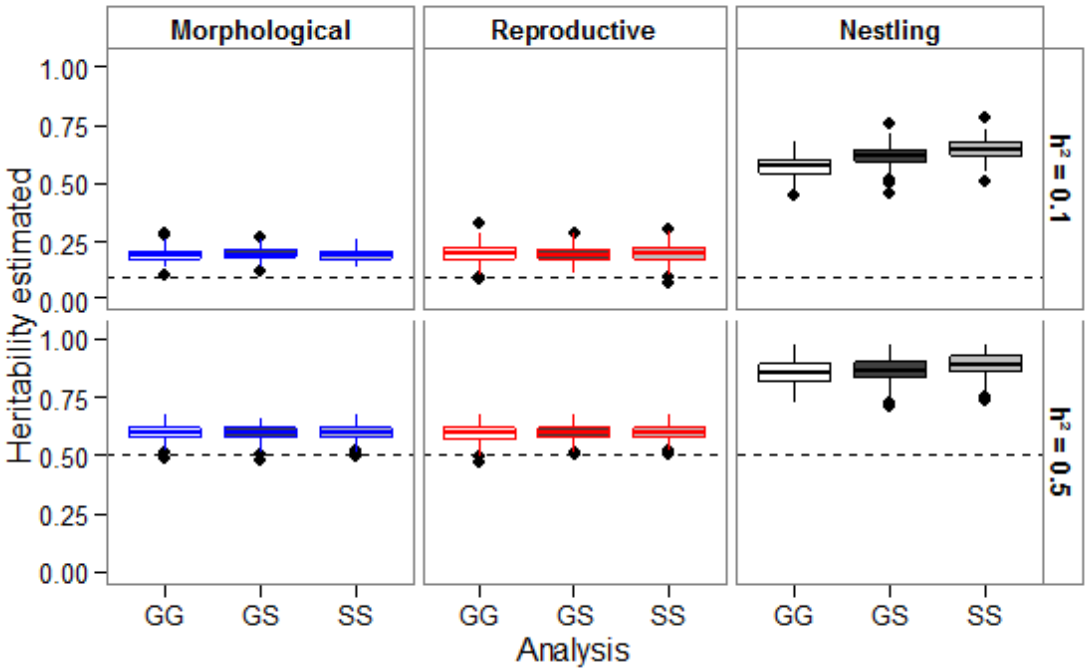

Figure S4.D7 Precision and accuracy of heritability estimated from animal models omitting an important cause of resemblance among individuals (V_{PE} or V_B), for morphological traits (blue), reproductive traits (red) and nestling traits (black) simulated in dataset 3. Distribution of these estimates are represented by boxplots (1st quartile, median, 3rd quartile) for 2 levels of heritability (dotted lines represent the h^2 true value of 0.1 and 0.5 simulated). Analysis types refer to datasets simulated with the genetic pedigree and analysed with both the genetic and the social pedigree (GG and GS analyses, respectively), and datasets simulated with the social pedigree and analysed with the social pedigree (SS analysis).

Appendix E: References

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Annexes Chapitre 5

Appendix A: Supplementary information

Equations

Selection gradient models for 3 traits, for linear (β_i) terms:

$$\omega = \alpha + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \varepsilon \quad \text{(S1)}$$

and non-linear (γ_i) and correlational (γ_{ij}) terms:

$$\omega = \alpha + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + (\gamma_1/2){x_1}^2 + (\gamma_2/2){x_2}^2 + (\gamma_3/2){x_3}^2 + \gamma_{12} x_1 x_2 + \gamma_{13} x_1 x_3 + \gamma_{23} x_2 x_3 + \varepsilon \quad \text{(S2)}$$

Table S5.A1 Comparison by AICc for each age class (2, 6, 12 and 16 days old) of six generalized linear mixed models (logit link and binomial error structure) assessing the effects of an environmental variable and its interaction with phenotypic trait values on fledging probability (0 or 1). Year, brood and farm identity were set as random effects.

