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IMPACTS EVOLUTIFS DE L'URBANISATION CHEZ LA MESANGE CHARBONNIERE :

DU PHENOTYPE AUX (EPI)GENOMES

Présentée par Aude CAIZERGUES

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UNIVERSITÉ DE MONTPELLIER

“C’est impossible, dit la Fierté,
C’est risqué, dit l’Experience,
C’est sans issue, dit la Raison,
Essayons, murmure le Cœur.”

William Arthur Ward

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– LISTE DES PUBLICATIONS ET COMMUNICATIONS –

Articles scientifiques:

Publiés

- **Caizergues, A.E.**, Charmantier, A., Lambrechts, M.M., Samuel Perret, S., Demeyrier, V., Lucas, A., & Arnaud Grégoire, A. (2021) An avian urban morphotype: how the city environment shapes Great tit morphology at different life stages. *Urban Ecosyst* https://doi.org/10.1007/s11252-020-01077-0
- **Caizergues, A. E**., Grégoire, A., & Charmantier, A. (2018). Urban versus forest ecotypes are not explained by divergent reproductive selection. *Proceedings of the Royal Society B: Biological Sciences*, 285(1882), 20180261. https://doi.org/10.1098/rspb.2018.0261
- Perrier, C., **Caizergues, A.E.** and Charmantier, A. (2020) Adaptation Genomics in Urban Environments. In *Urban Evolutionary Biology* (pp. 74-90). Oxford University Press.

En préparation

- **Caizergues, A. E.**, Grégoire, A., Choquet R., Perret S. & Charmantier, A. Are behavioural and stress responses to urbanization adaptive? Great tits as a case study. (*in prep for Journal of Animal Ecology*)
- **Caizergues, A.E.**, Le Luyer, J., Arnaud Grégoire, A., Szulkin, M., Señar, J.C., Charmantier, A. & Perrier, C. Testing for parallel genomic and epigenomic footprints of adaptation to urban life in a passerine bird (*in prep for PNAS*)

Communications orales:

- "Adaptive potential in urban habitats : a case study in Great tit." Parid meeting workshop, Strasbourg, 8 février 2018
- "Urban ecotypes are not explained by divergent reproductive selection." Poster présenté au congré international "Evolution" à Montpellier, 19-22 août 2018
- Ma thèse en 180 secondes finale régionale : « Mésange des villes, mésange des champs »

Enseignement et vulgarisation scientifique:

- Missions complémentaires d'enseignement au sein du département de Biologie Ecologie de l'Université de Montpellier (2017-2020, 64h par an, pour un total de 192h)
- Participation à un documentaire animalier sur la biodiversité urbaine, « Le Territorie des autres », réalisé par Léa Samson-Jousseaume pour le festival de Ménigoute 2020
- Fête de la science : stand de présentation des mésanges urbaines et oiseaux des jardins Zoo du Lunaret, 7 octobre 2020)
- Fête de la nature : stand d'échange avec le grand public sur la biodiversité (Zoo du Lunaret, 22 mai 2019)
- Ma thèse en BD : vulgarisation de ma thèse sous forme de bande dessinée (avec l'association co-science et le Collège Doctoral de l'Université de Montpellier, juin 2019)
- Participation à une émission de radio sur la biodiversité urbaine sur RCF (avril 2019)

– NOTE AU LECTEUR –

Ce manuscrit de thèse est rédigé sous une forme de thèse « sur articles » qui sont organisés en chapitres encadrés par une introduction, un chapitre de méthodes et une discussion générale.

Chaque chapitre débute par un résumé détaillant les objectifs et principaux résultats du (des) article(s) qui le constitue(nt). Les articles sont présentés tels quels, en anglais et avec leur propre bibliographie et structure interne qui dépend du journal dans lequel ils ont été publiés ou vont être soumis. Notons que pour la clarté du récit, le chapitre II fait référence à des articles présentés en chapitre III.

Le matériel supplémentaire associé aux publications déjà parues est disponible en ligne, sur les sites des compagnies de publications. Pour les manuscrits non publiés, les matériels supplémentaires sont présentés en annexe du présent document.

INTRODUCTION



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I. L'URBANISATION

I.1. L'expansion des zones urbaines

L'exploitation du territoire par l'Homme remonte aux premières étapes de sa sédentarisation. Déjà avant l'antiquité, l'installation d'une population humaine sur une zone peut conduire jusqu'à la destruction et déforestation massives de milieux naturels (Diamond 2011). Dans un premier temps, l'impact de l'Homme sur son environnement via l'urbanisation reste faible et très localisé car la croissance des villes et plus généralement de la population humaine est limitée par les lacunes technologiques (Davis 1955). Ce n'est qu'à l'aube de l'Anthropocène[1] que la démographie humaine commence à croitre significativement sur tout le globe puis à la révolution industrielle, débutant au XVIII° siècle, qu'elle entame le processus d'urbanisation intensif mondial tel que nous le connaissons aujourd'hui.

Ainsi depuis deux siècles, le processus d'urbanisation ne cesse de s'intensifier de telle sorte qu'en 2007, pour la première fois dans l'histoire de l'humanité, la population urbaine mondiale dépasse la population rurale (United Nations 2018). Chaque jour, dans le monde, 165 000 nouveaux urbains viennent renforcer ce chiffre. Bien qu'il n'existe pas de définition universelle de ce qui caractérise précisément une ville ou une zone urbaine, la plupart des pays prennent en compte au moins un critère démographique (densité ou nombre d'habitants), administratif, économique ou lié aux infrastructures (rues pavées, eau courante, assainissement, éclairage électrique) pour délimiter leur territoire urbain du rural. Toutes les définitions de ville semblent ainsi converger vers des zones densément peuplées, où les activités et les constructions humaines se regroupent et s'intensifient (United Nations 2018).

La croissance urbaine est généralisable à l'ensemble du globe (**Figure 1**), et entraine avec elle de nombreux changements liés directement ou indirectement aux activités humaines. Actuellement, les zones urbaines représentent environ 3% de la surface émergée de la planète (Aronson et al. 2014) et les images satellitaires révèlent qu'elle s'accroit de 110km² par jour, soit l'équivalent de la surface de Paris. A ce rythme, le cumul des aires urbaines mondiales aura triplé entre 2000 et 2030 (Schneider et al. 2009). L'urbanisation est donc un processus récent mais qui impacte la planète de façon colossale et croissante de sorte que certains considère que nous entrons dans l'aire des villes, « l'Urbanocène » (West 2017).

En France, on retrouve au 7ème rang des villes les plus peuplées, la ville de Montpellier, dans laquelle se déroule cette étude. A l'image de la tendance mondiale, l'aire urbaine montpelliéraine est en pleine expansion (**Figure 2**) et enregistre la croissance démographique la plus importante de France, avec un gain de 9300 habitants par an, soit +1.6% (INSEE 2018).

I.2. Un impact considérable sur l'environnement

L'expansion des villes a des effets directs sur l'environnement, comme la fragmentation des habitats et la conversion d'un milieu rural ou naturel en un ensemble d'infrastructures bétonnées. Ainsi, les prédictions annoncent que d'ici 2030, la croissance urbaine mettra en péril l'habitat de près de 25% des espèces d'amphibiens, de mammifères et d'oiseaux classées en danger ou en danger critique d'extinction sur la liste rouge de l'IUCN (International Union for the Conservation of Nature) (McDonald et al. 2013).

S'ajoutent à cela, les effets indirects des villes sur leur arrière-pays, car la croissance urbaine va de pair avec une demande accrue en ressources : bois, agriculture, élevage, transport… De tout temps, la fondation, la survie et le développement des villes dépend des ressources que l'Homme à la capacité de puiser dans l'arrière-pays. Si la limitation technologique a longtemps endigué la croissance des villes, les avancées récentes héritées de la révolution industrielle ont décuplé les capacités de l'Homme à extraire les ressources et à exploiter son environnement. Il bénéficie maintenant des ressources nécessaires à une croissance urbaine effrénée. A mesure que les villes grandissent, leur rayonnement s'agrandit. Les besoins en ressources nécessaires au développement des villes sont en

[1] La date précise du début de l'« anthropocène » varie selon les domaines d'étude. Pour les paléoclimatologues, il est marqué par le début de l'agriculture. Pour les météorologues il commence au milieu du XVIIIe siècle, avec l'invention de la machine à vapeur. Pour les autres, il commence à partir de la fin de la seconde guerre mondiale.

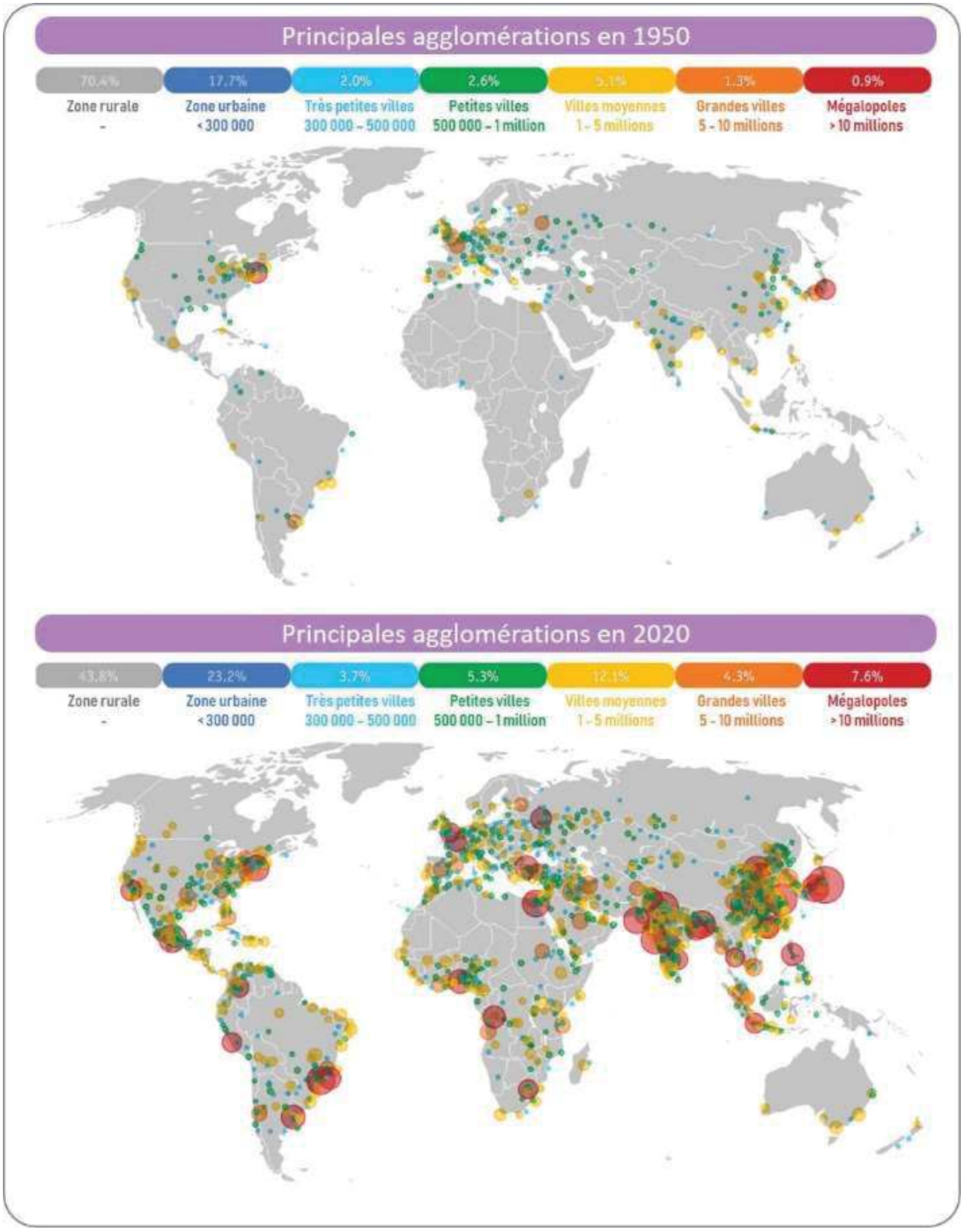


Figure 1 | Evolution des principales zones urbaines du globe entre 1950 et 2020. La couleur et la taille des points indiquent la population de la zone urbaine concernée. La frise colorée représente le pourcentage de la population mondiale vivant dans les différentes catégories de villes. Figure adaptée de "The Economist – Bright light, big cities, Urbanisation and the rise of the megacity (2015)".

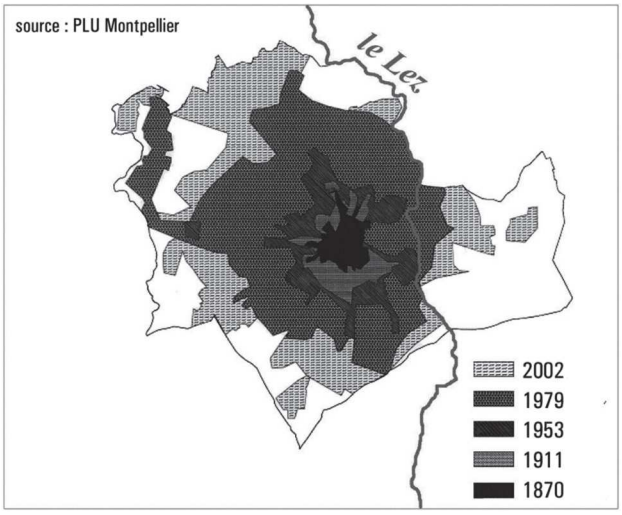


Figure 2 | Evolution de l'urbanisation de la ville de Montpellier entre 1870 et 2002. Echelle 1/60000ème. Source : Plan Local de l'Urbanisme 2017.

permanente augmentation, si bien qu'on ne les puise plus seulement dans les régions alentours, mais parfois à l'autre bout du monde (*e.g.* : utilisation de sable provenant des plages d'Asie ou des fonds marins pour l'industrie du bâtiment Européenne).

Les effets de l'urbanisation sont donc d'une amplitude planétaire. L'urbanisation participe à la déforestation et donc à l'augmentation du CO2 atmosphérique. De plus, les constructions humaines, représentent 190cm3 de béton coulé par seconde dans le monde, soit 6 milliards de mètres cubes de béton par an, ce qui en fait le produit manufacturé le plus consommé au monde après l'eau et une des sources principales d'émission de CO2 (Gartner 2004).

En conséquence, à l'instar du changement climatique, l'urbanisation est maintenant considérée comme l'une des plus grandes menaces pour la biosphère et la biodiversité (Maxwell et al. 2016).

I.3. L'habitat urbain : naissance d'un nouveau milieu

Le processus d'urbanisation représente un cas extrême de la transformation irréversible des milieux naturels (McKinney 2002). Il résulte en un vaste recouvrement de surfaces auparavant végétalisées, par des surfaces artificielles et souvent stériles (béton, goudron…), entrainant une perte ou/et une fragmentation d'habitat considérable pour de nombreuses espèces. Le nouvel habitat urbain remplaçant l'habitat originel est essentiellement façonné par l'Homme et ses activités, et présente un nouveau cocktail de conditions environnementales abiotiques et biotiques.

Au-delà des changements liés aux infrastructures *per se*, la concentration des différentes sources de pollutions est particulièrement élevée dans les zones urbaines. En effet, les niveaux de pollutions sonore (Berglund & Lindvall 1995; Slabbekoorn & Peet 2003) lumineuse (Rich & Longcore 2006) et chimique (Roux & Marra 2007; Isaksson 2010) y sont plus élevés qu'en milieu naturel. Les conditions météorologiques y sont aussi chamboulées avec la naissance de microclimats urbains. Par exemple, les surfaces bétonnées, le trafic et l'industrie sont responsables d'une hausse des températures de 2°c en moyenne (Ryu & Baik 2012), et perturbent les cycles hydrologiques (Shepherd 2005).

Le contrôle de l'Homme sur la structure des villes s'applique aussi à la végétation. Les espaces naturels sont considérablement réduits et la végétation naturelle est souvent remplacée par une végétation ornementale exotique. En conséquence, les vestiges d'habitat originel sont extrêmement restreints et fragmentés et la communauté végétale transformée (Rudnicky & McDonnell 1989).

Une autre composante majeure de l'écosystème urbain est l'omniprésence de l'Homme. Dans les zones urbaines la densité d'humain monte jusqu'à presque 3 000 hab./km² en France contre environ 117 hab./km² en moyenne sur l'ensemble du territoire (notons que dans le monde certaines villes dépassent largement les 10 000 hab./km² comme Manille aux Philippines avec 43 079 hab./km², ou Bombay en Inde avec 28 508 hab./km²) (INSEE 2018). Ces chiffres montrent par ailleurs que bien que l'urbanisation soit un phénomène global, sa rapidité et son impact sur les écosystèmes peut varier d'un pays à l'autre. En particulier, dans les pays en développement, la croissance des villes est souvent bien supérieure à celle observée dans les pays développés (Cohen 2004).

Sous nos yeux et entre nos mains, un nouveau milieu se développe, et bien que certaines espèces en soit chassées, il constitue une opportunité de vie pour d'autres.

I.4. Zones urbaines : un terrain de « jeu » récent pour les écologues

Les zones urbaines ont souvent été considérées comme stériles d'un point de vue écologique.

Pourtant, on retrouve de nombreuses espèces sauvages au cœur même des grandes agglomérations et la vaste majorité des grands groupes du vivant est affectée par l'urbanisation. Il devient donc de plus en plus pressant de comprendre les conséquences démographiques, écologiques et évolutives de la vie en milieu urbain pour les espèces sauvages.

Etonnamment, le domaine de l'écologie urbaine est un domaine relativement récent de la science. Jusqu'au milieu du XX° siècle, peu d'études s'intéressent à l'écologie dans des habitats modifiés par l'homme. En effet, la vision binaire opposant homme et nature est répandue dans la communauté scientifique de l'époque, et plus largement dans la société. De cette vision dichotomique résulte un non intérêt pour l'étude des milieux anthropisés, les écologues considérant ne pas être légitime à étudier ces écosystèmes « hors nature » (McDonnell 2011; notons que certains travaux sur des habitats d'origine anthropique furent tout de même réalisés, mais de façon anecdotique, voir Sukopp 2008). A partir des années 1950-1960, il devient apparent à l'ensemble de l'humanité que les activités anthropiques participent significativement à l'altération des écosystèmes locaux et régionaux (Thomas 1956). Un élément décisif dans la reconnaissance par les écologues de l'importance d'inclure l'humain comme composant des écosystèmes fut probablement la publication de la première étude concernant l'augmentation du CO2 atmosphérique au

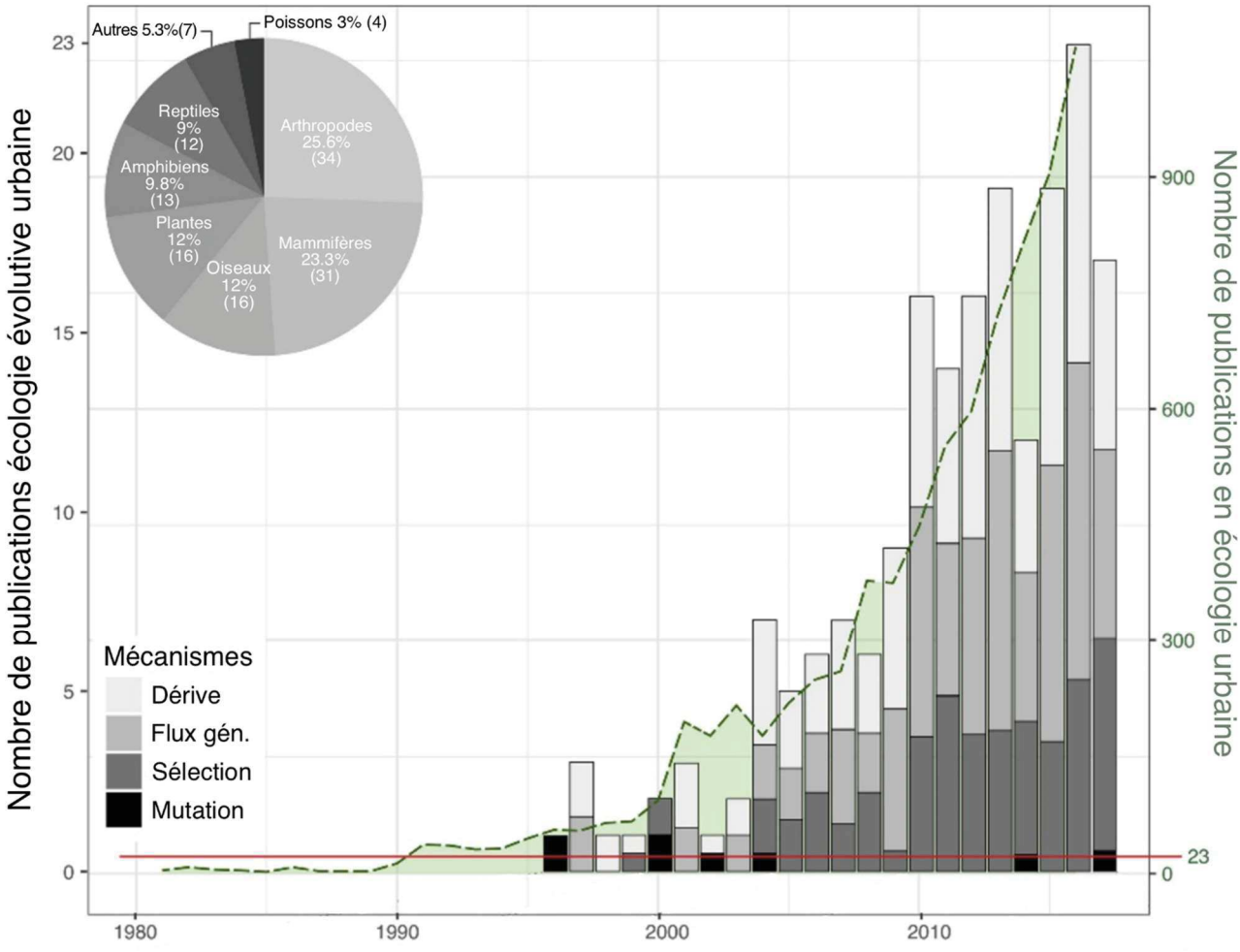


Figure 3 | Nombre de publications incluant les termes "écologie urbaine" ou "évolution urbaine" entre 1980 et 2017. L'axe de gauche (en noir), associé au diagramme à batons, montre le nombre de publications traitant d'évolution en milieu urbain par année. Chaque bâton est subdivisé selon la proportion d'études attribuant le mécanisme d'évolution à la derive génétique (gris clair), au flux génique (gris), à la selection (gris foncé) ou à la mutation (noir). L'axe de droite (en vert), associé à la ligne en pointillés, indique le nombre de publications en écologie urbaine par an. La ligne horizontale rouge indique le nombre maximum de publications en evolution urbaine par an relativement au nombre de publications en écologie urbaine. Le camembert représente les proportions des espèces étudiées appartenant à chaque taxon indiqué. Figure adaptée de Rivkin et al. 2018.

début des années 1960 (Keeling 1998). Avec la prise de conscience que la majorité des écosystèmes sont affectés par l'Homme, l'intérêt des scientifiques se tourne rapidement vers l'étude des impacts des activités humaines sur la biosphère, et notamment l'étude des écosystèmes associés au milieux anthropisés tels que le milieu urbain. Ainsi, la discipline de l'écologie urbaine nait au début des années 1970 (McDonnell et al. 2009).