Model	K	AICc	ΔAICc	AICcWt	Cum.Wt	LL
2 days old (Mass)						
Intercept	6	4044.09	0.00	0.51	0.51	-2016.04
Non-intensive – 5 km	8	4046.91	2.81	0.12	0.64	-2015.44
Density	8	4047.28	3.18	0.10	0.74	-2015.63
Intensive – 5 km	8	4047.36	3.26	0.10	0.84	-2015.67
Intensive – 500m	8	4047.58	3.48	0.09	0.93	-2015.78
Non-intensive – 500 m	8	4048.01	3.92	0.07	1.00	-2016.00
6 days old (Mass + Wing)						
Intercept	7	3237.69	0.00	0.51	0.51	-1611.83
Intensive – 5 km	10	3240.16	2.47	0.15	0.66	-1610.06
Intensive – 500 m	10	3240.16	2.48	0.15	0.81	-1610.06
Non-intensive – 5 km	10	3240.77	3.08	0.11	0.92	-1610.37
Density	10	3242.73	5.05	0.04	0.96	-1611.35
Non-intensive – 500 m	10	3243.03	5.35	0.04	1.00	-1611.50
12 days old (Mass + Wing)						
Density	10	1925.81	0.00	0.85	0.85	-952.89
Intercept	7	1930.31	4.50	0.09	0.94	-958.15
Non-intensive – 5 km	10	1933.02	7.21	0.02	0.96	-956.49
Intensive – 5 km	10	1933.48	7.68	0.02	0.98	-956.72
Intensive – 500 m	10	1934.11	8.30	0.01	0.99	-957.04
Non-intensive – 500 m	10	1935.62	9.81	0.01	1.00	-957.79
16 days old (Mass + Wing + Tarsus)						
Intercept	8	858.43	0.00	0.74	0.74	-421.20
Non-intensive – 500 m	12	862.83	4.40	0.08	0.82	-419.39
Intensive – 5 km	12	862.93	4.50	0.04	0.90	-419.44
Density	12	863.90	5.47	0.05	0.95	-419.92
Non-intensive – 5 km	12	864.65	6.22	0.03	0.98	-420.30
Intensive – 500 m	12	866.09	7.65	0.02	1.00	-421.02

Table S5.A2 Number of tree swallow nestlings sampled at each age for A) body mass, B) wing length and C) tarsus length, between 2007 and 2014 in our study system in southern Québec, Canada. 14 outlier values were removed from the final dataset (4 body mass over 30 grams at 16 days, 7 wing length over 10 mm at 6 days and 4 tarsus length over 14 mm at 16 days).

	2007	2008	2009	2010	2011	2012	2013	2014	Total
A) Body mass									
2 days old	1008	1020	886	872	790	808	662	1016	7062
6 days old	967	989	855	813	724	781	632	967	6728
12 days old	876	932	824	733	633	713	553	876	6140
16 days old	786	868	775	653	538	665	530	836	5651
B) Wing length									
6 days old	961	986	850	806	724	782	632	967	6708
12 days old	868	932	812	733	633	709	548	876	6111
16 days old	783	867	776	655	538	643	523	837	5622
C) Tarsus length									
16 days old	775	867	776	650	538	667	529	836	5638

Table S5.A3 Linear (*i*) and non-linear (*j*) selection differentials (±SE) for all traits measured for nestlings at 2, 6, 12 and 16 days old. Selection differentials were computed on raw data, and significant values are in bold (see Table 5.1 for standardized selection differentials).

Trait	**Age (days)**	***i* ± SE**	***j* ± SE**
Body mass	2	0.095 ± 0.009	-0.084 ± 0.010
	6	0.372 ± 0.016	-0.097 ± 0.007
	12	0.365 ± 0.010	-0.072 ± 0.004
	16	0.116 ± 0.005	-0.049 ± 0.002
Wing length	6	0.072 ± 0.007	-0.043 ± 0.008
	12	0.696 ± 0.027	-0.096 ± 0.005
	16	0.336 ± 0.017	-0.034 ± 0.002
Tarsus length	16	0.008 ± 0.001	-0.011 ± 0.003

Table S5.A4 Linear (β_i), non-linear (γ_i) and correlational (γ_{ij}) selection gradients (±SE) for all traits (body mass, wing and tarsus length) measured for nestlings at 6, 12 and 16 days old. Selection gradients were computed on raw data, and significant values are in bold (see Table 5.2 for standardized selection gradients).