L'essentiel des travaux de recherche sur le milieu urbain est initialement tourné vers la description de la diversité spécifique et des densités de populations. On constate alors l'homogénéisation biotique des communautés urbaines (McKinney 2006). En effet, la faune urbaine est assez similaire d'une ville à l'autre, même à large échelle géographique, et elle est souvent dominée par peu d'espèces présentes mais en forte densités. Par exemple, l'avifaune urbaine est typiquement dominée par des espèces granivores ou des omnivores de taille moyenne et par des espèces sédentaires (Bezzel 1985).

En ce qui concerne les études au niveau populationnel et individuel, bien que de rares études précoces situent le contexte (ex : Perrins 1965), celles-ci ne se généralisent que plus tard dans la littérature, au cours des années 1990. L'ensemble de ces études met en évidence de nombreuses différences entre les populations urbaines et rurales (voir section II.2), soulevant des questions quant aux processus impliqués dans cette différenciation et constituant un socle essentiel à la sous discipline de l'écologie évolutive urbaine. Ainsi, depuis le début des années 2000 (**Figure 3**) le domaine de l'écologie évolutive urbaine est en constante expansion (Rivkin et al. 2018; Szulkin et al. 2020b; Uchida et al. 2020). Malgré cela, le volume de publication des articles de recherches sur la biodiversité urbaine reste bas par rapport la vitesse de l'urbanisation et son impact toujours grandissant sur la biodiversité (Magle et al. 2012). Pourtant, le milieu urbain offre de nombreux réplicas sur l'ensemble du globe, et au-delà de l'étude de l'urbanisation *per se* il s'agit d'un bon modèle pour approfondir nos connaissances sur les capacités des espèces à s'adapter aux changements anthropiques rapides.

II. L'IMPACT DE LA VIE URBAINE SUR LA FAUNE

II.1. Des effets délétères mais aussi bénéfiques

Les nouvelles conditions environnementales urbaines ont des conséquences sur la vie des espèces sauvages. Bien qu'elles soient souvent détrimentaires, ces nouvelles conditions peuvent parfois constituer de nouvelles opportunités écologiques pour certaines espèces.

L'accès aux ressources alimentaires fait partie des aspects les plus chamboulés en milieu urbain : certaines ressources émergent alors que d'autres se raréfient. Par exemple, les déchets humains et le nourrissage volontaire constituent une source de nourriture importante pour certains commensaux[2] : pigeons, rats, ratons laveurs... A l'inverse, les espèces ne se nourrissant pas d'aliments produits par l'Homme ont parfois des difficultés à trouver de la nourriture. Notamment, la présence réduite de végétation native, remplacée par des essences exotiques, conduit à une diminution drastique de la diversité et de l'abondance des populations d'insectes phytophages, impactant directement les insectivores (Seress et al. 2018).

Autre point important, la structure géométrique urbaine qui affecte directement la disponibilité des lieux de reproduction/nidification. Par exemple, la multitude de façades verticales offre de nombreuses opportunités de nidification pour certaines espèces nichant sur des falaises (Jenkins et al. 2013; Reynolds et al. 2019). Vu sous un autre angle, les constructions humaines sont responsables d'une haute fragmentation des parcelles d'habitats naturels. Fragmentation qui peut conduire à une réduction drastique du flux génétique entre populations et donc à une perte de diversité génétique (Ruell et al. 2012), résultat inquiétant dans un contexte de conservation.

L'impact négatif de la lumière artificielle sur de nombreux taxons n'est plus à démontrer (Rich & Longcore 2006). Elle a potentiellement des effets complexes et subtils sur différentes composantes du comportement animal : l'orientation, la migration, la recherche de nourriture, et la communication (Longcore & Rich 2004). Un grand nombre de taxons

[2] Le commensalisme est une relation entre espèces qui est bénéfique pour l'une (l'espèce commensale) et neutre pour l'autre.

sont impacté par la lumière artificielle depuis les insectes (Eisenbeis 2006) jusqu'au oiseaux (Gauthreaux et al. 2006), en passant par les reptiles (Salmon 2006) et les mammifères (Schoeman 2016). Outre le comportement, la lumière artificielle impacte la reproduction, la croissance et le développement (Wise 2007). La luminosité diurne quasi-perpétuelle qui règne dans les agglomérations urbaines perturbe le rythme circadien et les patrons de sommeil des oiseaux, allant jusqu'à impacter leur valeur sélectives (Spoelstra & Visser 2013). Malgré tous ces effets néfastes, certains profitent de cette lumière artificielle. Par exemple, l'attrait qu'elle représente pour les insectes génère de véritables festins pour certains insectivores nocturnes (*e.g.* chez les chauves-souris, Jung & Kalko 2010).

Les sources de pollution chimique sont nombreuses en milieu urbain et les polluants qu'on y retrouve sont de natures très variées (par ex. métaux lourds, gaz à effet de serre, composés organiques, nanoparticules). Bien que les polluants semblent, selon leur nature, avoir un impact particulier sur les organismes urbains (Kekkonen 2017), de manière générale, ils impactent négativement la physiologie, et sont responsables d'un stress oxydatif accru au niveau cellulaire. Ainsi, la pollution élevée en milieu urbain peut avoir des répercussions importantes sur le développement, la survie et la reproduction des individus (Isaksson 2010). Par ailleurs, au-delà des impacts directs sur la physiologie des individus la pollution participe à l'augmentation de la température ambiante des villes. En milieu tempéré, l'effet « îlot de chaleur » peut constituer une aubaine pour les populations urbaines en particulier en hiver ou il réduit la mortalité et la dépense énergétique engagée dans la production de chaleur corporelle. Au contraire, en saison estivale ou en milieu tropical, il est responsable d'un stress thermique important (Angilletta et al. 2007).

Ainsi, la présence ou l'absence d'une espèce en milieu urbain dépend grandement de la capacité de celle-ci à tolérer et/ou tirer profit des conditions environnementales urbaines. On distingue ainsi 3 types d'espèces en fonction de leur affinité pour l'habitat urbain : celles qui profitent du milieu urbain, les « urban exploiters », celles qui l'évitent « urban avoiders » et les espèces intermédiaires qui y vivent sans pour autant y prospérer, les « urban adapters » (détaillé dans l'**Encadré 1**, Blair 1996; McKinney 2002; Shochat et al. 2006, mais voir Lambert et al. 2020). On retrouve en ville les « urban exploiters » et les « urban adapters ». Que l'on soit un « urban exploiter » ou un « urban adapter », les conditions de vie urbaine sont si particulières qu'il n'est pas étonnant d'observer des différences de phénotype[3] entre les populations restant dans le milieu naturel et les populations s'étant établies en ville (Hendry et al. 2008).

II.2. De nombreux changements phénotypiques

Il a été largement démontré que les actions de l'Homme sur la transformation des milieux naturels conduisent à de forts changements phénotypiques chez les espèces sauvages (Hendry et al. 2008). Bien évidemment, l'habitat urbain, avec toutes les nouvelles contraintes et opportunités qu'il présente, ne déroge pas à cette règle (Alberti et al. 2017). En effet, on constate de manière globale que les populations urbaines présentent un phénotype différent des populations occupant des milieux naturels sur de nombreux types de traits : morphologiques, phénologiques, comportementaux et physiologiques. Dans cette section, je me suis limitée à l'illustration des différences phénotypiques observées principalement chez les oiseaux, permettant de placer le contexte en lien avec le modèle d'étude utilisé dans ce présent travail, mais il est cependant nécessaire de garder à l'esprit que des différences phénotypiques sont aussi présentes chez de nombreux autres taxons. Par ailleurs, l'objectif ici n'est pas de faire une liste exhaustive de toutes les différences répertoriées entre milieu urbain et milieu « naturel », mais d'illustrer la diversité des réponses connues et les grandes tendances observées. J'aborderai ensuite plus en détails les effets spécifiques et mécanismes connus dans les chapitres correspondants.

Au niveau morphologique, la taille globale des individus, leur masse ainsi que leur condition corporelle font partie des traits les plus étudiés par les écologues urbains. Et pour cause, ils sont directement liés au taux métabolique et donc à certains traits d'histoire de vie (fécondité, longévité…) affectant

[3] Phénotype : ensemble des caractères observables d'un individu.

Encadré 1 | « Urban exploiters », « urban adapters» et « urban avoiders » : définitions.

Les espèces sauvages peuvent être classées en 3 catégories en fonction de leur réaction et tolérance face à l'urbanisation :

- Les « **urban exploiters** » : traduit littéralement : les espèces qui « exploitent le milieu urbain ». Ce sont des espèces qui prospèrent particulièrement bien en milieu urbain car commensales de l'Homme. Elles utilisent les ressources d'origine humaine au point d'en devenir souvent dépendantes. Elles présentent une association de caractéristiques qui leur permet de réussir en ville : régime alimentaire (ex : tolèrent les ressources d'origine humaine comme les oiseaux granivores), possède une flexibilité et capacité d'innovation leur permettant d'exploiter de nouvelles ressources, souvent sédentaires et sociales, tolèrent la présence de l'Homme.
- Les « **urban adapters** » : les espèces qui « s'adaptent au milieu urbain ». On retrouve les espèces « urban adapters » en milieu urbain, mais elles évitent en général les zones extrêmement urbanisées. Elles ne dépendent pas des ressources humaines mais les utilisent de temps en temps. Notons ici que la notion d'« adaptation » n'est pas nécessairement de l'adaptation au sens évolutif du terme, mais reflète plutôt l'idée que ces espèces sont « capables » de vivre en ville et de tolérer les nouvelles conditions environnementales.
- Les « **urban avoiders** » : les espèces qui « évitent le milieu urbain ». Elles représentent les espèces les plus sensibles à l'urbanisation. Parce qu'elles sont sensibles au stress ou qu'elles ne trouvent pas leurs ressources en ville, on les retrouve très rarement en ville.

La classification des espèces dans ces trois catégories détermine en partie la manière dont elles sont étudiées et les prédictions associées les concernant. En particulier, les études sur les "urban avoiders" sont les moins fréquentes et abordent essentiellement des aspects de conservation liées à la destruction de leur habitat. Les "urban adapters" sont probablement les espèces les plus intrigantes, car à l'inverse des "urban exploiters" qui dépendent des humains et dont les populations ont des dynamiques comparables à des phénomènes d'invasion biologique (Kark et al. 2007), il est parfois difficile de comprendre comment elles arrivent à s'établir et/ou persister en ville malgré qu'elles ne semblent pas particulièrement bénéficier des activités humaines dans leur globalité. L'utilisation du terme "adapter" pour les classifier est d'ailleurs controversée car peut porter à confusion. En effet, il suggère que ces espèces aient évolué et se soient adaptés au milieu urbain, ce qui n'est bien souvent pas démontré (Lambert et al. 2020).

ainsi directement la dynamique écologique des écosystèmes (Brown et al. 2004). En particulier, la règle de Bergmann formule l'hypothèse selon laquelle la taille des animaux serait contrainte par (et donc négativement corrélée à) la température ambiante en raison de la corrélation entre coûts métaboliques et volume corporel (Bergmann 1848; Kleiber 1932). Ainsi, l'îlot de chaleur que constitue la ville conduirait à la diminution de la taille des individus les peuplant. Cette tendance a été montrée chez de nombreux taxons (Liker et al. 2008; Merckx et al. 2018), mais ne semble pourtant pas systématique (*e.g.* Marini et al. 2017; Putman & Tippie 2020). Par ailleurs, la masse et la condition corporelle reflètent l'état nutritionnel des individus et renseignent donc sur la qualité et/ou la quantité de nourriture disponible dans le milieu. Chez l'ensemble des oiseaux urbains, aucun patron général de variation de ces deux traits n'est mis en évidence (condition plus élevée : Auman et al. 2008 ; plus faible : Liker et al. 2008), bien que pour le cas particulier des passereaux urbains la majorité des études relèvent une condition corporelle plus faible (*e.g.* Isaksson et al. 2007) ou égale (*e.g.* Bókony et al. 2012). De plus, au sein d'une même espèce, la divergence morphologique associée à l'urbanisation dépend parfois de la ville concernée. Par exemple, Biard et collaborateurs (2017) rapportent que les mésanges charbonnières urbaines ne sont pas significativement plus petites que les rurales quand elles vivent dans une ville de petite taille comme Niort, mais ils constatent une réduction significative de leur taille quand elles vivent dans des villes plus grandes comme Paris. Ainsi, l'étendue des villes semble avoir un impact direct sur le phénotype, rendant la généralisation compliquée. Par ailleurs, taille, masse et condition corporelle ne sont pas les seuls traits morphologiques variant en fonction du

taux d'urbanisation. Chez les oiseaux par exemple, la forme du bec et la taille des ailes peuvent aussi diverger entre populations urbaines et rurales (Giraudeau et al. 2014; Seress et al. 2020).

Les traits physiologiques sont parmi les premiers à être modifiés face à la dégradation des habitats (Chown & Gaston 2008), et subissent donc des changements importants en lien avec la vie en milieu urbain. En effet, l'habitat urbain est un environnement particulièrement stressant (présence humaine, pollution, densité de population, alimentation) et la variété des stress et des réponses à ces stress dépendent souvent de l'espèce étudiée. Par exemple, certaines espèces présentent des taux de corticostérone (hormone liée au stress) plus faibles en milieu urbain traduisant une habituation à la présence de l'homme (Partecke et al. 2006), alors que d'autres présentent au contraire des niveaux de stress plus élevés (*e.g.* Beaugeard et al. 2019). Par ailleurs, la pollution chimique (entre autre) augmente le stress oxydatif et donc la demande en antioxydants assimilés, ce qui représente un défi pour le système immunitaire (Isaksson et al. 2005). Enfin, la nourriture d'origine humaine est aussi responsable de changements physiologiques comme l'augmentation du cholestérol sanguin et de la glycémie qui peuvent avoir un effet direct sur la valeur sélective (Ruiz et al. 2002; Ishigame et al. 2006).

A plus large échelle sur la vie des individus, l'urbanisation impacte largement les traits d'histoire de vie, c'est-à-dire la distribution des évènements majeurs au cours de la vie des individus (naissance, croissance, âge à la première reproduction, taille et nombre des descendants, etc). En particulier, certains effets sont visibles dès les premiers stades du développement, avec par exemple, une perturbation de la croissance des jeunes (Seress et al. 2020). Ces impacts développementaux ont souvent des répercussions directes sur la reproduction des individus car intrinsèquement liés à la survie juvénile. Ainsi, de nombreuses espèces présentent un succès reproducteur amoindri en zone urbaine (Chamberlain et al. 2009). Outre le succès reproducteur, la phénologie[4] de la reproduction est aussi largement impactée. Chez les oiseaux par exemple on constate une avancée globale de la date de ponte chez de nombreuses espèces (Chamberlain et al. 2009). Cette anticipation est attribuée à différents facteurs tels que la chaleur plus élevée, la perturbation du rythme circadien par la lumière artificielle ou encore le maintien d'une bonne condition corporelle pendant l'hiver. De plus, la date de ponte a une importance capitale sur le succès reproducteur chez de nombreuses espèces car elle détermine l'abondance de nourriture qui sera disponible au moment de la croissance des jeunes. Pondre trop tôt ou trop tard résultant en un manque de nourriture pour élever ses petits, il est donc important de « choisir » quand pondre.

Les traits comportementaux font partie des traits les plus étudiés en milieu urbain. En effet, ils sont en première ligne pour l'adaptation aux changements rapides puisqu'ils peuvent changer au cours de la vie d'un individu en réponse à des stimuli divers. On constate une large gamme de changements comportementaux en milieu urbain partant de simples comportement d'habituation aux nouvelles conditions et allant jusqu'à des innovations comportementales poussées, témoin de capacités cognitives élevées. Ainsi, parmi les premiers changements comportementaux observés chez les populations urbaines se trouvent les comportements liés à la façon de s'ajuster au dérangement humain. En général, on constate des distances de fuite réduites face à la proximité humaine (Atwell et al. 2012) ou à l'inverse, des modifications des horaires et lieux d'activité pour éviter ce dérangement (Baker et al. 2007; Dowding et al. 2010). Certaines espèces sont contraintes d'ajuster leurs méthodes de communication. On constate par exemple des changements de chants en réponse à la pollution sonore. Par exemple, le chant des mésa nges charbonnières urbaines est modifié vers des fréquences sonores plus aigües (Slabbekoorn & Peet 2003). Par ailleurs, en lien avec la modification des ressources disponibles et de leur accès, de nouveaux comportements de recherche de nourriture s'observent en ville (Sol et al. 2012a). Ces comportements sont parfois associés à des prises de risque plus élevées notamment par l'utilisation de nouvelles ressources alimentaires jusqu'alors inconnues (Sol et al. 2012b) ou en ignorant le risque

[4] Phénologie : récurrence annuelle des processus biologiques (migration, reproduction, hibernation, floraison, germination) et plus largement des traits d'histoire de vie liés à la saisonnalité du climat.

de prédation (Tsurim et al. 2010). Pour certaines espèces, les nouvelles stratégies comportementales vont jusqu'au développement de comportements extrêmement complexes comme par exemple l'utilisation du trafic routier par les corbeaux pour casser des noix (Nihei 1995).

De manière générale il est fréquent que de multiples aspects du comportement soient corrélés entre eux et forment un « syndrome comportemental » (Sih et al. 2004), par exemple les individus au comportement téméraires sont souvent plus agressifs que les individus plus timides qui, eux, ont tendance à être plus dociles (Réale et al. 2007). La notion « syndromes » ne s'arrête pas nécessairement aux traits comportementaux. Les syndromes de rythme de vie (« pace-of-life syndrome ») sont par exemple particulièrement étudiés dans le domaine de l'écologie évolutive (*e.g.* Tieleman 2009). L'hypothèse d'une existence de ces syndromes prédit la coévolution de traits d'histoire de vie, de traits de comportement et de traits physiologiques. Ainsi, d'un côté les individus « lents » ont des traits d'histoire de vie ralentis (*e.g.* survie élevée, reproduction retardée), des comportements timides et un métabolisme lent, et de l'autre côté, les individus « rapides » ont des traits d'histoire de vie rapides, un comportement téméraire et un taux métabolique élevé (**Figure 4**, Réale et al. 2010) et l'évolution de ces différentes stratégies est intrinsèquement liée à l'hétérogénéité environnementale. Par exemple, dans des habitats à fort taux de prédation, les individus téméraires ayant des comportements avec prise de risque élevés sont contre sélectionnés (Lapiedra et al. 2018). En milieu urbain, les oiseaux semblent évoluer vers un rythme de vie plus lent en terme de traits d'histoire de vie et de physiologie (**Figure 4**, Sepp et al 2017). Pourtant, de façon surprenante les prédictions et les observations concernant leurs comportements sont à l'opposé des comportements associés au rythme de vie lent, et les individus les plus téméraires, agressifs et explorateurs sont attendus à mieux s'établir et tolérer le milieu urbain (Evans et al. 2010; Atwell et al. 2012). Ainsi, la structure des syndromes de rythmes de vie semble pouvoir être altérée par les conditions environnementales urbaines (Sol et al. 2018).

II.3. Quelle(s) origine(s) de ces changements phénotypiques ?

L'origine de ces changements phénotypiques (plastique/génétique) et leurs implications évolutives, bien que fréquemment discutées dans la littérature, restent le plus souvent inconnues.

II.3.a. Origine génétique ou plastique ?

Les divergences phénotypiques entre populations urbaines et rurales peuvent être liées à différents processus biologiques. Cas différences peuvent avoir une origine génétique, c'est-à-dire être induites par des différences au niveau des séquences d'ADN et être héritables entre générations. De manière générale dans la nature, une grande partie de la variation de caractère observée entre individus réside dans l'existence de différentes versions des gènes, appelées allèles (voir **Encadré 2**). Ensuite, plusieurs processus peuvent être responsables de différences génétiques entre populations et *in fine* d'évolution. En particulier, parmi ces processus on retrouve : la mutation, la sélection, la dérive et la migration. La mutation est l'apparition, par modification de la séquence d'ADN, de nouveaux allèles. Il s'agit de la source fondamentale de la variation génétique. La sélection est le processus par lequel les allèles sont avantagés ou désavantagés (*i.e.* augmentent ou diminuent en fréquence dans la population) en fonction de l'impact qu'ils ont sur la valeur sélective des individus (c'est-à-dire de leur capacité à survivre et se reproduire et ultimement la proportion de descendants qu'ils laissent dans la génération suivante). La dérive génétique est la fluctuation aléatoire des allèles dans la population au cours des générations. Elle est essentiellement due à l'échantillonnage aléatoire des gamètes et est donc plus forte dans les petites populations. Enfin la migration est l'introduction d'allèles nouveaux par flux d'individus d'une population à l'autre. Ce processus peut participer à la divergence génétique et phénotypique entre population s'il n'est pas aléatoire et si certains génotypes/phénotypes dispersent plus que d'autres (Lenormand 2002).

Dans le contexte urbain, il est relativement peu probable que des mutations *de novo* soient responsables de l'ensemble de la diversité des réponses phénotypiques observées (Johnson & Munshi-South 2017). En effet, les mutations sont des événements rares et l'augmentation en fréquence

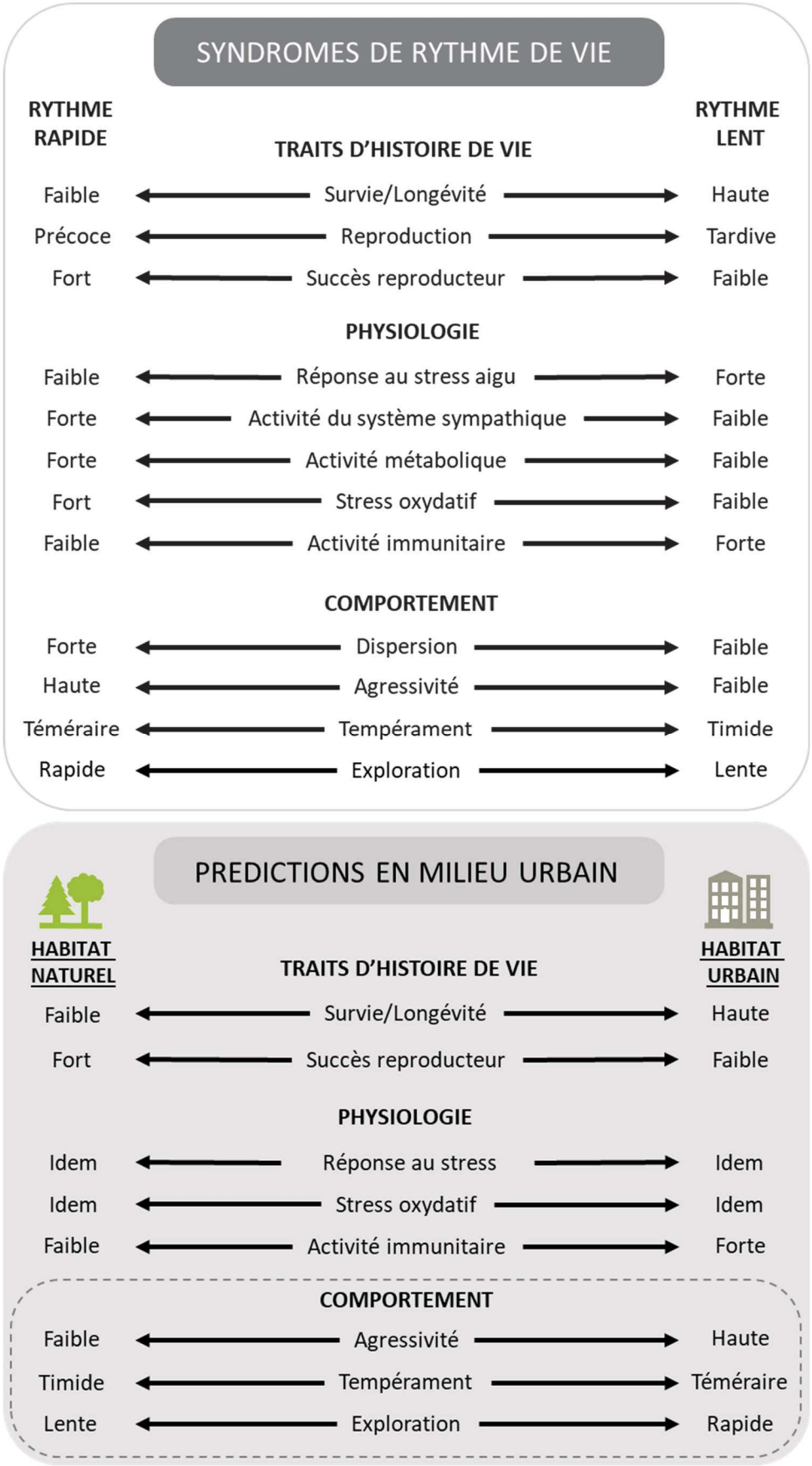


Figure 4 | Schéma de la variation potentielle de différents traits le long d'un continuum de rythme de vie et prédictions associées au milieu urbain. Les flèches illustrent la variation continue théorique des strategies d'histoire de vie, des traits physilogiques et des traits comportementaux entre individus. Noter que le sens de variation des traits comportementaux en milieu urbain est oppose aux attendus théoriques généraux. Figure adaptée de Réale et al. 2007 et Sepp et al. 2017.

d'un nouvel allèle dans une population (sous l'effet de la dérive ou de la sélection) est longue et nécessite de nombreuses générations. Ainsi, elle agit à des échelles de temps bien plus longues que celle de l'urbanisation. Alternativement, si la différence est d'origine génétique, elle repose probablement essentiellement sur de la diversité génétique déjà disponible dans les populations (« standing genetic variation »). D'une part, la sélection peut agir sur cette diversité génétique et conduire à des divergences phénotypiques entre populations. Par exemple, le morphe mélanique (=couleur foncée) chez la phalène du bouleau, qui constitue un avantage en terme de camouflage dans les milieux industriels où les troncs d'arbre sont noirs de pollution à l'inverse du morphe ancestral gris clair qui est plus facilement repérable et prédaté (Hof et al. 2016), est dû à l'insertion d'un élément transposable dans le génome, apparu une trentaine d'années avant le début de l'urbanisation intensive. D'autre part, à partir de cette diversité génétique préexistante, d'autres processus que la sélection naturelle peuvent être responsables de différenciation entre les populations urbaines et rurales : l'effet fondateur (= colonisation des villes par un petit nombre d'individus représentant une petite proportion de la diversité génétique totale de l'espèce), la dérive (= fixation de certains allèles sous l'effet du hasard, avec un effet exacerbé dans les petites populations) ou encore l'isolement entre populations, empêchant les flux génétiques (Evans et al. 2009; Munshi-South 2012).

Par ailleurs, ces différences phénotypiques peuvent avoir une origine plastique. La plasticité phénotypique est définie comme la capacité pour un génotype donné d'exprimer un phénotype différent en fonction de l'environnement (Pigliucci 2001; DeWitt & Scheiner 2004). La plasticité est souvent impliquée dans les réponses phénotypiques rapides induites par les changements humains. En effet, les différences phénotypiques observées sont si nombreuses, conséquentes et rapides à se mettre en place que la génétique seule ne peut être totalement responsable (Hendry et al. 2008). En particulier, il est souvent mentionné que seules les espèces ou les individus particulièrement plastiques sont capables de s'établir dans de nouveaux environnements tels que la ville. Par exemple, les comportements de chants en milieu urbain sont très probablement plastiques et dépendent de l'environnement sonore (Slabbekoorn 2013) mais ça n'est pas le cas pour la témérité, qui ne s'ajuste pas systématiquement au niveau d'urbanisation (Sprau & Dingemanse 2017). Les mécanismes sous-jacents de la plasticité sont multiples. Parmi les mécanismes mesurables, on trouve les mécanismes épigénétiques[5], et en particulier la méthylation de l'ADN (**Encadré 2**). La méthylation de l'ADN est responsable de la modulation de l'expression des gènes, et elle est largement influencée par l'environnement (Martin & Fry 2018). Elle constitue donc un bon candidat pour expliquer les différenciations phénotypiques rapide constatée en milieu urbain.

Enfin, le phénotype n'est pas seulement façonné par le génotype et l'environnement dans lequel vit l'individu lui-même, mais peut être aussi impacté par l'environnement dans lequel les parents et en particulier la mère, ont vécu. L'ensemble de ces phénomènes est appelé « effets maternels ». Par exemple, chez la mésange charbonnière, la concentration en plomb à laquelle fait face la femelle est négativement corrélée avec la quantité d'antioxydants transmis dans le jaune d'œuf disponible pour l'embryon, et une fois l'embryon développé et l'œuf éclos cette différence d'origine maternelle peut avoir un impact considérable sur l'immunité (Hargitai et al. 2016).

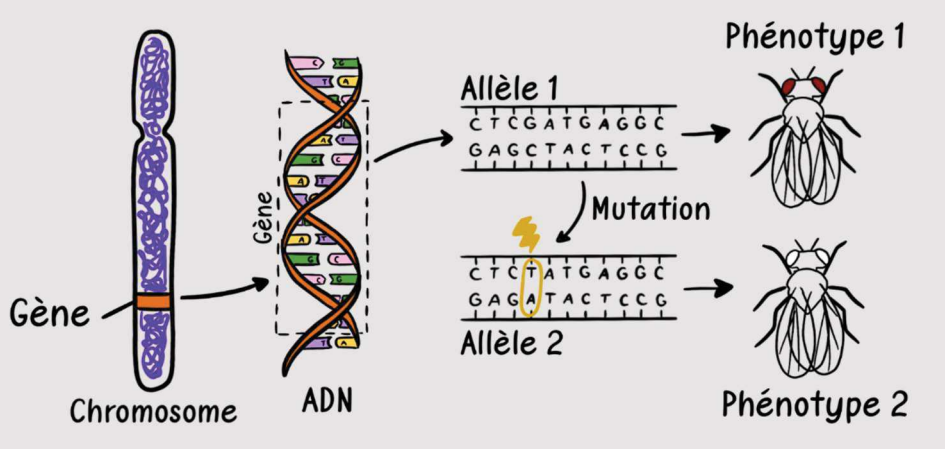
Dans la pratique, comprendre quelle part du phénotype est d'origine génétique, quelle part est liée à de la plasticité et quelle part est induite par des effets maternels nécessite de nombreuses données de pédigrées ou des approches expérimentales conséquentes. En particulier, les expériences de jardin commun, consistant en élever des individus originaires de milieux différents en milieu contrôlé, permettent de tester si les différences phénotypiques sont maintenues et donc génétiques, ou disparaissent, et sont donc plastiques (environnementales). Alternativement, il est possible de faire des expérimentations de transplantations réciproques, c'est-à-dire d'échanger des très jeunes individus

[5] Modification de l'ADN ou de la chromatine qui n'impliquent pas de changement de la séquence d'ADN en elle-même. Les mécanismes épigénétiques incluent notamment : la méthylation de l'ADN, la modification de la structure en 3D de la chromatine et l'état des histones.

Encadré 2 | Détail des mécanismes moléculaires pouvant induire de la variation phénotypique entre individus étudiés dans cette thèse : les changements de séquences d'ADN et la méthylation.

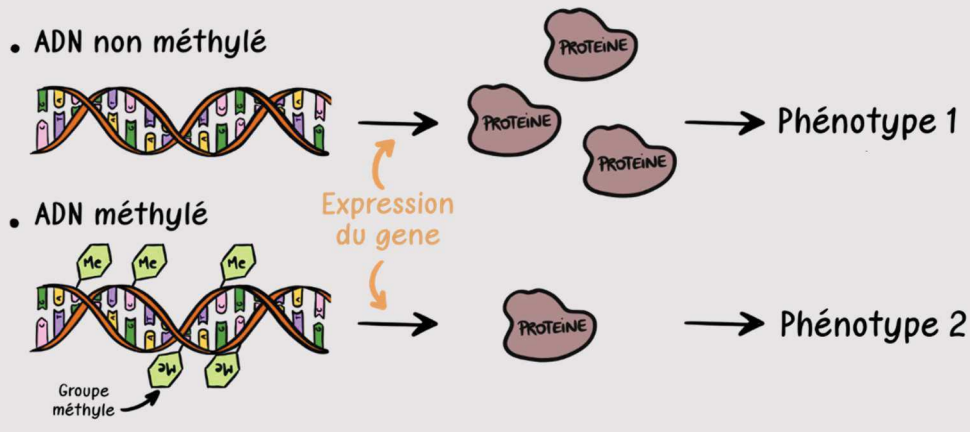
- **Les modifications de séquence d'ADN :**

Toute l'information génétique permettant le développement, le fonctionnement et la reproduction d'un individu est stockée sous forme d'ADN dans les cellules. Chaque brin d'ADN est composé de 4 types de nucléotides : A, T, C et G suivant un ordre donné appelé séquence. La séquence d'ADN des gènes est traduite et transformée en protéine. La nature de chaque protéine et sa structure conditionnent sa fonction dans l'organisme et plus largement l'expression des caractéristiques des individus. Il arrive cependant que des erreurs apparaissent dans la séquence d'ADN (mutation), entrainant une modification de la protéine impliquée et donc potentiellement du phénotype de l'individu comme dans le schéma ci-dessous.



- **La méthylation de l'ADN :**

La méthylation de l'ADN est un processus épigénétique dans lequel certaines bases nucléotidiques peuvent être modifiées par ajout d'un groupe méthyle (CH3). Ce processus ne modifie pas la séquence d'ADN en elle-même mais peut changer l'expression du gène concerné car il modifie directement la capacité d'accrochage de la molécule responsable de la transcription de l'ADN. Alors qu'un faible taux de méthylation favorise en général la transcription (et donc l'expression) d'un gène, une forte méthylation a tendance à l'inhiber. Le processus de méthylation est géré par une enzyme appelée l'ADN-méthyltransférase ; c'est un processus réversible à tout moment de la vie d'un individu.
Outre son implication dans la plasticité phénotypique, la méthylation de l'ADN est grandement responsable de la différenciation cellulaire. En effet, toutes les cellules contiennent l'ensemble de l'information génétique d'un individu, pourtant une cellule du cœur n'exprime pas les mêmes gènes qu'un neurone, et cette différence d'expression résulte essentiellement de la méthylation.



entre familles d'habitats différents pour mesurer leurs phénotypes dans le nouvel habitat. Ces expérimentations se révélant couteuses en moyens, en temps et discutables d'un point de vue éthique sur les modèles animaux, elles ne restent, dans les faits, que relativement peu pratiquées sur des modèles vertébrés. Malgré cela, des études associées à l'urbanisation ayant réalisé des expériences de jardin commun suggèrent une origine génétique probable de la variation des traits de personnalité chez le merle noir *Turdus merula* (Miranda et al. 2013).

II.3.b. Adaptatif ou non adaptatif ?

Les multiples constats de différences phénotypiques observées en milieu urbain posent des questions sur leurs implications évolutives. Par exemple, il devient essentiel de comprendre si la taille réduite fréquemment observée chez les oiseaux urbains constitue une adaptation (= les petits oiseaux urbains survivent mieux et/ou ont un meilleur succès reproducteur que les grands) ou s'il s'agit d'un réponse non-adaptative (par exemple, la taille des oiseaux urbains serait contrainte par un manque de nourriture durant leur croissance les empêchant d'atteindre la taille qu'ils auraient atteint dans un milieu où la nourriture serait plus abondante). Quelle que soit la nature du changement phénotypique (morphologique, phénologique, comportemental…) ou son origine (génétique ou plastique), il peut représenter une adaptation (= un avantage en valeur sélective) ou non, et alors être non-adaptatif (= neutre du point de la valeur sélective) ou mal-adaptatif (= entrainer une baisse de la valeur sélective). En effet, le caractère adaptatif d'un trait se définit comme l'avantage en valeur sélective qu'il apporte, c'est-à-dire si ce trait permet d'augmenter le nombre de descendants ou la survie de l'individu ou non. Par ailleurs, il apparait clair que l'origine génétique ou plastique du trait (décrite dans le paragraphe précédent) aura une importance capitale sur son évolution. Ainsi, pour que la sélection naturelle agisse, il est nécessaire que la variation du trait impliquant une variation de valeur sélective soit héritable entre générations. Les variations de traits d'origine plastique peuvent ainsi constituer des adaptations au sein d'une génération, mais puisqu'elles ne sont (en théorie) pas transmises à la descendance, la sélection naturelle ne peut agir dessus. Pourtant, la plasticité peut avoir un rôle très important dans l'adaptation d'une population à un environnement donné. Par exemple, s'il n'existe pas encore de base génétique permettant la modification du trait (ou si ce trait est faiblement héritable), la plasticité phénotypique peut jouer un rôle temporaire dans l'acclimatation (= processus d'ajustement à un nouvel environnement se produisant au cours de la vie des individus) des populations, leur permettant de survivre et de se reproduire dans les nouvelles conditions environnementales en attendant que les autres traits évoluent. C'est ce qu'on appelle l'effet Baldwin (Simpson 1953).

Plusieurs mécanismes peuvent être à l'origine d'adaptation, de mal-adaptation ou encore de divergence neutre entre populations. Premièrement, comme évoqué précédemment, la sélection naturelle est le principal moteur d'adaptation. En effet, par ce processus, les valeurs de traits héritables conférant un avantage à leur porteur dans un environnement donné augmentent en fréquence au cours des générations, générant une différence phénotypique entre populations si les deux populations présentent des pressions de sélection divergentes. L'adaptation est parfois limitée par l'existence de compromis évolutifs : bien qu'un trait puisse être hautement avantageux dans un certain contexte, s'il représente un coût trop élevé, son développement et la sélection agissant dessus seront limités. De même, un flux génétique important entre populations peut limiter l'adaptation en empêchant la montée en fréquence d'un phénotype (ou génotype) avantageux via l'introduction continuelle de phénotypes (ou génotypes) non avantageux dans la population. Par ailleurs, certains traits peuvent être corrélés entre eux (voir plus haut la notion de syndrome) et la sélection sur un des traits peut être limitée si un trait associé est, de son côté, contre-sélectionné. L'augmentation en fréquence de traits avantageux ne passe pas uniquement par de la sélection naturelle, par exemple dans les cas d'acclimatation plastique, l'acclimatation peut être nécessaire au développement voire à la survie d'un individu dans un milieu, conférant un avantage notable au sein d'une génération, mais non transmis à la suivante (il ne s'agit donc pas d'adaptation au sens strict, bien que conférant un avantage).