	6 days	**12 days**	**16 days**
β_{MASS}	0.060 ± 0.003	0.041 ± 0.002	0.021 ± 0.001
β_{WING}	-0.059 ± 0.006	0.005 ± 0.001	0.005 ± $3.5\cdot10^{-4}$
β_{TARSUS}			-0.008 ± 0.005
γ_{MASS}	-0.006 ± 0.001	-0.010 ± 0.001	-0.008 ± 0.001
γ_{WING}	0.005 ± 0.004	$-5.0\cdot10^{-4}$ ± $1.6\cdot10^{-4}$	$1.0\cdot10^{-4}$ ± $7.6\cdot10^{-5}$
γ_{TARSUS}			-0.002 ± 0.012
$\gamma_{MASS\text{-}WING}$	0.007 ± 0.004	$7.0\cdot10^{-4}$ ± $3.1\cdot10^{-4}$	$-1.0\cdot10^{-3}$ ± $1.6\cdot10^{-4}$
$\gamma_{MASS\text{-}TARSUS}$			0.010 ± 0.002
$\gamma_{WING\text{-}TARSUS}$			$-6.4\cdot10^{-5}$ ± $7.3\cdot10^{-4}$

Table S5.A5 Sequential model building assessing variation in selection on nestling morphological traits between low and high density environments. Models including (m_1) or not (m_0) an environmental interaction were fitted by age, and compared using LRTs for both linear and non-linear terms of selection gradients (except at 2 days where selection differentials were compared). At 12 days, the m_1 model was also compared with models without the interaction with mass ($m_{1\text{-MASS}}$) or wing length ($m_{1\text{-WING}}$).

Age (days)	**Model**	**Linear**				**Non-linear**			
		df	Log-L	χ^2	*P*-value	df	Log-L	χ^2	*P*-value
2	m_0	7	-2015.8			9	-1998.2		
	m_1	8	-2015.6	0.35	0.56	10	-1995.6	5.10	0.024
6	m_0	8	-1611.6			12	-1605.4		
	m_1	10	-1611.4	0.54	0.76	14	-1605.0	0.66	0.72
12	m_0	8	-957.05			12	-942.53		
	m_1	10	-951.34	11.41	0.003	14	-938.10	8.86	0.012
	$m_{1\text{-MASS}}$	9	-956.88	11.08	<0.001	13	-941.08	5.97	0.015
	$m_{1\text{-WING}}$	9	-952.02	1.35	0.24	13	-938.68	1.17	0.28
16	m_0	9	-420.97			15	-415.74		
	m_1	12	-419.28	3.38	0.33	18	-412.97	5.54	0.14

Table S5.A6 Difference in linear (β_i), non-linear (γ_i) and correlational (γ_{ij}) selection gradients (±SE) between low and high density environments for body mass and wing length measured for 2 and 12-day-old nestling. Selection gradients were computed on both raw and standardized data, and significant values (bold) are based on sequential model building results (see Table S5.A5).

	2 days		**12 days**	
	Low density	High density	Low density	High density
Selection gradients (raw values)				
β_{MASS}	0.051 ± 0.007	0.043 ± 0.006	0.047 ± 0.002	0.036 ± 0.002
β_{WING}			0.004 ± 0.001	0.005 ± 0.001
γ_{MASS}	-0.047 ± 0.007	-0.036 ± 0.006	-0.012 ± 0.001	-0.009 ± 0.001
γ_{WING}			$-6.0 \cdot 10^{-4}$ ± $2.4 \cdot 10^{-4}$	$-4.4 \cdot 10^{-4}$ ± $2.2 \cdot 10^{-4}$
$\gamma_{MASS\text{-}WING}$			$10.9 \cdot 10^{-4}$ ± $4.6 \cdot 10^{-4}$	$4.1 \cdot 10^{-4}$ ± $4.4 \cdot 10^{-4}$
Standardized selection gradients				
β_{MASS}	0.071 ± 0.009	0.065 ± 0.008	0.130 ± 0.007	0.102 ± 0.006
β_{WING}			0.037 ± 0.007	0.038 ± 0.006
γ_{MASS}	-0.094 ± 0.014	-0.078 ± 0.014	-0.062 ± 0.010	-0.070 ± 0.009
γ_{WING}			-0.018 ± 0.011	-0.024 ± 0.01
$\gamma_{MASS\text{-}WING}$			0.009 ± 0.008	0.012 ± 0.008

Table S5.A7 Estimated variance components (±SE) from A) univariate animal models, B) multivariate animal models including only morphological traits and C) multivariate animal models including morphological trait and relative fitness, for 4 age-classes (2, 6, 12 and 16 days old). Variance components are additive genetic variance (V_A), broods (V_B), year (V_Y) and residual variance (V_R).

Age (days)	Trait	Variance component											
		V_A			V_B			V_Y			V_R		
A) Univariate													
2	Mass	0.097	±	0.042	1.305	±	0.056	0.015	±	0.012	0.587	±	0.029
	Fitness	0.067	±	0.009	0.174	±	0.008		-		0.051	±	0.006
6	Mass	1.033	±	0.236	5.324	±	0.248	0.248	±	0.152	2.382	±	0.157
	Wing	0.041	±	0.027	1.012	±	0.043	0.027	±	0.018	0.448	±	0.020
	Fitness	0.033	±	0.006	0.150	±	0.007		-		0.052	±	0.004
12	Mass	1.471	±	0.241	4.382	±	0.223	0.449	±	0.258	1.621	±	0.151
	Wing	1.941	±	0.884	36.086	±	1.569	3.113	±	1.794	10.714	±	0.607
	Fitness	0.012	±	0.004	0.079	±	0.004	-	±	-	0.043	±	0.003
16	Mass	0.976	±	0.170	2.131	±	0.128	0.074	±	0.050	1.319	±	0.108
	Wing	1.298	±	0.953	37.713	±	1.702	3.557	±	2.046	12.298	±	0.666
	Tarsus	0.056	±	0.010	0.086	±	0.006	0.008	±	0.005	0.098	±	0.007
	Fitness	0.001	±	0.002	0.026	±	0.002		-		0.032	±	0.001
B) Multivariate – Morphological traits													
6	Mass	1.049	±	0.231	5.302	±	0.246	0.259	±	0.158	2.376	±	0.154
	Wing	0.042	±	0.027	1.019	±	0.044	0.027	±	0.018	0.446	±	0.020
12	Mass	1.536	±	0.241	4.356	±	0.222	0.450	±	0.258	1.584	±	0.151
	Wing	2.120	±	0.892	36.253	±	1.579	3.104	±	1.783	10.594	±	0.610
16	Mass	0.975	±	0.168	2.145	±	0.128	0.074	±	0.050	1.317	±	0.107
	Wing	1.289	±	0.949	37.625	±	1.697	3.581	±	2.047	12.307	±	0.664
	Tarsus	0.053	±	0.010	0.089	±	0.006	0.008	±	0.005	0.100	±	0.007
C) Multivariate – Morphological traits + Relative fitness													
2	Mass	0.137	±	0.043	1.294	±	0.056	0.015	±	0.012	0.564	±	0.030
	Fitness	0.068	±	0.009	0.174	±	0.008		-		0.051	±	0.006
6	Mass	1.145	±	0.233	5.386	±	0.250	0.255	±	0.154	2.318	±	0.154
	Wing	0.045	±	0.027	1.020	±	0.044	0.027	±	0.017	0.445	±	0.020
	Fitness	0.034	±	0.006	0.150	±	0.007		-		0.052	±	0.004
12	Mass	1.548	±	0.242	4.880	±	0.247	0.468	±	0.263	1.588	±	0.151
	Wing	2.373	±	0.916	38.230	±	1.683	3.191	±	1.816	10.499	±	0.622
	Fitness	0.012	±	0.004	0.080	±	0.004		-		0.043	±	0.003
16	Mass	0.857	±	0.166	2.662	±	0.151	0.078	±	0.050	1.422	±	0.107
	Wing	1.047	±	0.962	42.731	±	1.951	3.505	±	1.998	12.808	±	0.681
	Tarsus	0.054	±	0.010	0.092	±	0.006	0.008	±	0.005	0.099	±	0.007
	Fitness	0.002	±	0.002	0.026	±	0.002		-		0.032	±	0.001