Deuxièmement, l'adaptation d'une population à son environnement peut agir via le choix, par les individus, d'un environnement donné

qui correspond le mieux à leur valeur de trait (en anglais « habitat-phenotype-matching, Edelaar et al. 2008; Jacob et al. 2015). Par exemple, chez l'accenteur mouchet, seuls les individus téméraires capables de tolérer les habitats fortement dérangés par l'Homme colonisent ce type d'habitat, conduisant à une divergence phénotypique importante entre habitats dérangés et habitats non dérangés (Holtmann et al. 2017).

Troisièmement, des cas de mal-adaptations peuvent émerger dans les conditions environnementales totalement nouvelles présentes dans le milieu urbain. Par exemple, dans la nature, de nombreux traits sont calqués sur des indices environnementaux reflétant la qualité de l'environnement ou la disponibilité en ressources. Or le nouvel environnement urbain peut conduire à un découplage entre les indices environnementaux et ce qu'ils sont supposés indiquer (Robertson et al. 2013; Demeyrier et al. 2016). Et un tel découplage peut facilement causer des mal-adaptations. Par exemple, de nombreuses espèces ont une période de reproduction calée sur leur rythme circadien, lui-même modulé par la photopériode, puisqu'une augmentation de la durée du jour indique l'arrivée du printemps (Farner & Lewis 1971; Dawson et al. 2001). Or, en ville la lumière perpétuelle augmente artificiellement la durée du jour mais n'est plus synonyme d'arrivée du printemps créant ainsi un découplage entre l'indice « durée du jour » et l'arrivée du printemps. Ce découplage peut avoir pour conséquence d'engager un effort de reproduction précoce pouvant être non-optimal car les conditions climatiques/nutritives ne sont pas encore en place pour permettre le bon développement de jeunes. Par ailleurs, certains phénotypiques peuvent constituer un avantage sur un aspect mais, un désavantage sur un autre. Par exemple, l'habituation au dérangement humain induit des comportements téméraires chez de nombreuses espèces d'oiseaux et cette témérité est essentielle à la poursuite de leurs activités (notamment la recherche de nourriture) malgré un fort dérangement humain. Pourtant, elle est par ailleurs désavantage si elle contribue aussi en une diminution de l'attention portée aux prédateurs (Geffroy et al. 2015), ce qui rejoint la notion de compromis évolutif.

Enfin, de façon intermédiaire entre mal-adaptation et adaptation, certains phénotypes paraissant spécifiques au milieu urbain peuvent aussi se révéler neutres du point de vue de la sélection naturelle pour plusieurs raisons. Premièrement, comme évoqué plus haut, certains traits sont corrélés entre eux (dans un contexte de syndrome comportemental par exemple), et l'altération d'un trait Z_1 sous l'effet de la sélection, peut entrainer avec lui un changement chez un autre trait Z_2 (Lande & Arnold 1983). Deuxièmement, le hasard peut aussi amener à de la divergence entre population, n'ayant aucun impact sur la valeur sélective, notamment par des effets de dérive génétique. Troisièmement, la ville peut agir comme un filtre dans l'établissement de certains individus en son sein (Lomolino 1984), sans pour autant que le trait soit adaptatif une fois installé dans l'environnement.

Ainsi, toutes les différences phénotypiques propres au milieu urbain ne constituent pas nécessairement des adaptations. Il est donc nécessaire d'étudier de manière fine les liens entre un trait et la variation de valeur sélective qu'il induit (via des analyses de sélection, Lande & Arnold 1983) pour comprendre quels changements phénotypiques constituent des adaptations ou des mal-adaptations, et ainsi pouvoir appréhender l'impact évolutif de la vie en milieu urbain. De telles études reposent sur des suivis de populations à long terme s'étalant sur de nombreuses années, pour lesquelles l'accès aux paramètres de valeur sélective sont essentiels, et requièrent une puissance statistique conséquence. De telles contraintes rendent les analyses de sélection rares à ce jour dans le contexte urbain. Pourtant, face à l'ampleur et la rapidité de l'urbanisation, il devient de plus en plus pressant de comprendre les implications évolutives que constituent de tels changement pour les espèces sauvages. En parallèle et à une bien plus petite échelle, une palette d'outils d'analyses moléculaires reposant sur le séquençage de l'ADN permet de chercher des traces d'adaptations dans les génomes, donnant un accès direct aux gènes concernés (Perrier et al. 2020). Si cette approche nécessite un travail de terrain moins important, les couts de séquençage d'ADN restent encore très élevés (bien qu'en diminution continuelle grâce à des innovations technologiques de plus en plus efficaces), ce qui limite leur application systématique et à très large échelle.

L'origine biologique (génétique ou plastique) des différences phénotypiques induites par

le milieu urbain ainsi que les processus qui sont à l'origine de ces différences (sélection, dérive, filtre…) sont multiples, complexes et intrinsèquement liés. Il est donc essentiel d'étudier conjointement ces deux facettes pour comprendre l'impact de l'urbanisation sur les trajectoires évolutives des espèces.

III. ENJEUX ET OBJECTIFS

III.1. Etude de l'évolution en milieu urbain : des lacunes importantes

Comme évoqué précédemment, l'urbanisation fait partie des changements planétaires rapides d'origine anthropique. La nature des changements environnementaux qu'elle induit ainsi que la rapidité à laquelle se produisent ces changements en font une des principales menaces pour la biodiversité. L'étude de l'impact de l'urbanisation sur les espèces sauvages est donc essentielle dans un contexte de conservation de la biodiversité. Par ailleurs, l'étude de l'évolution darwinienne en milieu urbain offre une opportunité aux biologistes de l'évolution de comprendre dans quelle mesure les espèces sont capables de répondre à des changements environnementaux rapides. Ensuite, identifier la nature des réponses observées est crucial pour pouvoir appréhender dans quelle mesure les activités humaines influencent les trajectoires évolutives des populations sauvages. Malgré les enjeux pressants liés à ces problématiques et la multiplication des articles scientifiques dans le domaine, les connaissances quant à la manière dont les espèces évoluent en milieu urbain restent restreintes (Lambert et al. 2020).

II.2. Objectifs et plan du projet

Ce présent travail se place donc dans un contexte où l'impact de l'urbanisation sur les populations sauvages n'est plus à démontrer mais dont les origines et implications évolutives restent toujours largement méconnues. En effet, bien que l'on rapporte de nombreux changements phénotypiques liés à l'urbanisation, l'origine de ces changements (génétique/plastique) ainsi que l'impact qu'ils ont sur la valeur sélective des individus demeurent largement indéterminés.

L'objectif global de cette thèse est de comprendre comment les populations sauvages sont affectées par la vie en milieu urbain et d'identifier quelle est la dimension évolutive des nombreux changements phénotypiques observés : morphologiques, de traits d'histoire de vie, comportementaux et de réponse au stress. En utilisant la mésange charbonnière (*Parus major*) comme modèle biologique je tente de comprendre quelle peut être l'origine des différences phénotypiques observées en milieu urbain par rapport au milieu forestier en suivant le raisonnement illustré **Figure 5** (et détaillé **Chapitre V**).

Ainsi, après un bref premier chapitre décrivant les méthodes générales utilisées dans ce manuscrit, je consacre le second chapitre à la description de la variation phénotypique observée entre population de mésange charbonnière urbaine et forestière. Trois types de traits sont étudiés ici : des traits morphologiques, des traits d'histoire de vie et des traits comportementaux. Au vu des résultats précédemment rapportés dans la littérature (décrits plus hauts, section II.2) sur une grande variété d'espèces je pars de l'hypothèse que la vie en milieu urbain affecte une majorité de ces traits. En particulier, j'émets les prédictions suivantes : (1) les traits morphologiques sont globalement plus « petits » en milieu urbain, (2) la date de ponte est précoce en ville et la taille de ponte réduite (contrairement aux attentes du syndrome de rythme de vie) et (3) les individus urbains présentent des comportements en moyenne plus exploratoires et plus agressifs et ont une réponse au stress plus élevée que leurs homologues forestiers.

Ensuite, dans un troisième chapitre je mesure la direction et la force de la sélection naturelle agissant sur ces traits et compare cette sélection entre milieu forestier et milieu urbain. J'émets l'hypothèse qu'étant données les particularités environnementales que présente le milieu urbain, de nouvelles pressions de sélection émergent et agissent sur les traits étudiés. Il est difficile cependant de prédire direction et magnitude de la sélection naturelle attendue sur chaque trait car, à ma connaissance, malgré les prédictions entrant en compte dans la théorie des syndromes de rythme de vie, seule une poignée d'études ont réalisé ce type d'analyses sans trouver de patron global (*e.g.* Rodewald et al. 2011; Senar et al. 2014). Cependant, si le phénotype urbain est adaptatif, alors les analyses révèleront un alignement entre la divergence phénotypique entre ville et forêt et la direction de la

sélection. Par exemple, les oiseaux urbains étant plus petits (Liker et al. 2008), on s'attend à une sélection favorisant cette réduction de taille en ville, soit du fait que les oiseaux plus petits survivent mieux, soit qu'ils ont un meilleur succès de reproduction.

Dans un quatrième chapitre, je m'intéresse à l'origine potentiellement génomique et épigénomique des particularités phénotypiques urbaines. En effet, il est possible de détecter des traces d'adaptation dans les génomes. De plus, le rôle des processus épigénétiques dans l'adaptation face aux changements environnementaux est de plus en plus suggéré dans la littérature (Bossdorf et al. 2007). Dans ce chapitre j'explore donc les différences génomiques et épigénomiques observées entre individus urbains et ruraux et je regarde si certains marqueurs (épi)génomiques sont associés à des gènes pouvant être impliqués dans la divergence phénotypique.

En cinquième partie, je présente un chapitre de manuel auquel j'ai participé. Ce chapitre constitue une feuille de route pour tout chercheur voulant étudier la sélection en milieu urbain et plus particulièrement le développement d'une approche génomique pour étudier l'adaptation locale.

Enfin, car à mes yeux le partage et la diffusion du savoir sont la pierre angulaire du travail de recherche, un chapitre « bonus » contient une partie des travaux de vulgarisation scientifique auxquels j'ai eu la chance de contribuer ou que j'ai réalisés de ma propre initiative. Il est présenté après le chapitre de discussion générale. J'invite tout lecteur intéressé à le feuilleter en adoptant un regard naïf et ouvert, et je serai ravie d'écouter avec attention toute suggestion me permettant d'améliorer ce travail et de le rendre plus accessible et percutant.

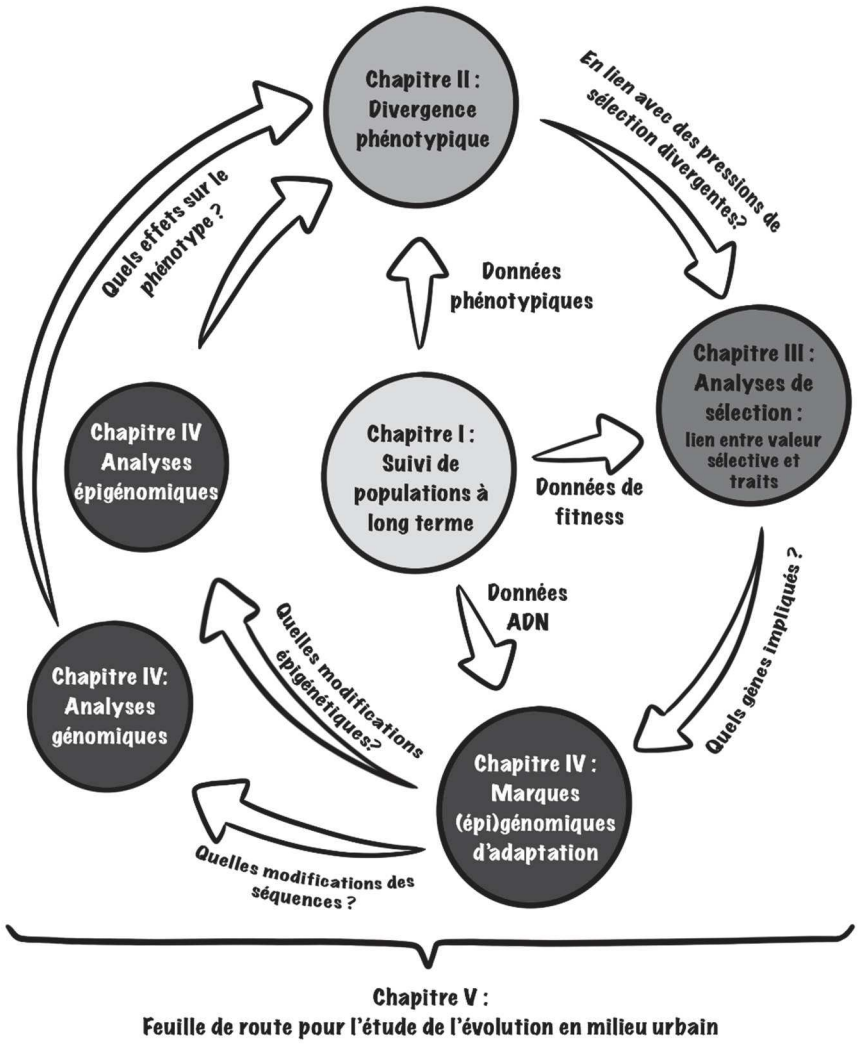


Figure 5 | Schéma circulaire du plan de déroulement de cette these. Chaque cercle représente une chapitre (ou une partie de chapitre) ayant pour but de répondre à la question qui le précède, représentée sous forme de flèche.

CHAPITRE 1

MÉTHODES GÉNÉRALES
MODÈLE D'ÉTUDE ET SUIVI DE POPULATION



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I. MODELE D'ETUDE : LA MESANGE CHARBONNIERE

I.1. Description générale

La mésange charbonnière (*Parus major*) (**Figure 1A**), appartenant à la famille des passéridés, est la plus commune des mésanges européennes. Répartie sur la quasi-totalité du contient eurasiatique, elle fréquente tout type de milieux naturels boisés : forêts de résineux, caducifoliées et mixtes ; elle est aussi très présente dans des habitats anthropisés : vergers, jardins, et zones urbaines (del Hoyo et al. 2007). C'est une espèce territoriale cavicole occupant des cavités préexistantes dans lesquelles elle construit un nid en coupe à base de brindilles, de mousse et souvent de poils d'origine animale. La femelle pond en général entre 5 et 12 œufs par nichée et peut produire jusqu'à deux nichées par an pour lesquelles les deux parents participent à l'élevage des poussins. Alors que l'alimentation des poussins est essentiellement composée d'arthropodes (en majorité des chenilles et des araignées) l'adulte a un régime alimentaire plus généraliste et devient partiellement granivore/opportuniste en hiver (del Hoyo et al. 2007).

I.2. Une espèce très étudiée et largement présente en ville

La mésange charbonnière est l'une des modèles biologiques les mieux étudiées en Europe (McKenzie & Robertson 2015) et on la retrouve largement dans les milieux urbanisés comme le démontrent de nombreuses études s'intéressant aux mésanges urbaines à travers l'Europe (Isaksson & Andersson 2007; Biard et al. 2017; Senar et al. 2017; Corsini et al. 2017; Seress et al. 2020). Son affinité pour les nichoirs artificiels, sa facilité d'observation et son abondance en font un modèle de choix pour les chercheurs. En particulier, l'accès aux paramètres démographiques (suivi capture-marquage-recapture) et aux traits d'histoire de vie (parmi lesquels le nombre de poussins produits et recrutés) permet d'estimer la valeur sélective de chaque individu suivi, donnée essentielle pour les études évolutives. Ainsi, de nombreux travaux se sont penchés sur son écologie, sa physiologie, son comportement ou encore son évolution, offrant un corps de littérature conséquent nécessaire à la compréhension des mécanismes éco-évolutifs dans leur globalité.

II. SITES DE SUIVI

L'essentiel du présent travail s'appuie sur le suivi et la comparaison de deux populations de mésanges charbonnières du sud de la France métropolitaine, en région méditerranéenne : une population forestière et une population urbaine, décrites ci-après.

II.1. Site forestier

Le site d'étude forestier se situe à 20km au Nord-Ouest de Montpellier, dans la forêt de la Rouvière appartenant à la commune de Montarnaud (43°39'51.0''N, 3°40'13.6''E, **Figure 1B**). Sur ce site, une population de mésanges charbonnières et une population de mésanges bleues (*Cyanistes caeruleus*) sont suivies depuis 1991 grâce à la mise en place de nichoirs adaptés à chaque espèce (trou d'entrée de 32mm pour les mésanges charbonnières et de 28mm pour les mésanges bleues), le nombre de nichoirs à mésanges charbonnières fluctuant entre 51 et 91 selon les années. Afin d'éviter le chevauchement des territoires et ainsi réduire la compétition entre couples reproducteurs, les nichoirs sont positionnés à 50m au minimum les uns des autres.

La forêt de la Rouvière est une forêt méditerranéenne mature composée essentiellement de chênes verts (*Quercus ilex*) et de chênes blancs (*Quercus pubescens*). Le dérangement humain et l'artificialisation du milieu y sont très faibles et limités à quelques chemins forestiers empruntés par d'occasionnels randonneurs ou chasseurs. Ce site est donc considéré comme un habitat naturel, et sert de point de comparaison tout au long de cette étude.

II.2. Site urbain

II.2.a. Description générale

Le suivi de population de mésanges charbonnières urbaines est réalisé dans la ville de Montpellier (43°36'43''N, 3°52'36''E) au moyen de 203 à 223 nichoirs répartis dans 8 zones de la ville (**Figure 1C & D**). Comme évoqué précédemment, Montpellier est la ville française enregistrant la plus forte croissance démographique. En 2017 on dénombrait 285 121 habitats sur la municipalité même (soit

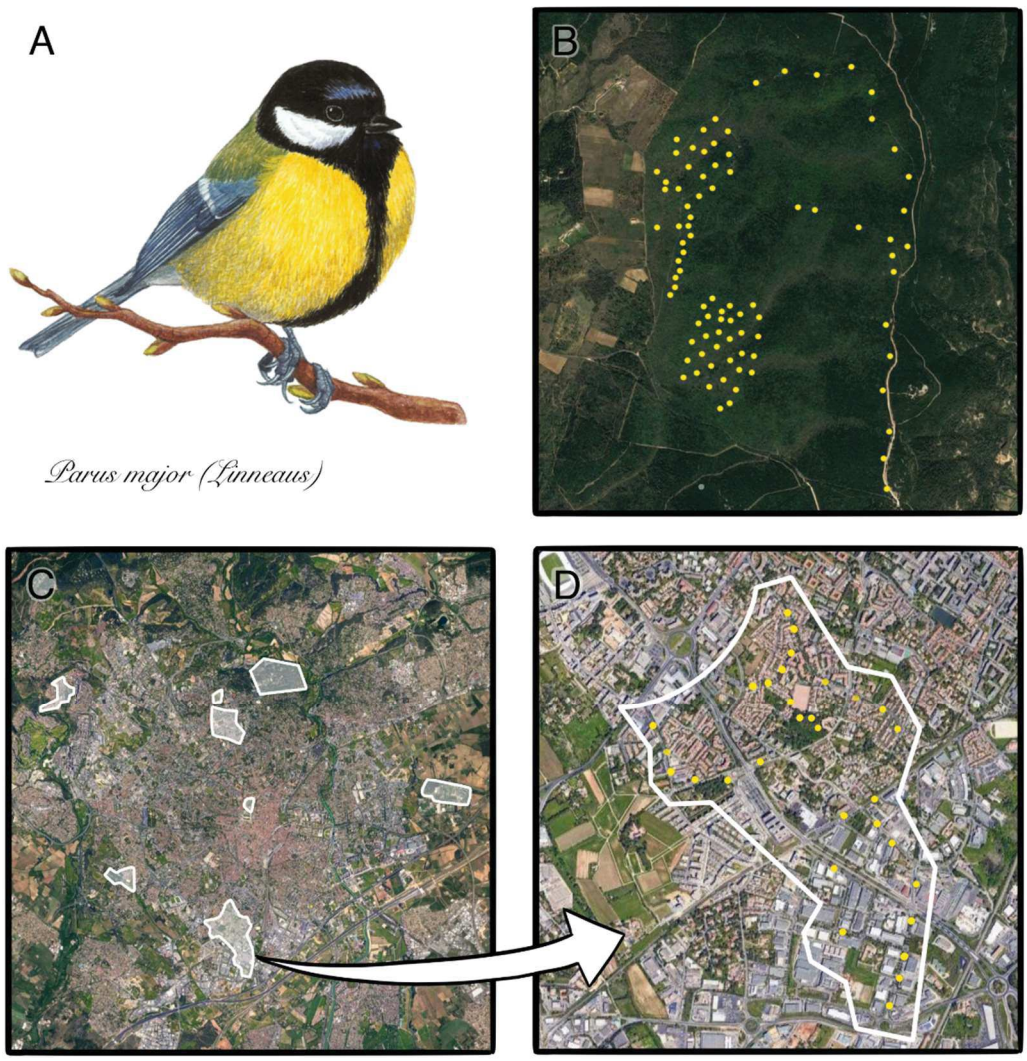


Figure 1 | (A) Dessin d'une mésange charbonnière (*Parus major*) mâle. (B) Image satellite du site d'étude forestier de la Rouvière. (C) Localisation des 8 sites d'études urbains dans la ville de Montpellier. (D) Image satellite rapprochée d'un des sites d'étude urbain : Mas Nouguier. Dans (B) et (D), chaque point jaune représente un nichoir à mésange charbonnière. Images satellites issues de Google Earth.