Table S5.A8 Statistical comparisons of predicted vs. observed evolutionary responses (*R*) for all trait-age combinations. Predicted evolutionary responses were assessed with breeder's equation (BE) or secondary theorem of selection (STS), see the main text for more details.

Trait	**Age**	**Comparisons with BE**			**Comparisons with STS**		
		t-value	df	*P*-value	t-value	Df	*P*-value
Body mass	2	0.87	7	0.41	1.91	7	0.10
	6	3.12	7	0.017	3.65	7	0.008
	12	2.90	7	0.023	2.81	7	0.026
	16	1.85	7	0.11	1.47	7	0.18
Wing length	6	1.29	7	0.24	1.62	7	0.15
	12	2.45	7	0.044	2.59	7	0.036
	16	2.39	7	0.048	2.32	7	0.054
Tarsus length	16	2.93	7	0.022	4.55	7	0.003

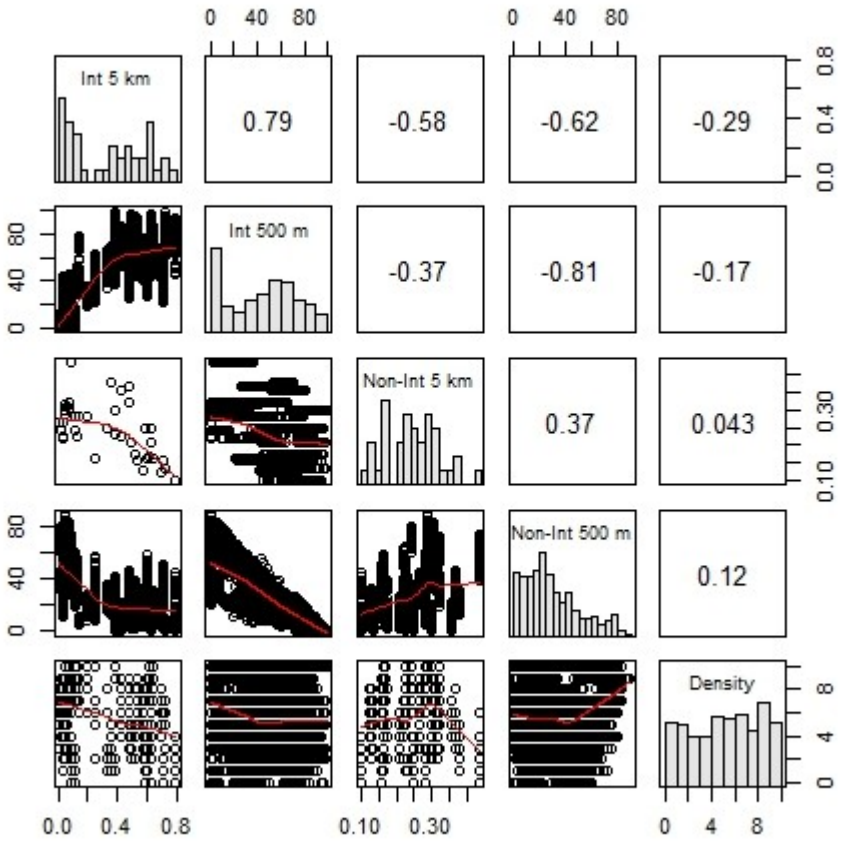

Figure S5.A1 Relationships among environmental variables (intensive cultures at 5 km, intensive cultures at 500 m, non-intensive cultures at 5 km, non-intensive cultures at 500 m, tree swallow density). Distributions of environmental observations are on the diagonal (gray bars) while they are plotted together below the diagonal (red lines are lowess smoothing curves). Pearson's correlation coefficients are presented above the diagonal.