56.88km²) et 616 296 sur la totalité de l'aire urbaine (INSEE 2018).

La disposition des nichoirs dans les zones urbaines a été choisie de manière à couvrir une large gamme de niveaux d'urbanisation. Ainsi, plus de 200 nichoirs ont été placés en ville en automne 2010 (de 203 à 223, nombre fluctuant au cours des années pour cause de vols ou actes de vandalisme), répartis sur un gradient d'urbanisation allant de parcs arborés jusqu'à des zones industrielles extrêmement artificialisées et pauvres en végétation, en passant par des quartiers résidentiels aux niveaux d'urbanisation intermédiaires. La disposition choisie permet également de capter un gradient de perturbation anthropique à l'échelle du nichoir (fréquence des piétons, fréquence des véhicules motorisés et présence de lumière artificielle).

L'existence de deux sites de suivi aux conditions environnementales distinctes ainsi que la répartition des nichoirs en ville permettent d'avoir deux types d'approches différentes dans l'étude de l'impact de l'urbanisation. Dans un premier temps, il est possible d'effectuer une comparaison entre ville et habitat naturel pour étudier l'impact global de l'urbanisation sur la mésange. Et dans un second temps, il est possible d'étudier, à plus fine échelle, l'impact du niveau d'urbanisation au sein même de la ville.

II.2.b. Mesures d'urbanisation à fine échelle

Pour pouvoir estimer l'impact de l'urbanisation à fine échelle, les conditions environnementales ont été quantifiées dans un rayon de 50 m autour de chaque nichoir à travers 4 mesures (adapté de Demeyrier et

al. 2016): (1) le couvert végétal, (2) la pollution lumineuse, (3) la fréquence de piétons et (4) le trafic de véhicules motorisés.

L'estimation du couvert végétal a été réalisée à l'aide d'images satellites traitées avec le logiciel Q-gis (version, citation), et sert classiquement de proxy de la disponibilité en ressources de l'habitat. Les végétaux de toutes strates confondues ont été considérés comme couvert végétal, car les mésanges nourrissent leurs poussins avec des insectes quelles cherchent dans les arbres, les arbustes ou directement sur la strate herbacée (Betts 1955), en opposition aux surfaces artificielles, qu'elles soient bétonnées ou non (trottoirs, chemins de terre, routes bétonnée, etc). La mesure de pollution lumineuse est effectuée en estimant le pourcentage de recouvrement de chaque territoire autour de chaque nichoir par le halo lumineux estimé produit par les lampadaires publics à l'aide de Q-Gis. La fréquence en véhicules motorisés, proxy de la pollution chimique et sonore, et la fréquence en piétons, proxy du dérangement humain, ont été estimés, lors du baguage des poussins, en dénombrant le nombre de véhicules ou piétons aux pieds de chaque nichoir pendant 5 minutes.

Pour résumer l'ensemble des informations fournies par ces 4 mesures environnementales, une Analyse en Composantes Principales (ACP) a été réalisée et a révélé deux principaux axes synthétiques (**Figure 2**). Le premier axe, expliquant 42.4% de la variance, décrit un degré de naturalité de l'habitat, car positivement corrélé au couvert végétal et négativement corrélé à la pollution lumineuse et au trafic automobile (**Figure 2A & 2B**). Le deuxième axe, expliquant quant à lui 25.4% de la variance, représente un gradient de perturbation piétonnière dans la mesure où il est essentiellement expliqué par la fréquence en piétons (**Figure 2A & 2C**). Ainsi, les coordonnées des nichoirs sur chacun de ces deux axes leur donne un indice de d'urbanisation le long d'un « gradient de naturalité » et un indice de perturbation le long d'un « gradient de fréquence piétonnière ».

Notons que les données d'urbanisation à fine échelle sont utilisées uniquement dans le chapitre II Manuscrit 1 qui décrit la variation morphologique le long du gradient d'urbanisation. Le lien entre gradient d'urbanisation et traits d'histoire de vie ou comportement a précédemment été publié (Charmantier et al. 2017) et n'a donc pas été traité dans cette thèse. Les analyses de sélection présentées chapitre III nécessitant une puissance statistique bien plus élevée n'ont pas permis la prise en compte du gradient d'urbanisation.

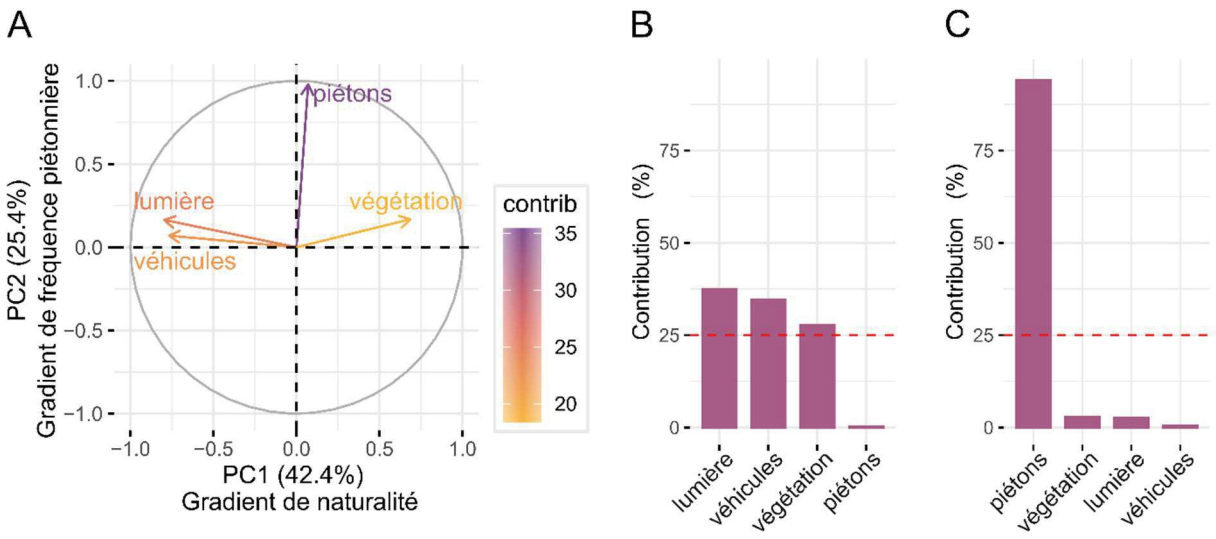


Figure 2 | Résultat de l'Analyse en Composantes Principales réalisée sur les données environnementales autour de chaque nichoir : couvert végétal, trafic de véhicules motorisés, pollution lumineuse et fréquence en piétons. (A) Représentation graphique de la contribution de chaque variable aux 2 axes synthétiques retenus : PC1 « gradient de naturalité » et PC2 « gradient de fréquence piétonnière ». (B) Contribution détaillée des variables à l'axe PC1. (C) Contribution détaillée des variables à l'axe PC2.

II.3. Collaborations européennes

Comme décrit plus haut, la mésange charbonnière est l'un des modèles biologiques les plus usités dans le domaine de l'écologie évolutive. Ainsi de nombreuses équipes de recherche ont mis en place des suivis similaires à celui présenté dans cette thèse dans différentes villes européennes. Il est donc possible d'établir des collaborations à large échelle, particulièrement intéressantes pour travailler sur des réplicas et ainsi vérifier si les réponses à l'urbanisation sont similaires entre milieux urbains ou au contraire dépendantes du milieu urbain local et de son environnement bioclimatique.

Ainsi, le chapitre IV de ce présent travail est le fruit d'une collaboration avec une équipe basée à Varsovie (Pologne) dirigée par Marta Szulkin et une équipe basée sur Barcelone (Espagne) dirigée par Juan-Carlos Senar. Grace à des échantillons de sang fournis par ces deux équipes j'ai pu réaliser un travail comparant les différences génomiques et épigénomiques observées entre populations urbaines et rurales pour 3 villes d'Europe.

III. PROTOCOLE DE SUIVI ET TRAITS ETUDIES

II.1. Suivi de la reproduction

Au cours de la saison de reproduction s'étalant de mars à juillet, chaque nichoir est suivi hebdomadairement de manière à suivre aussi précisément que possible l'évolution de chaque nichée, de la construction du nid à l'envol des poussins. Dans un premier temps ce suivi donne accès à l'essentiel de la phénologie de reproduction de chaque couple : date de ponte, taille de ponte et nombre d'œufs éclos. La date de ponte est enregistrée comme le jour où la femelle pond son premier œuf. Elle est facile à estimer puisqu'une femelle pond en général un œuf par jour jusqu'à atteindre la taille de ponte finale. C'est un trait particulièrement étudié chez les mésanges car il est d'une importance capitale pour la survie d'une nichée et donc la valeur sélective des individus (Lack 1966; Blondel 1985). En effet, chez ces espèces, une bonne croissance des poussins dépend entièrement de l'abondance de nourriture disponible au moment de leur besoin en nutriments. Or, de manière générale, l'abondance en arthropodes et plus précisément en chenilles suit une distribution normale avec un pic d'abondance particulièrement fort au cours du printemps. La synchronie entre le pic de demande de nourriture par les poussins et le pic d'abondance en chenilles est donc particulièrement important pour la croissance et la survie des poussins. La taille de ponte, quant à elle, est un proxy de l'investissement parental dans la reproduction.

Dans un second temps, le suivi des nichées donne accès au succès d'envol et au nombre de poussins envolés qui sont des composants importants de la valeur sélective des parents. L'accès à des composantes de valeur sélective individuelle est indispensable aux études éco-évolutives les mesures de sélection naturelle en dépendent.

II.2. Baguage et mesures individuelles

Lorsque les poussins sont âgés de 9 à 15 jours, les parents de chaque nichée sont capturés pour être identifiés et mesurés. La capture est réalisée directement dans le nichoir lorsqu'un parent entre pour nourrir les poussins, soit à l'aide d'une trappe mécanique qui bloque le trou d'entrée déclenchée par l'oiseau, soit en obstruant manuellement le trou à l'aide d'un bâton. Chaque oiseau est équipé d'une bague à identifiant unique délivrée par le Museum National d'Histoire Naturelles qu'il gardera toute sa vie. Une série de mesures morphologiques est réalisée en routine sur tous les oiseaux capturés : la mesure du tarse-métatarse avec un pied à coulisse (précision de 0.02mm), la prise du poids avec un peson ou une balance électronique (précision 0,5g), la mesure de l'aile avec un réglet à butée (précision 0.5mm, mesure aile pliée, du poignet jusqu'à la rémige primaire la plus longue) et la mesure des plumes de la queue avec un réglet simple (précision 0.5mm, du croupion aux bout les rectrices). Un prélèvement de sang de 10µL est réalisé dans une venue du cou et stocké dans du « Queen's buffer », un tampon anticoagulant.

A l'âge de 15 jours, les poussins ayant fini leur croissance osseuse (Lindström 1999), sont mesurés de la même manière que les adultes (tarse et poids) et bagués avant d'être remis dans leur nichoir.

II.3. Mesure de traits comportementaux

Additionnellement aux traits morphologiques mesurés sur chaque adulte, trois traits comportementaux ont été caractérisés chez une majorité d'individus urbains et forestiers depuis 2014 (Charmantier et al. 2017): l'agressivité en main,

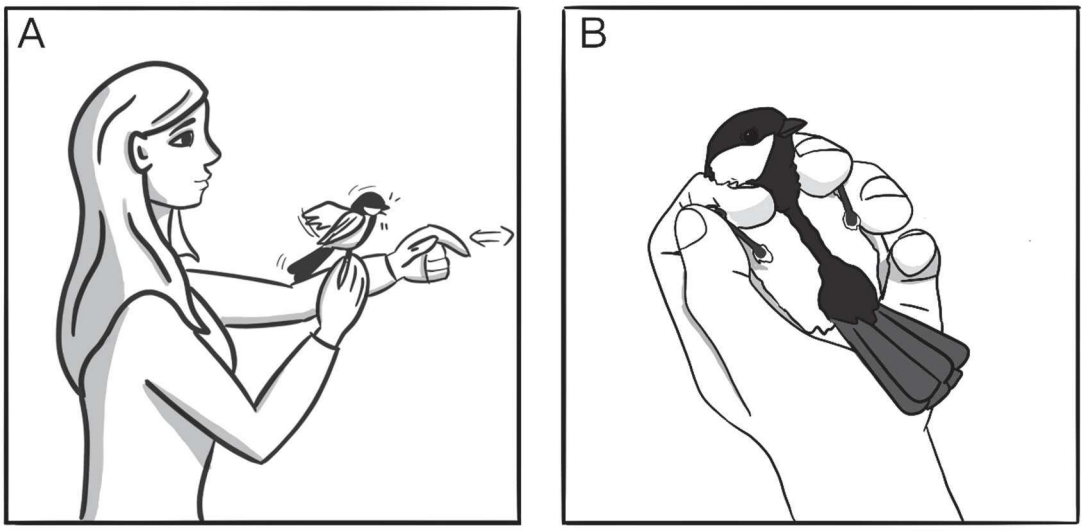


Figure 3 | (A) Dessin de la position du manipulateur et de l'oiseau pour le test d'agressivité en main. (B) Dessin de l'oiseau maintenu sur le dos pour la mesure du rythme respiratoire.

le rythme respiratoire et le comportement exploratoire dans un environnement nouveau. Ces trois traits sont liés à la personnalité des individus (Réale et al. 2007). Par ailleurs, le rythme respiratoire étant étroitement lié à la physiologie du stress il peut donc aussi être considéré comme un trait physiologique (Carere & van Oers 2004).

L'agressivité en main est mesurée par le bagueur juste après la capture au nichoir. L'oiseau est tenu debout par les pattes, dos au manipulateur (**Figure 3A**) et taquiné avec un doigt de l'autre main de façon standardisée (avancer 5 fois son doigt horizontalement vers le bec de l'oiseau sans le toucher, puis l'avancer 5 autres fois de façon plus agressive en touchant le bec pour le provoquer). Après environ 15 secondes d'observation, le manipulateur attribue un score d'agressivité à l'oiseau sur une échelle de 0 (oiseau non réactif) à 3 (oiseau attaque le doigt à chaque fois et écarte ses ailes et ses plumes de queue), avec un pas de 0.5 (**Table 1**). Après cette manipulation, l'oiseau est placé au calme dans un sac en tissu pendant 5 minutes.

Puis, après 5 minutes de repos dans le sac en tissu, l'oiseau est sorti du sac et maintenu sur son dos par le manipulateur (**Figure 3B**) qui procède à la mesure de son rythme respiratoire. Entre 2014 et 2016, la mesure de rythme respiratoire consistait à compter le nombre de mouvements de poitrine de l'oiseau pendant 30 secondes. Depuis 2017, le protocole a été mis à jour et constitue maintenant à mesurer le temps mis pour 30 mouvements de poitrine. Chaque comptage est reproduit deux fois et la moyenne de ces deux mesures constitue la mesure finale de rythme respiratoire. Les mesures faites avant 2017 ont été converties pour obtenir une estimation du temps nécessaire à compléter 30 respirations, en accord avec la nouvelle mesure. L'impact du changement de protocole est faible mais significatif, la première mesure ayant tendance à surestimer le temps mis à réaliser 30 inspirations ($moyenne_{(ancien\ protocole)}$=14.182, $moyenne_{(nouveau\ protocole)}$=12.998 ; t-test : t=8.2, P<0.001), résultat logique car en 30 secondes de mesure, l'oiseau a le temps de se calmer pour reprendre un rythme respiratoire plus calme. Dans la suite des analyses cette différence est prise en compte en utilisant les résidus de régression entre la mesure brute du rythme respiratoire et le protocole utilisé.

Table 1 | Barème d'attribution du score d'agressivité en main. Noter que lorsqu'un individu présente une reaction spécifique d'un score et une autre réaction spécifique d'un autre score, le score final atribué est la moyenne des deux.

Score	Donne des coups de becs	Ecarte la queue	Ecarte les ailes
0	Non	Non	Non
1	Oui, mais si provoqué	Non	Non
2	Oui, spontanement	Oui	Non
3	Oui, spontanement	Oui	Oui

Enfin, l'oiseau est soumis à un test d'«environnement nouveau» à l'aide d'une arène expérimentale standardisée construite en suivant Stuber et al. (2013), appelée cage open-field. Ce test est classiquement utilisé pour évaluer le comportement exploratoire chez les mésanges (Stuber et al 2013, Dubuc-Messier et al 2016). Juste après la mesure du rythme respiratoire, l'oiseau est placé dans un sas d'acclimatation attenant à l'open-field pendant 2 minutes avant d'être lâché dans l'arène d'exploration.

Le comportement de l'oiseau est alors filmé pendant 4 minutes. Les vidéos sont ensuite analysées à l'aide du logiciel BORIS (Friard & Gamba 2016) paramétré pour compter ne nombre de sauts et de vols réalisés par l'oiseau en 4 minutes. Ce nombre de sauts et vols ayant subi une transformation racine carrée est utilisé comme proxy du comportement exploratoire de l'oiseau et est appélé « score d'exploration » (Dingemanse et al 2002).