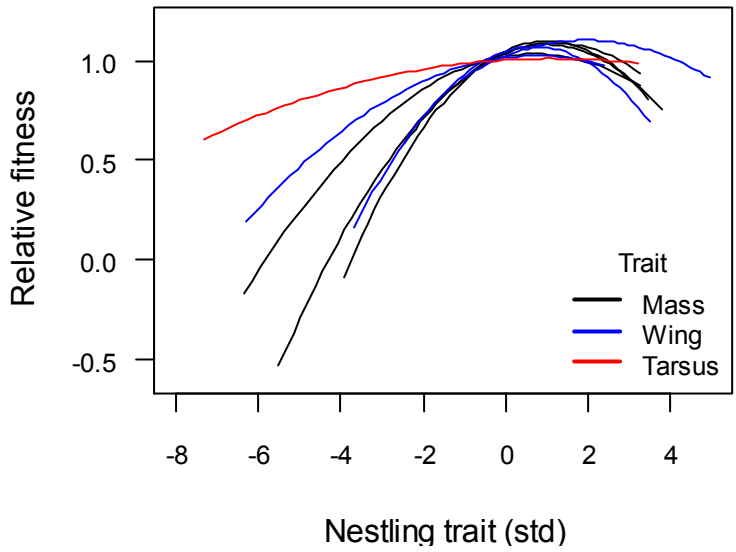

Figure S5.A2 Graphic representation of non-linear selection differentials, for body mass (4 models, black lines), wing length (3 models, blue lines) and tarsus length (1 model, red line).

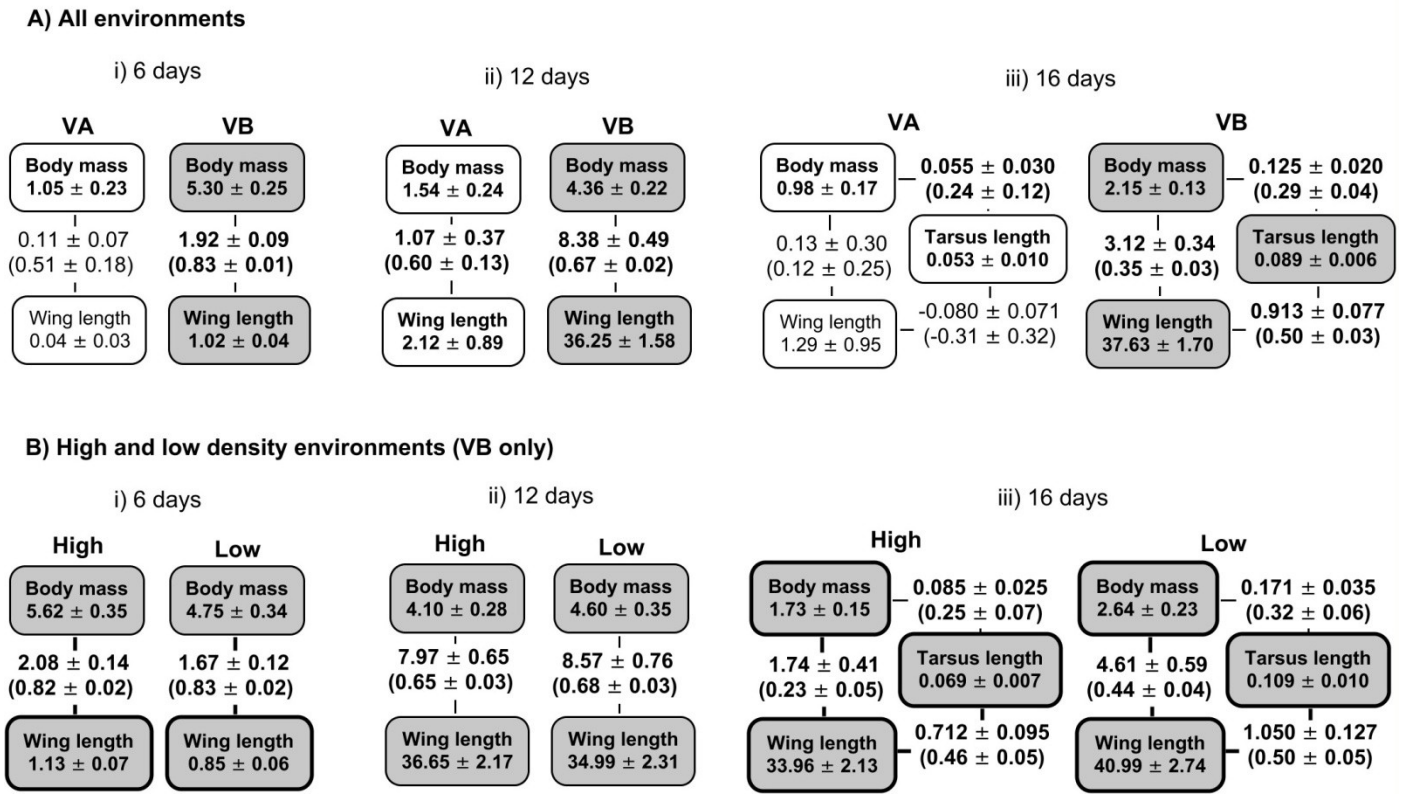

Figure S5.A3 Schematic representation of additive genetic (white boxes) and brood (gray boxes) (co)variances (±SE) obtained from multivariate animal models within A) all environments and B) high or low density environments, for nestlings at i) 6 days old, ii) 12 days old iii) 16 days old. Within each box are reported the variance component of a trait and the covariance for the two related traits are presented on lines between boxes (correlations are in parentheses). Values significantly different from zero are in bold.

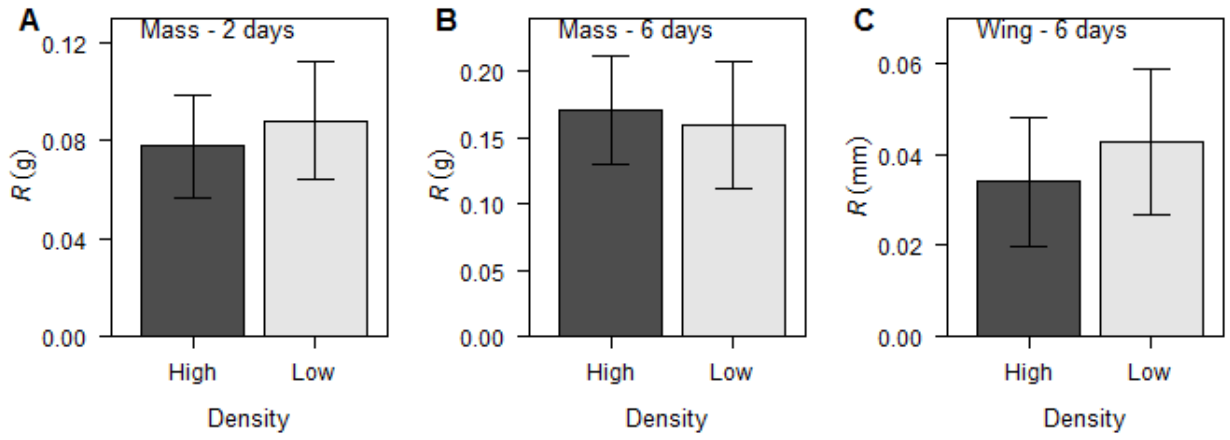

Figure S5.A4 Predicted evolutionary changes (*R*) with the secondary theorem of selection (STS) approach for 3 studied trait-ages (A-C) in low and high density environments. *R* represents the change in mean trait between 2 generations and is expressed in the trait measurement unit. Errors bars represent standard errors.