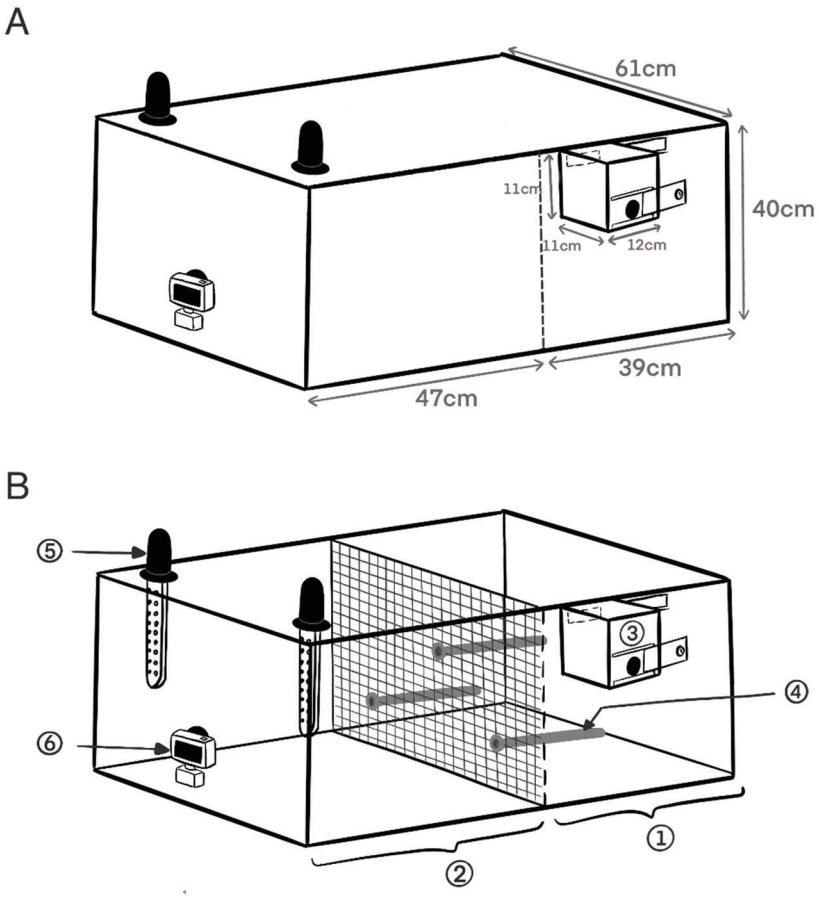
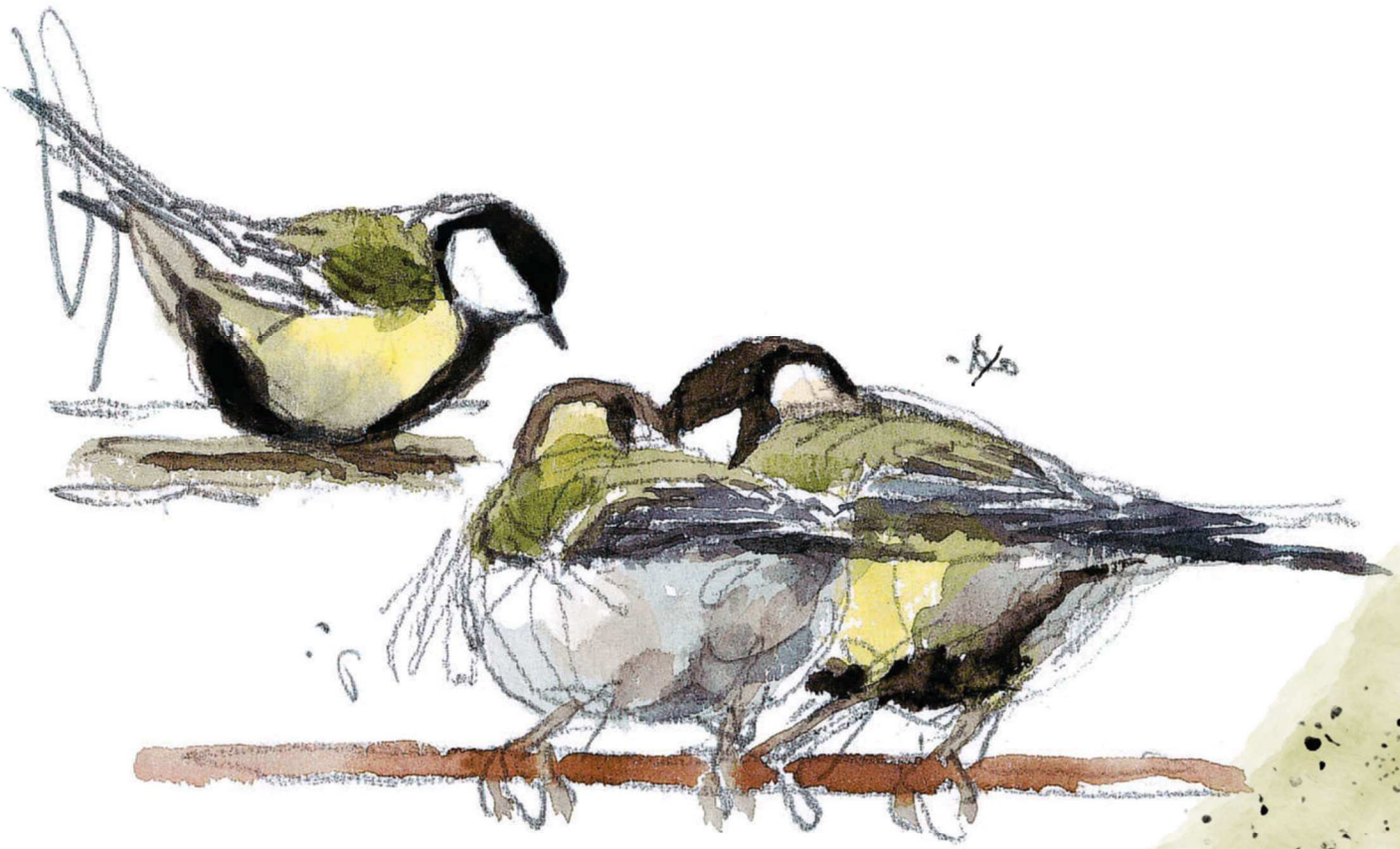


Figure 4 | Représentation schématique de la cage d'open-field (A) Vue extérieure et dimensions (B) Vue en transparence de la structure interne de la cage. 1 : arène d'exploration. 2: zone vide inaccessible à l'oiseau 3: sas d'acclimatation. 4: perchoir. 5: lampe. 6: camera. Une porte coulissante connecte le sas d'acclimatation et l'arène d'exploration. Les parois externes sont construites avec des plaques de PVC expansé blanc (l'oiseau ne perçoit pas l'environnement extérieur à la boîte).

CHAPITRE II

DIVERGENCE PHÉNOTYPIQUE ENTRE HABITATS URBAIN ET FORESTIER



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– RÉSUMÉ DU CHAPITRE II –

La première étape dans l'étude de l'impact de l'urbanisation sur les processus évolutifs affectant les populations sauvages est de caractériser les différences phénotypiques observées entre populations urbaines et rurales. L'objectif principal de ce chapitre est donc de décrire la divergence phénotypique existant entre nos populations de mésanges charbonnières urbaines et forestières, ainsi que la variation le long d'un gradient d'urbanisation pour différent types de traits. De nombreux travaux dans la littérature rapportent déjà que les passereaux urbains ont tendance à être globalement plus petits, mais pas nécessairement en moins bonne condition corporelle que leurs comparses ruraux (*e.g.* Liker et al. 2008), qu'ils ont une reproduction plus précoce et pondent moins d'œufs (Chamberlain et al. 2009), et qu'ils sont en moyenne plus explorateurs, plus agressifs, mais pas systématiquement plus stressés (Martin & Réale 2008; Evans et al. 2010; Sepp et al. 2017). Ainsi, je prédis ici que les mésanges urbaines présenteront des traits morphologiques plus petits, une ponte précoce et réduite en taille, ainsi qu'un comportement plus agressif et plus explorateur, et enfin un rythme respiratoire plus rapide.

A l'aide de modèles linéaires généralisés j'ai exploré la variation de 4 types de traits: morphologiques (**Manuscrit 1** : taille du tarse, condition corporelle, longueur de l'aile et de la queue), d'histoire de vie (**Manuscrit 2**, présenté dans le **Chapitre III** : date de ponte et taille de ponte), comportementaux et physiologique (**Manuscrit 3**, présenté dans le **Chapitre III**, agressivité en main, exploration et rythme respiratoire). La taille du tarse ainsi que la condition corporelle sont étudiées chez les individus reproducteurs ainsi que chez les poussins de 15 jours. Notons que la variation le long du gradient d'urbanisation des traits d'histoire de vie et comportementaux n'est pas présentée ici car son étude a déjà fait l'objet d'une publication antérieure à mes travaux de thèse (Charmantier et al. 2017).

L'analyse des données morphologiques révèle une réduction de la taille des adultes urbains par rapport à ceux évoluant en milieu rural. En effet, en milieu urbain les individus présentent des tarses significativement plus petits, et des ailes et une queue significativement plus courtes (Figure 1A). Au contraire, la condition corporelle (« Scaled Mass Index », Figure 1A) des individus ne montre pas de différence significative entre habitats (noter ici que le résultat sur la condition corporelle dépend de l'indice utilisé, par exemple, en utilisant la masse corrigée par le tarse en effet fixe du modèle comme proxy, voir Chapitre III Manuscrit 2, la différence de condition est significative). Chez les poussins, on constate de même une réduction de la taille des tarses chez les urbains, mais pas de différence de condition corporelle (Figure 1B). Par ailleurs, en regardant la variation des traits à plus fine échelle, le long d'un gradient d'urbanisation on constate une diminution significative de la taille du tarse dans les zones les plus urbanisées seulement pour une année sur les sept étudiées. De plus, à cette échelle on observe une corrélation positive entre condition corporelle et degré de naturalité de l'habitat, mais seulement chez les oiseaux de deux ans ou plus. Concernant les traits du plumage, la taille de l'aile augmente significativement avec la naturalité de l'habitat chez les males. Enfin, aucune corrélation significative n'est détectée entre la taille de queue et le gradient de naturalité. Ensuite, nos résultats indiquent de plus une date de ponte significativement plus avancée en ville ainsi qu'une taille de ponte réduite (Figure 1C, Manuscrit 2). Concernant les traits comportementaux, les individus urbains sont en moyenne significativement plus agressifs que les ruraux et aussi significativement plus explorateurs (Figure 1D, Manuscrit 3). De plus, ils présentent un indice de rythme respiratoire plus faible, signifiant qu'ils respirent plus vite que les individus ruraux.

De nombreuses hypothèses concernant l'origine de ces divergences phénotypiques ont été

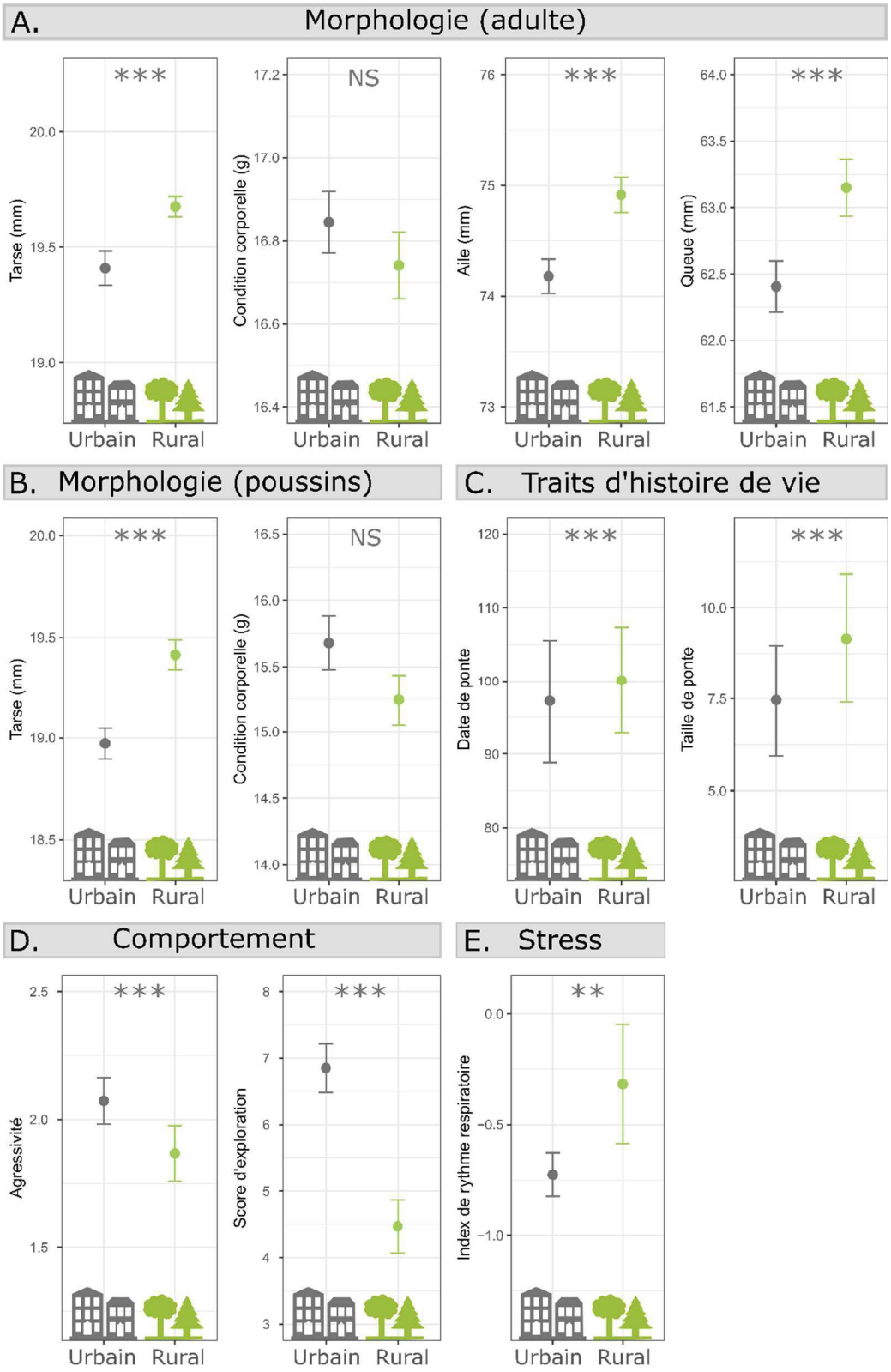

Figure 1 | Divergences phénotypiques entre mésanges urbaines (en gris) et mésanges forestières (en vert) pour les traits : (A) morphologiques (au stade adulte), (B) morphologique (au stade poussin), (C) d'histoire de vie, (D) comportementaux et (E) de réponse au stress. Les points représentent la moyenne de la population pour chaque trait et les barres d'erreur, les erreurs standard autour de la moyenne. Les étoiles indiquent le seuil de significativité de la différence de moyenne entre les habitats (*** : P<0.001 ; ** : P<0.01 ; * : P<0.05 ; NS : P>0.05). Notez que pour l'indice de rythme respiratoire (E), une valeur plus élevée indique un rythme respiratoire plus lent.

émises dans la littérature. Par exemple, la réduction de taille en milieu urbain est souvent attribuée à une plus faible disponibilité et qualité des ressources alimentaires en ville (Seress et al. 2012 ; voir aussi l'impact probable de l'effet îlot de chaleur, Bergmann 1848), hypothèse pouvant être étendue aux caractères du plumage, couteux à produire. Mais, l'absence de différence significative de condition corporelle entre populations urbaine et rurale n'écarte pas l'hypothèse que la petite taille (et les ailes et queue plus courtes), plutôt que d'être contrainte par le manque de nourriture, puisse être adaptative en ville. Par ailleurs, plusieurs conditions environnementales sont soupçonnées d'induire la précocité de reproduction (luminosité, température ; Dhondt & Eyckerman 1979) et l'importance que constitue la date de ponte pour le développement et la survie des poussins rend crucial de comprendre si ce décalage de date de ponte constitue un avantage ou non. En effet, si la disponibilité en ressources pour les poussins est plus précoce en ville, alors les individus précoces auront un meilleur succès reproducteur que les tardifs. A l'inverse, si la période de disponibilité en ressources n'est pas avancée en ville alors que les indices environnementaux (en particulier la température printanière) induisent une ponte précoce, alors les individus précoces auront un succès reproducteur réduit. La taille de ponte est aussi un trait majeur lié au succès reproducteur. Et alors qu'une ponte réduite en taille peut refléter une contrainte de l'environnement, on peut aussi émettre l'hypothèse qu'elle soit adaptative. En effet, en cas d'environnement pauvre, pondre moins d'œufs pourrait permettre de produire moins de descendants mais des descendants de bonne qualité alors que pondre plus d'œufs contraindrait à avoir des descendants de moins bonne qualité, avec des chances de survie réduites.

Les changements comportementaux observés ont aussi été décrits dans la littérature (*e.g.* Evans et al. 2010). Les comportements plus agressifs et plus exploratoires sont caractéristiques de personnalités plus téméraires (Réale et al. 2007) et la témérité est souvent associée à la capacité des individus à tolérer la nouveauté et le dérangement humain (Møller 2008), particulièrement intéressant en milieu urbain. De plus, les individus urbains ont un rythme respiratoire plus rapide en milieu urbain, traduisant une réponse au stress plus élevée. L'origine de ces comportements en milieu urbain est discutée, certaines études suggèrent une origine plastique et l'apparition de divergence par « acclimatation[6] » des individus face aux conditions urbaines (Cavalli et al. 2018), d'autre suggère un que seuls les individus téméraires puissent s'installer dans les milieux urbanisés (Holtmann et al. 2017) et d'autres encore soulèvent l'hypothèse de l'adaptation (Riyahi et al. 2017).

En conclusion, ce chapitre décrit des différences phénotypiques multiples entre populations urbaine et rurale. S'il offre parfois des pistes de réflexion quant à l'origine de ces différences, il surligne surtout l'importance majeure de comprendre si elles constituent des adaptations au milieu urbain, pour comprendre l'impact de l'urbanisation sur la dimension évolutive de ces variations.

Manuscrit associé au chapitre :

- **Manuscrit 1:** Caizergues, A.E., Charmantier, A., Lambrechts, M.M., Samuel Perret, S., Demeyrier, V., Lucas, A., & Arnaud Grégoire, A. (2021) An avian urban morphotype: how the city environment shapes Great tit morphology at different life stages. *Urban Ecosyst* https://doi.org/10.1007/s11252-020-01077-0

- Les analyses concernant les traits d'histoire de vie et les traits comportementaux sont présentés dans les **Manuscrits 2** et **3**, **Chapitre III**.

[6] Changement(s) phénotypique(s) plastiques se produisant à l'échelle de la vie d'un individu lui permettant de s'ajuster à des changements environnementaux et de maintenir ses performances biologiques.

– MANUSCRIT 1 –

An avian urban morphotype: how the city environment shapes great tit morphology at different life stages

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– ABSTRACT – Urbanization is a worldwide phenomenon associated with tremendous modifications of natural habitats. Understanding how city dwelling species are affected by those changes is becoming a pressing issue. We presently lack fine scale spatio-temporal studies investigating the impact of urbanization across different life stages and along urbanization gradients. Based on 8 years of monitoring of urban and forest great tits (*Parus major*), we investigated how city life shapes morphological characteristics at different life stages in the city *versus* the forest, and within the urban habitat (along naturalness and pedestrian frequency gradients). We found that urban nestlings were significantly smaller than forest ones, but not in lower body condition. Urban breeders showed reduced tarsus, wing and tail lengths compared to forest birds. Within the city, variation in nestling tarsus length and body condition along the naturalness gradient highly depended on the year, with no consistent pattern. For breeders, tarsus length and body condition were positively correlated to the naturalness gradient, although only in 2019 for tarsus, and only in older individuals for body condition. Finally, we found that males had smaller wing lengths in more urbanized parts of the city. These results suggest that urbanization affects morphology early on in development, influencing many morphological attributes. While the mechanisms underlying the urban morphotype remain to be determined, we discuss the potential origins for the documented differences between forest and urban morphotypes, and argue that they most probably result from urban environmental constraints linked to food availability.

– KEYWORDS – avian life stages | morphological variation | plumage allometry | urbanization gradient | urban morphotype

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INTRODUCTION

Urbanization is an increasing worldwide phenomenon driving massive environmental alterations (Marzluff 2001; Gaston et al. 2015). The process of converting land into cities results in new environmental conditions. For instance, compared to non-urbanized habitats, urbanized areas are associated with higher human densities and more impervious surfaces (tarmac roads, concrete ground and buildings) with the remaining green areas often covered with exotic plant species mainly used as decoration (Forman and Godron 1986). Hence, for many organisms, breeding sites, nature and abundance of food resources and human-associated disturbances differ drastically between urban and non-urban habitats (Seress and Liker 2015). Because of these novel environmental features, it is pervasively expected that urban-dwelling organisms should differ from their conspecifics living in less urbanized environments in many of their features, in particular in their development and morphology. Birds are one of the taxa that have most extensively been studied in an urban context, offering the rare opportunity to identify urban-associated drivers of phenotypic divergence (Marzluff 2017).

Availability and abundance of food resources are key factors determining the phenotype of organisms in a given environment. For temperate forest insectivorous passerines, the timing and abundance of resources are important determinants of nestling body condition (Lack 1958; Naef-Daenzer et al. 2001). Reduced vegetation densities and replacement of native plants by exotic ones are responsible for a drastic reduction of insect abundance in urban areas (Jones and Leather 2012; Seress et al. 2018). This lack of food resources can negatively affect birds' morphology, especially nestlings' (Seress et al. 2020). The urban habitat provides numerous novel sources of food (Chamberlain et al. 2009) but the combined effects of lower levels of insect abundance and access to new types of food (e.g. anthropogenic food) might be detrimental for birds during specific stages of their life while being beneficial during others. For instance, human-provided food resources (e.g. seeds in feeders) could be beneficial for adult great tits with a broad diet, but not for their nestlings that rely on insect resources (e.g. Pagani-Núñez et al. 2017). Since many morphological traits are labile throughout life, on a yearly basis (e.g. wing and tail length) or shorter term (e.g. body mass), this might potentially result in different morphotype outcomes in breeders relative to offspring.

The abundance of food resources is not the only constraint on bird development and morphology in cities. Intensified human activities might result in higher ambient temperatures, higher densities of chemical pollutants, and more artificial light and noise, all possibly affecting bird phenotypes directly or indirectly (e.g. Warren et al. 2006, Longcore 2010, Hargitai et al. 2016, Ruiz et al. 2016). For instance, in urban areas with high levels of motorized traffic, heavy metals are common pollutants (Dauwe et al. 2005). Oxidative stress due to these pollutants and the stress induced by human presence have consequences on nutrients assimilation (Dauwe et al. 2005; Müller et al. 2006; Koivula et al. 2011). High levels of oxidative stress could inhibit growth and energy storage, inducing a reduction of body mass and size (Müller et al. 2006; Koivula et al. 2011). Whether oxidative stress has age-dependent effects is still largely unknown. Artificial lights represent another important urban feature influencing morphology since they can induce earlier laying and desynchronization between the peak date of prey availability and the date of maximum nestling prey demand (Kempenaers et al. 2010), resulting in a constrained nestling development. Hence local environmental factors, such as light or chemical pollution, can have behavioral and physiological consequences on bird physiology, foraging or oxidative stress response, all potentially influencing nestling and adult morphology (Müller et al. 2006; Koivula et al. 2011). Importantly, anthropogenic factors might differentially affect birds during different stages of their life, reinforcing the need to study multiple life stages to understand the overall impact of urbanization on city dwelling bird species.

In the context of studying phenotypic responses to a drastically new and constraining environment, such as cities, morphological traits are particularly interesting because i) they are relatively easy to measure, ii) many morphological measures are functionally different hence provide non-redundant information (e.g. regarding health status, dispersal capacities or sexual attractiveness), iii) different morphology-associated traits are often

determined at different life stages (i.e. bone size is determined at nestling stage (Gebhardt-Henrich and van Noordwijk 1991) while feathers are renewed every year), and (iv) many morphological traits are closely related to survival or reproduction (Lindström 1999). As a result, a large panel of literature already suggests the existence of an urban morphotype in birds. For instance, several studies on great tits, blue tits (*Cyanistes caeruleus*), house sparrows (*Passer domesticus*) and common blackbirds (*Turdus merula*), have found that urban nestlings and adults have lower body condition, associated with lower fledging success, compared to forest birds (e.g. Perrins 1965, Liker et al. 2008, Ibañez-Alamo and Soler 2010, Seress et al. 2012, Bailly et al. 2016). While tarsus length and body condition (or body mass) are classically compared between urban and forest birds, plumage morphometrics, especially wing and tail lengths, are far less studied than size-linked traits. However, one study on great tits found divergence in nestling wing length (Seress et al 2020), while another study in urban vs. rural mountain chickadees (*Poecile gambeli*) revealed no such difference in adults (Marini et al. 2017).

One limitation of previous investigations on morphotypes in urban habitats is that they have mainly compared habitats in a binary way, such as forests versus urban parks (e.g. Björklund et al. 2010; Hedblom and Söderström 2012; Markowski et al. 2013; but see Marini et al. 2017; de Satgé et al. 2019). The urban landscape is however very heterogeneous and outside the heavily studied parks it also offers a wide range of more or less urbanized habitats (Amrhein 2013). This important heterogeneity in urban biophysical conditions could translate into contrasted environmental constraints (Alberti 2015), and hence in within-city phenotypic differentiation linked to various human activities.

The present 8-year study aims at improving our understanding of how urban environmental features shape the morphology of wild urban great tits. Our objective was to compare multi-trait morphometrics (tarsus length, body condition and relative wing and tail lengths) at different life stages, between a pair of urban and forest habitats as well as along an urbanization gradient. Our measures include a size and weight measure, but also plumage allometry (wing and tail), in order to explore late development as well as flight aerodynamism (Thomas 1996; Pennycuick 2008). We monitored great tit breeding in nest-boxes in the city of Montpellier and in a nearby oak forest ca. 20 km from the city. The nest-box distribution in the urban habitat was designed specifically to take into account the wide variety of urban landscapes, as recommended by Amrhein (2013): the monitoring set-up was conducted in areas composed of both parks and residential or commercial streets.

Based on previous findings, we first hypothesized that birds from urban environments would be smaller and display lower body condition compared to forest birds (Caizergues et al. 2018). Comparing morphological divergence at nestling and breeding life stages was instructive to test whether differences in morphology between forest and urban birds were already expressed during growth, and whether urbanization differentially affected individuals during different life stages. Second, we tested whether parent and nestling body condition and morphology also declined across the urban gradient within the city.

MATERIALS AND METHODS

Study sites

Between 2012 and 2019, a population of wild urban great tits was monitored annually from March to July in the city of Montpellier, in the south of France (43°36’43’’N, 3°52’38’’E). Birds bred in nest-boxes installed during the winter 2010-2011. The number of nest-boxes fluctuated between 183 and 222 because of theft or acts of vandalism. Nest-boxes were placed in eight areas of the city (see Figure S2A), in various urban environments from city parks to highly artificialized areas. Within most of the eight urban sites, nest-boxes were distributed in both green areas and along streets (on trees near pavements, roads and street lamps, Figure S2B). A long-term monitoring of great tits and blue tits from an oak forest site ca. 20 km from the city of Montpellier (La Rouvière forest, 43°64’82’’N, 3°69’43’’E, Figure S2C) provided similar breeding data during the same period of time (51 to 94 nest-boxes available for great tits across the eight years, Lambrechts et al. 2016).

Different types of nest-boxes were used in the monitoring: wooden squared-based nest boxes of three different sizes (small, medium and large) chosen within the ranges of dimensions accepted by

great tits for the purpose of previous study (e.g. Lambrechts et al. 2010, Demeyrier et al. 2016) and more commonly used concrete Schwegler nest-boxes 1B, with a round base. The different types of nest-boxes were distributed randomly across the study area. To limit intraspecific competition, a distance of at least 100 meters separated each nest box from its neighbour.

Breeding monitoring

From the beginning of the breeding season until the fledgling phase, nest-boxes were visited at least weekly. Because quality and quantity of resources required for nestling growth are expected to significantly change across the breeding season (e.g. Nager and Van Noordwijk 1992), we only focused in this study on nestling data from first clutches, i.e. clutches initiated less than 30 days after the first egg was laid in a given year. Data from second broods or replacement clutches after a failed first breeding attempt were thus not considered. We also excluded broods involved in field experiments, such as brood size manipulation or food-supplementation (Demeyrier et al. 2017). Based on this data filtering, this study included data on 914 (467 females and 447 males) breeding birds trapped between 2013 and 2019 inside nest-boxes while they were feeding 9 to 15 days-old nestlings (hatching is day 0 and age of the brood is the age of the eldest nestling) and on 1576 urban and 1211 forest nestlings. Parents and nestlings were ringed with uniquely numbered metal leg rings provided by the Centre de Recherche Biologique sur les Populations d'Oiseaux (Paris, France). Breeders were sexed based on the presence /absence of a brood patch, and the colour of their wing coverts (Svensson 1992) was used to age them (birds born in the previous breeding season = yearling vs. bird of more than 2 years old = adult). In breeders, we measured the right tarsus length with a calliper (±0.01mm), body mass with a Pesola balance (±0.1g), and wing length and tail length with a ruler (±0.5mm). Wing length was measured from the radius-ulna / carpometacarpus fold (=wirst) to the end of the longest primary remige. For ca 15-day old nestlings, we measured the right tarsus length and body mass using the same field procedures as those applied for breeders. Note that because of fieldwork constraints and of asynchronous hatching, while the majority of nestlings were measured at 15 days, nestling age at measurement varied between 13 and 17 days.

Estimation of urbanization level

The level of urban disturbance and the potential food resources for great tits was quantified for each nest box, using a radius of 50 meter representing the area where breeders forage more frequently (Perrins 1979).

The measures described below were collected in 2011 (see Demeyrier et al 2016) and repeated in 2018. Since these measures were very stable across time (see Supplementary information 1), we used in this study the 2018 data only, which were collected for all nest-boxes with avian phenotypic measures. Note that while Demeyrier et al (2016) used three measures of vegetation cover (oak cover, tree cover and total vegetation cover), these three measures were highly correlated (Demeyrier et al. 2016: Figure 4) hence only total vegetation cover is used here.

Within 50 meters around each nest box, we measured total vegetation cover (canopy and grass) as proxy for prey availability (Hinsley et al. 2002; Mackenzie et al. 2014). The surface of vegetation cover around each next box was measured on satellite pictures analysed with Q-Gis (version 2.18.16). We also counted motorized traffic and the number of pedestrians to quantify respectively noise and chemical pollution, and human presence (Katti and Warren 2004; Hedblom and Söderström 2012; Aronson et al. 2014). This count was made once for each nest-box during 5 minutes, when ringing nestlings. Finally, light pollution was estimated as the spatial surface of artificial light within 50 meters from each box (Kempenaers et al. 2010) by overlaying a satellite map of each nest-box 50m radius area with a satellite map of referenced lampposts and their radius of influence with the Q-gis software. A Principal Component Analysis was run on these urban factors and revealed two major axes: PC1 (42.4% of variance explained) represented the degree of habitat artificiality, named "naturalness gradient" (positive values associated with higher vegetation cover, lower light pollution and lower vehicle traffic), and PC2 (25.4% of variance explained) represented the "pedestrian frequency" (see Sup Mat Figure S1 for PCA results).

Statistical analyses

We focused our analyses on four morphological traits: (1) tarsus length, which is used as a proxy of overall body size (because highly correlated with overall bone size, Freeman and Jackson 1990), (2) body condition, estimated via the Scaled Mass Index (Peig and Green 2009) which adjusts the mass of individuals as if they all had the same size, and relative (4) wing and (4) tail lengths (measures corrected by tarsus length for each individual see below). To analyze variation in all these traits between habitats and within the urban matrix, we used Linear Mixed-Effect Models (LMM) run with R packages lme4 and lmerTest (Bates et al. 2015, Kuznetsova et al. 2017; R version 3.5, R Core Team 2018), with a normal error distribution for all nestling and breeder traits. These models allow to use all data collected while accounting for the non-independence of repeated measures on the same individual. Two different sets of models were conducted: first, a global model included the data from the city and the forest to analyze inter-habitat variation and second, an urban model focused on variation within the city. Nestlings and adults were analyzed separately.

We exploited the data from 1576 urban nestlings (363 broods) and 1211 forest nestlings (186 broods) measured between 2012 and 2019. When modeling nestling morphology, explanatory variables were habitat (urban/forest) or naturalness gradient (PC1) and pedestrian frequency (PC2), age (in days), date (as a continuous variable, 1= 1st of March, standardized per habitat per year), year and the interactions between urban parameters and year (habitat*year or naturalness gradient*year and pedestrian frequency*year). Hour of measure was also added as a continuous fixed effect in the body condition model since weight can vary across daytime (Lange and Leimar 2004). Nest-box identity was included as a random effect in all models since the majority of nest-boxes were occupied more than once across breeding seasons. Mother ring number was also included as a random effect, to account for sibling relatedness as well as maternal environmental/genetic effects. Finally, measurer identity was also included as a random effect to account for differences due to measurement bias.

The analyses on breeder morphology (nurban=640/868; nforest=265/414 for number of individuals/measurement events) were based on data collected between 2013 and 2019. In models on adult morphology, explanatory variables in the full model were: habitat (urban/forest) or naturalness gradient (PC1) and pedestrian frequency (PC2), sex, age (yearling vs. adult), date (as a continuous variable, 1= 1st of March, standardized per year), year, and all biologically relevant double interactions (habitat*sex, habitat*age, habitat*year or naturalness gradient*sex, naturalness gradient*age, naturalness gradient*year, pedestrian frequency*sex, pedestrian frequency*age, pedestrian frequency*year, age*sex, and sex*year). Tarsus length was included as a fixed effect in the models explaining wing length and tail length. Thus wing length and tail length hereafter refers to “relative wing length” or “relative tail length”. As explained above, daily hour of measure was also added in this model of body mass. Individual ring number was included as a random effect, since numerous breeders were trapped at least twice across breeding seasons (nurban=164 and nforest=99 captured at least twice). This individual random effect also encompasses variation in micro-habitats since most individuals breed in the same or very close nest-boxes across their reproductive life. Finally, measurer identity was also included as random effect. In all models, random effects were set on the intercept, and were non-nested.

For model selection, we followed a backward stepwise procedure, consisting in removing one non-significant (p-value > 0.05) fixed-effect (simple or in interaction) at each step, starting from the full model (summary of best models are presented in Supplementary Tables S1 to S4). Significance of fixed effects was tested with an analysis of variance (F-test, type III ANOVA), and degrees of freedom were automatically computed with default settings.

When the final model after the above procedure included at least one interaction with habitat, naturalness gradient or pedestrian frequency, we used emmeans (if categorical) or emtrends (if continuous) functions (R package emmeans) to obtain estimates per group, and applied post-hoc Tukey HSD tests to assess the significance of a difference between groups (all these results are detailed in Supplementary Tables S5 to S18).

Table 1 | Mean and standard deviation of morphological traits in nestlings and breeder great tits in forest and urban habitats, calculated from the outputs of the best linear mixed models displayed in Table 2 & 3 using emmeans() R function with default parameters. Tarsus wing and tail lengths are measured in millimetres and body condition in grams. Wing length and tail length are corrected for tarsus length in the linear models. P-values are extracted from the best LMM model habitat effect (see Tables 2, 4, S1 & S3).

Life stage	Trait	Forest		Urban		P
		mean	SE	mean	SE	
Nestling	Tarsus length	19.415	0.074	18.972	0.076	**<0.001**
	Body condition	15.242	0.193	15.680	0.203	0.072
Breeder	Tarsus length	19.676	0.044	19.409	0.036	**<0.001**
	Body condition	16.741	0.080	16.845	0.074	0.141
	Wing length	74.915	0.161	74.179	0.154	**<0.001**
	Tail length	63.150	0.214	62.406	0.193	**<0.001**

In order to check whether models respected linear regression basic assumptions, we ran several tests. First, we tested for multicolinearity between variables via the calculation of the variance inflation factor of models, using vif() function of car package (Fox et al. 2007). Multicolinearity was not an issue since the maximum value of VIF was below 2 for all models. We found no autocorrelation between residuals (Dubin-Watson test, $1.5 < d < 1.99$ for all models). Finally, we graphically checked homoscedasticity of residuals, which was respected for all models.

RESULTS

Forest nestlings overall had longer tarsi than urban nestlings (P<0.001, Table 1 & 2 and Figure 1). However, the magnitude of the habitat effect depended on the year as indicated by the significant habitat by year interaction (P<0.001; Table 2 and Figure 1). Forest nestlings had longer tarsi than urban nestlings in 2012 (est.±SE=0.857±0.244, P=0.035, Table S6), 2017 (est.±SE=0.589±0.143, P=0.004, Table S6) and 2018 (est.±SE=0.779±0.143, P<0.001, Table S6), but the difference was not significant for the other years (see Table S6). In addition, the habitat effect on nestling body condition varied across years (significant habitat by year interaction; P<0.001; Table 1 and Figure 1C; see ESM Figure S4 for details) and forest nestlings displayed a significantly lower body condition compared to urban nestlings in 2015 (est.±SE=-1.505±0.440, P=0.046, Table S6).

Urban breeders had shorter tarsi but not lower body conditions than forest breeders (respectively: P<0.001 & P=0.141, Table 1 & 4 and Figure 2A & 2B). Urban breeders also had smaller relative wing lengths compared to forest breeders (P<0.001, Table 1 & 4 and Figure 2C), and the magnitude of the urban-forest difference depended on age (significant habitat by age interaction; P =0.009; ESM Table S3). The difference in relative wing length was more pronounced in yearlings (est.±SE=+0.993±0.174 for forest birds, P<0.001; Table S10) than in birds of 2 years or more (est.±SE=+0.479±0.160, P=0.011; Table S10).

Table 2 | F-statistics and associated P-values, variance components and sample sizes from final mixed models comparing great tit nestling tarsus length and body condition between forest and urban habitats (type III ANOVA). Detailed outputs and estimates from these models are presented Table S1.

	Tarsus length				Body condition			
Fixed effects	**sum sq.**	**d.f.**	**F**	**P**	**sum sq.**	**d.f.**	**F**	**P**
Habitat	6.654	1	21.686	**<0.001**	6.353	1	3.31	0.072
Year	12.087	7	5.628	**<0.001**	57.481	7	4.279	**<0.001**
Habitat x Year	9.867	7	4.594	**<0.001**	107.587	7	8.009	**<0.001**
Random effects	**Var.**	**Sample Size**			**Var.**	**Sample Size**		
Mother identity	0.230	Nb mothers = 401			1.391	Nb mothers = 399		
Nest-box identity	0.047	Nb nest-boxes = 202			0.278	Nb nest-boxes = 200		
Observer	0.031	Nb observer = 23			0.256	Nb observer = 23		
Residuals	0.307	N = 2792			1.919	N = 2769		

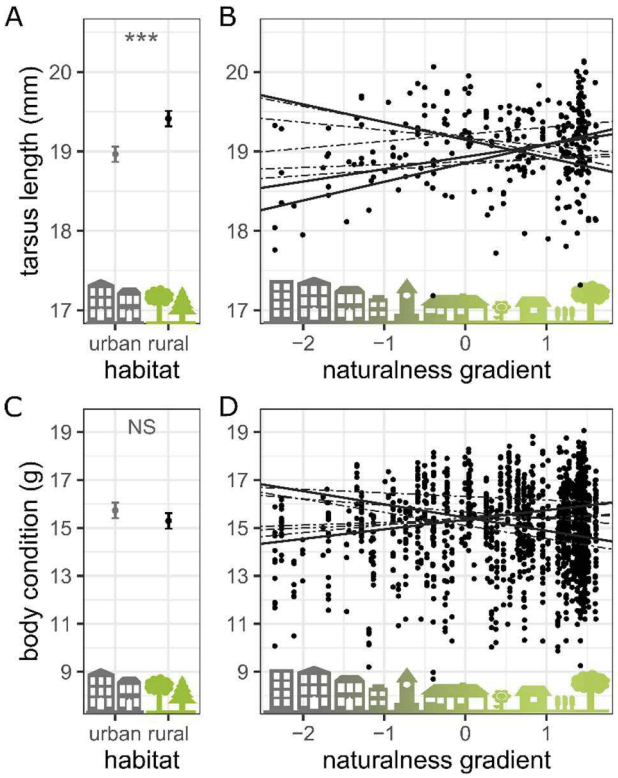

Figure 1 | Morphological divergence in nestling tarsus length and body condition between urban and forest habitats (mean±SE, A and C) and along an urbanization gradient (B and D). In B and C, points represent individual predicted values output from the best LMM models presented Tables 2 and 3. Intercept and slopes of lines are detailed in supplementary Table S7. , In panels B and D, each line represents a year and solid lines represent slopes for which 95% confidence intervals did not include zero (for B: 2016, 2018 & 2019; for D: 2015, 2019).

Relative tail length was also overall shorter in urban breeders (P<0.001, Table 1 & 4 and Figure 2D), but post-hoc tests revealed that the difference was significant only for 2015 (est.±SE=1.866 ±0.416, P<0.001; Table S12). Finally, yearling breeders had overall smaller morphometrics than older breeders (body condition: est.±SE=-0.257±0.049, P<0.001; wing length: est.±SE=-1.076±0.184, P<0.001; tail length: est.±SE=-1.884±0.146, P<0.001 ; Table 2 and ESM Table S3), except for tarsus length.

Within the city, the effect of the degree of urbanization on nestling tarsus length varied across years, as indicated by the statistically significant interaction between the naturalness gradient and year (P=0.0004; Table 7 and Figure 1B). Estimates of the regression slopes between naturalness gradient and tarsus length varied between -0.223 (±0.128) in 2016 and +0.235 (±0.107) in 2019 (see Figure 1B; for

Table 3 | F-statistics and associated P-values, variance components and sample sizes from final mixed models comparing nestling tarsus length and body condition along gradients of urbanization and pedestrian disturbance (type III ANOVA). Naturalness gradient and pedestrian frequency are synthetic variables obtained by a PCA on measures of vehicles densities, pedestrian count, light pollution and green cover around each nest-box (see supplementary Figure S1). Detailed outputs and estimates from these models are presented Table S2.