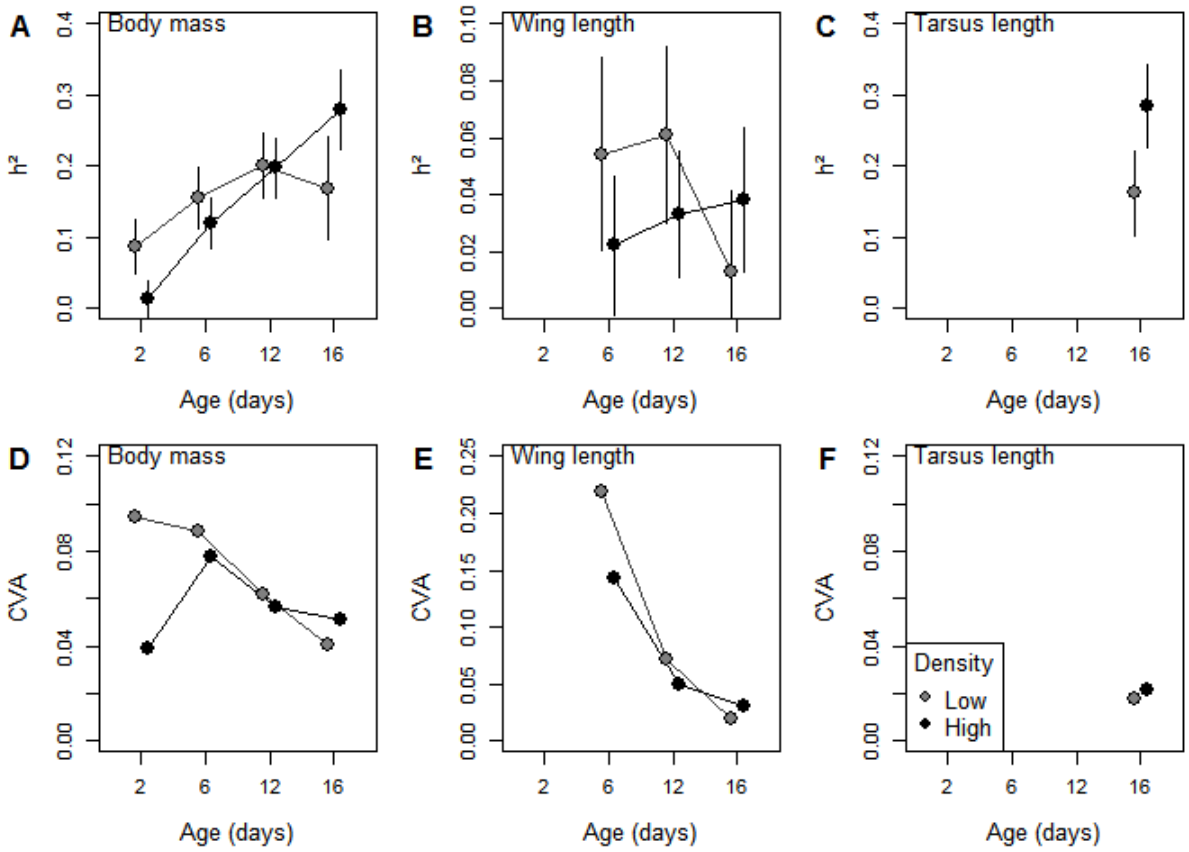

Figure S5.A5 Difference in h^2 (A-C) and CV_A (D-F) between low (gray dots) and high (black dots) density environments, for body mass (A,D), wing length (B,E) and tarsus length (C,F). Error bars for h^2 estimates are SE.

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