	Tarsus length				Body condition			
Fixed effects	**sum sq.**	**d.f.**	**F**	**P**	**sum sq.**	**d.f.**	**F**	**P**
Naturalness gradient (PC1)	$1.5x10^{-6}$	1	$<1x10^{-22}$	0.998	0.607	1	0.275	0.601
Pedestrian frequency (PC2)	0.014	1	0.041	0.840	n.s.	n.s.	n.s.	n.s.
Year	3.459	7	1.483	**<0.001**	19.478	7	1.263	**0.268**
Naturalness gradient x Year	9.061	7	3.885	**<0.001**	44.644	7	2.893	**0.006**
Pedestrian disturbance x Year	4.912	7	2.106	**0.043**	n.s.	n.s.	n.s.	n.s.
Random effects	**Var.**	**Sample Size**			**Var.**	**Sample Size**		
Mother identity	0.214	Nb mothers = 255			1.385	Nb mothers = 255		
Nest-box identity	0.041	Nb nest-boxes = 116			0.226	Nb nest-boxes = 116		
Observer	0.031	Nb observer = 14			0.524	Nb observer = 14		
Residuals	0.333	N = 1556			2.204	N = 1554		

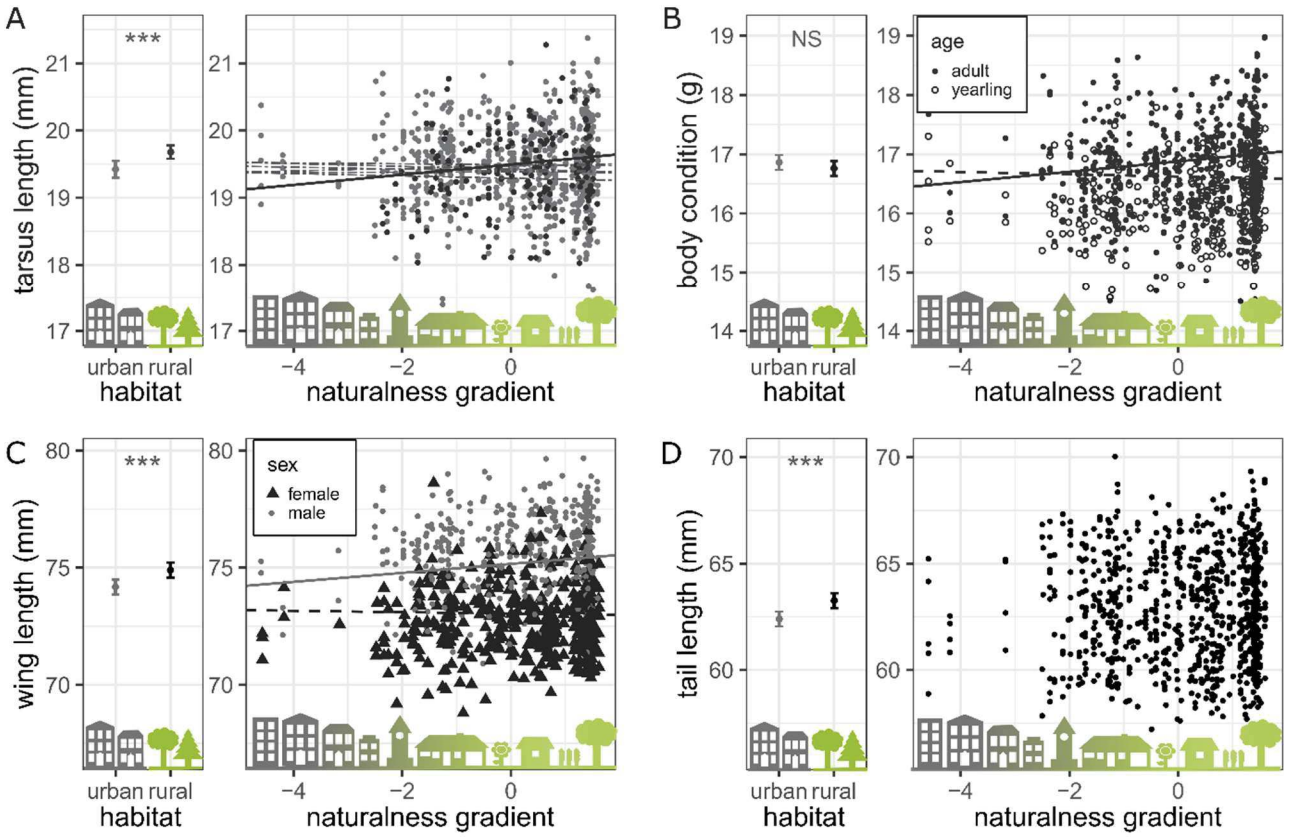

Figure 2 | Morphological divergence between urban and forest breeders and along an urbanization gradient for (A) tarsus length, (B) body condition, (C) relative wing length and (D) relative tail length. Points represent individual predicted values output from models presented Tables 4 and 5. Detailed intercepts and slopes are presented in Supplementary Table S4 & S9 to S18. Solid lines represent slopes for which 95% confidence intervals did not include zero. In A, the black color represents 2019, and grey represent all other years. In B, solid dots and the solid slope represent adult breeders while empty dots and the dashed line represent yearling breeders. In C, grey points and the grey line represent males and black, females.

detailed estimates see ESM Table S7), and were significantly different between 2016 and 2018 (P=0.003; post-hoc tests presented in Table S8), and 2016 and 2019 (P=0.001; Table S8). Additionally, there was a significant effect of the naturalness gradient in interaction with year (P<0.001; Table 4; Figure 1D). When looking at detailed slopes per year, only two of them had 95% credibility intervals non overlapping zero (see Table S7). We also detect a significant effect of pedestrian frequency on nestling tarsus length in 2019 (est.±SE=0.201±0.097, Table S7; P=0.043, Table 3). In 2015, nestlings were in better body condition in more natural parts of the city (est.±SE= 0.398±0.264, Table S7) whereas the reverse was found in 2019 (est.±SE=-0.551±0.155, Table S7), and this difference in slope was confirmed by post-hoc tests between years (ESM Table S2).

Interestingly, significant annual variation was also found for the effect of the naturalness gradient on breeder tarsus length (significant naturalness gradient x year: P=0.035; Table 5). The regression slope between tarsus length and the naturalness gradient was positive for 2019 and it was the only year for which the standard error of the estimate did not include zero (est.±SE=0.076±0.029, P<0.001; Table S13; see Table S14 for post-hoc between years comparisons of annual slopes), meaning that for this year breeders were smaller in the more urbanized parts of the city (Figure 2A).

For breeder body condition, the naturalness gradient by age interaction was statistically significant (P=0.023, Table 5). Body condition of adult urban breeders significantly decreased in more urbanized areas while it was not the case for yearlings for which the slope standard error included zero (est.adult±SE =0.087±0.034, est.yearling±SE =-0.019±0.037; P=0.24; Figure 2B, Tables S15 & S16). Finally, urban breeders were in better physical condition in the highly crowded areas of the city given that there was a statistically significant positive

Table 4 | F-statistics and associated P-values, variance components and sample sizes from final mixed models comparing breeders' tarsus length, body condition, relative wing and tail lengths between urban and forest habitats. Detailed outputs and estimates from these models are presented Table S3.

	Tarsus length				Body condition				Wing length				Tail length			
Fixed effects	**sum sq.**	**d.f.**	**F**	**P**	**sum sq.**	**d.f.**	**F**	**P**	**sum sq.**	**d.f.**	**F**	**P**	**sum sq.**	**d.f.**	**F**	**P**
Tarsus length	-	-	-	-	-	-	-	-	107.699	1	66.955	**<0.001**	28.806	1	7.663	**<0.001**
Habitat	1.197	1	40.003	**<0.001**	n.s.	n.s.	n.s.	n.s.	48.875	1	30.384	**<0.001**	48.421	1	12.882	**<0.001**
Sex	6.142	1	205.233	**<0.001**	n.s.	n.s.	n.s.	n.s.	547.002	1	340.050	**<0.001**	1263.218	1	336.057	**<0.001**
Age	n.s.	n.s.	n.s.	n.s.	9.845	1	27.773	**<0.001**	427.993	1	266.066	**<0.001**	601.489	1	160.016	**<0.001**
Year	1.417	6	7.891	**<0.001**	19.534	6	9.185	**<0.001**	42.664	6	4.420	**0.002**	139.156	6	6.170	**<0.001**
Hour	-	-	-	-	2.883	1	8.132	**<0.001**	-	-	-	-	-	-	-	-
Habitat x Age	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	10.967	1	6.818	**0.009**	n.s.	n.s.	n.s.	n.s.
Habitat x Year	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	55.914	6	2.480	**0.022**
Sex x Age	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	16.897	1	10.504	**0.001**	n.s.	n.s.	n.s.	n.s.
Random effects	**Var.**		**Sample size**		**Var.**		**Sample size**		**Var.**		**Sample size**		**Var.**		**Sample size**	
Identity	0.285		Nb indentity = 912		0.491		Nb indentity = 905		1.287		Nb indentity = 910		2.589		Nb indentity = 893	
Observer	0.008		Nb observer = 18		0.027		Nb observer = 18		0.178		Nb observer = 18		0.188		Nb observer = 18	
Residuals	0.030		N = 1282		0.354		N = 1264		1.609		N = 1277		3.759		N = 856	

Table 5 | Outputs from final mixed models comparing breeder tarsus length, body condition, relative wing and tail lengths across gradients of naturalness and pedestrian frequency in the urban habitat. Naturalness gradient and pedestrian disturbance are synthetic variables obtained by a PCA on measures of vehicles densities, pedestrian count, light pollution and green cover around each nest-box (see supplementary Figure S1). Detailed outputs and estimates from these models are presented Table S4.

	Tarsus length				Body condition				Wing length				Tail length			
Fixed effects	**sum sq.**	**d.f.**	**F**	**P**	**sum sq.**	**d.f.**	**F**	**P**	**sum sq.**	**d.f.**	**F**	**P**	**sum sq.**	**d.f.**	**F**	**P**
Tarsus length	-	-	-	-	-	-	-	-	79.892	1	53.516	**<0.001**	28.423	1	7.383	**<0.001**
Naturalness gradient (PC1)	0.003	1	0.118	0.732	0.513	1	1.554	0.213	3.481	1	2.488	0.11521	n.s.	n.s.	n.s.	n.s.
Pedestrian frequency (PC2)	n.s.	n.s.	n.s.	n.s.	1.672	1	5.06	**0.025**	n.s.	n.s.	n.s.	n.s.	15.374	1	3.994	**0.046**
Sex	3.556	1	126.981	**<0.001**	n.s.	n.s.	n.s.	n.s.	272.950	1	195.042	**<0.001**	790.560	1	205.361	**<0.001**
Age	n.s.	n.s.	n.s.	n.s.	4.889	1	14.795	**<0.001**	363.304	1	259.606	**<0.001**	454.322	1	118.018	**<0.001**
Year	1.291	6	7.683	**<0.001**	11.475	6	5.787	**<0.001**	n.s.	n.s.	n.s.	n.s.	115.142	6	4.985	**<0.001**
Hour	-	-	-	-	3.539	1	10.709	**0.001**	-	-	-	-	-	-	-	-
Nat. grad. x Age	n.s.	n.s.	n.s.	n.s.	1.704	1	5.157	**0.023**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Nat. grad. x Sex	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	6.482	1	4.632	**0.032**	n.s.	n.s.	n.s.	n.s.
Nat. grad. x Year	0.386	6	2.297	**0.035**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Sex x Age	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	10.406	1	7.436	**0.007**	n.s.	n.s.	n.s.	n.s.
Random effects	**Var.**		**Sample size**		**Var.**		**Sample size**		**Var.**		**Sample size**		**Var.**		**Sample size**	
Identity	0.309		Nb indentity = 640		0.506		Nb indentity = 636		1.643		Nb indentity = 638		2.688		Nb indentity = 634	
Observer	0.006		Nb observer = 12		0.014		Nb observer = 12		0.275		Nb observer = 12		0.221		Nb observer = 12	
Residuals	0.028		N = 868		0.33		N = 859		1.399		N = 866		3.850		N = 856	

effect of pedestrian frequency on breeder body condition (est.±SE=+0.076±0.034; P=0.025; Table 5 & S4).

For breeder relative wing length, there was a statistically significant positive interaction between the naturalness gradient and breeder sex (P=0.032, Table 5). In city males, relative wing length increased with increasing habitat naturalness (est.±SE=0.192±0.074 Figure 2C, Table S4, S17 & S18) whereas this effect was close to zero for city females (est.±SE=-0.029±0.072, Figure 2C, Table S4, S17 & S18). Finally, relative tail length was not correlated with the naturalness gradient. However, breeders had longer tails in more crowded locations (pedestrian frequency effect: est.±SE=0.19±0.095; P=0.046; Table 5 and Table S4).

DISCUSSION

In this study, we aimed to assess the impact of the degree of urbanization on a suite of morphological traits in great tits from southern France (Montpellier), at three life stages: nestling, yearling breeder and older breeder. First, as expected based on previous reports, urban birds were smaller than forest birds. At each life stage, great tits had shorter tarsi in the city than in the forest, which is consistent with findings in previous studies of body size in urban passerines (Liker et al. 2008; Meillère et al. 2015; Biard et al. 2017) although not all (Marini et al. 2017). However, in contrast to a previous study in house sparrows (Meillère et al. 2017), we found no consistent pattern of variation in tarsus length along the naturalness gradient, since the sign of the correlation between tarsus length and the degree of urbanization varied among years in both nestlings and breeders (Figure 1B & 2A). Second, we did not detect a significant difference between urban and forest breeders regarding body condition, which contrasts with previous findings of thinner urban individuals (Caizergues et al. 2018, but note the use of a different proxy for body condition) but is in line with results found in other species (e.g. Bókony et al. 2012). However within the city, urban breeders had a lower body condition in the more urbanized areas, as reported by Liker et al. (2008), but only in birds of 2 years or older, not in yearlings. In addition, we found a significant influence of the degree of urbanization on nestling body condition in interaction with year, when comparing urban versus forest nestlings as well as along the naturalness gradient, with estimates changing direction among years. Similar inconsistent results have been reported in the literature (Isaksson and Andersson 2007, Meillère et al. 2017, but see Biard et al. 2017). Surprisingly, however, nestling tarsus length was correlated with pedestrian frequency for the year 2019, and we did not find any report of similar results in the literature.

Finally, urban birds had reduced relative wing and tail lengths compared to forest birds (Figure 2C & 2D). In addition, male breeders showed reduced relative wing length with increasing urbanization. To our knowledge, such findings on plumage morphometric traits have rarely been reported in the literature (but see for instance Seress et al. 2012 for evidence of shorter wings in urban house sparrow nestlings).

Consequences of living in cities for nestlings

In birds, bone size, and thus tarsus length, becomes structurally fixed towards the end of the nestling stage (Lindstrom 1999), and poor environmental conditions during development are often associated with smaller structural size (e.g. Seress et al 2012). Thus, reduced size of urban nestlings could result from developmental constraints linked to urban habitat quality (Seress et al. 2018, 2020). For instance, Riddington and Gosler (1995) found lower heritability for tarsus length in great tits living in sub-urban gardens compared to woodland great tits, suggesting that urbanization might limit nestling growth. However, we also observed that urban nestlings were not consistently in lower body condition than forest nestlings (urban nestlings were thinner than forest nestlings in 2012, 2017 and 2018, but no difference was detected during other years). Perhaps spatial variation in the availability of high-quality food might explain why urban nestlings are smaller, but not necessarily in worse physical condition compared to forest nestlings (Isaksson and Andersson 2007). For instance, García-Navas et al. (2013) reported a positive correlation between the proportion of spiders in the diet of nestlings on the one hand and nestling tarsus length on the other hand, which was attributed to the fact that spiders are richer in calcium and taurine than caterpillars. Hence a change in diet constrained by different environmental conditions might result in a change in bone

development but not necessary in body condition, which in our case could explain why urban nestlings had shorter tarsi but not always reduced body condition. In addition, growth and bone development might also be affected by chemical pollution and stress. Indeed, heavy metals combined with acidifying agents can reduce calcium assimilation in organisms (Dauwe et al. 2005).

Interestingly, we found that morphology not only expressed significant spatial variation, but also varied substantially between study years. For instance, the difference in body condition between urban and forest nestlings varied among years. In the same line, tarsus length that varied along the naturalness gradient also displayed strong variation across breeding seasons. Hence, inter-annual variation in environmental conditions is probably important in our system and should be further explored via the quantification of both food quantity and quality across years and study areas. For example, a recent review highlighted that resources and habitat structure are more stable between years in urban than in natural habitats (Leveau 2018). Finally, nestling tarsus within the city was positively correlated with the pedestrian frequency gradient. This could either be associated with pedestrian-associated resources (wastes) or result from an alteration of parental care patterns and time constraints in the presence of human pedestrians. For instance, humans might scare away bird predators and birds habituated to the presence of humans might therefore be given more time to look for insect prey required to feed the nestlings (Møller 2012).

Consequences of living in cities for breeders

As discussed above, the shorter tarsi of urban birds were already observed at the nestling stage, suggesting that constraints during nestling development affect breeders' morphology. Interestingly, the difference in tarsus length between forest and urban birds was larger for nestlings than for breeders (mean differences of 0.443 mm for nestlings versus 0.265 mm for adults, Table 1). While great tit tarsi have usually reached their final size when measured on 14-15 days old nestlings (Orell 1983), data on nestling growth in urban areas might reveal that the time needed to reach an asymptotic size is longer in the city. Alternatively, a larger difference in size between urban and forest nestlings compared to adults could also result from survival selection favouring taller nestlings in the city. Future demographic field studies will allow to test whether smaller city fledglings have lower survival.

In addition, we did not detect a difference in body condition between urban and forest breeders which contrast with other studies in this and other populations of great tit (Caizergues et al. 2018). This result suggests that urban great tits could compensate for fewer resources by being smaller and hence maintain a similar body condition compared to forest great tits. However, within the city, body condition of adult birds increased significantly in more natural areas which is not the case for yearlings that have a lower and constant body condition all along the gradient (Figure 2B). This result indicates that food availability might be reduced in more urbanized parts of the city. For instance, Hinsley et al. (2008) reported that in patchy urban habitats the daily energy expenditure of female great tits increased by 64% per nestling. This indicates that increased parental foraging effort during the nestling stage can lower body condition of breeders facing a depleted urban habitat. One hypothesis for the difference observed between adults and yearlings is that more experienced adult birds are able to better exploit their environment and maintain high body condition but only in the greener areas of the city. While one-year-old birds are too novice to optimally explore their habitat or secure high-quality territories and thus present lower body condition in all parts of the city. Future studies will be required to examine whether the urban morphotype is adaptive. For instance, body condition might be associated with resource predictability (Cuthill et al. 2000), with e.g. high food predictability associated with a significant reduction in body reserves (Shochat 2004). Knowledge on the composition of the urban great tit diet and its spatio-temporal variation would once again be instrumental in this context. We therefore currently do not reject the hypothesis that a reduction in body condition can be plastically or genetically adaptive in more urbanized habitats. While previous selection analyses did not reveal any reproductive advantage for breeders with lower body condition in the city (Caizergues et al. 2018), survival advantages could occur and need to be evaluated.

Similarly, the urban great tit morphotype was characterized by shorter relative wing and tail lengths

compared to forest birds, as well as shorter relative wing length along the naturalness gradient (in males only). To our knowledge, very few studies have investigated the link between urbanization and plumage length in passerines. Marini et al. (2017), found no relation between level of urbanization and wing and tail lenghts in mountain chickadee. On the contrary, a recent study of Seress et al. (2020) found shorter wings in great tit nestlings. Differences in wing and tail lengths could result from urban constraints or be adaptive. In the first scenario, shorter feathers in city birds could be explained by increased diet-associated constraints in the city since feather growth is costly, especially in a context of protein deprivation (Saino et al. 2013). Previous investigations used feather quality as a proxy to assess the nutritional and energetic constraints acting during molt or breeding (Nilsson and Svenssonn 1996). In addition, Meillère et al. (2017) reported a decrease in feather quality (i.e. density) along the naturalness gradient in juvenile birds. In an alternative scenario, rather than resulting from urban constraints, smaller wings and tails might be adaptive in cities. Previous selection analyses relating reproductive success to wing and tail lengths did not reveal any breeding selection on these traits in the city nor in the forest (see Table 3 in Caizergues et al., 2018); however, it is likely that natural selection could act via survival rather than reproductive success. For instance, plumage traits are closely linked to flight aerodynamics, and thereby can be important features determining predator or obstacle avoidance (Rayner 1988). In particular, shorter wings confer higher maneuverability (Rayner 1988, Thomas 1996), which could be crucial to avoid collision with moving vehicles or to escape from cats. For instance, in a population of cliff swallows (*Petrochelidon pyrrhonota*) nesting near a highway, individuals collected after collision deaths had longer wings than the rest of the study population which suggested survival selection favoring shorter-winged swallows (Brown and Bomberger Brown 2013). In line with these findings, we found that male great tits had shorter wings in areas with denser traffic (Table 5). Survival studies using capture-recapture histories will be required to further explore the association between wing length and traffic density.

Going further

This study examined in a multi-dimensional way the urban morphotype and we mainly discuss the urban constraints that could result in a plastic morphotype. However, our study is of correlative nature, and future studies will need to (1) identify environmental drivers explaining the urban morphotype, (2) test for a plastic versus genetic origin in the forest / city differences described here, and (3) test for the adaptive nature of these differences (Perrier et al. 2020). Experimental tests manipulating different environmental condition (pollution, access to resources, disturbance) and comparing forest and city birds raised in similar conditions (cross-fostering and/ or common garden) will be most efficient to tackle these questions.

Also, our comparative field study focused on a single pair of study populations. Because different cities share many environmental features, they offer multiple opportunities to replicate our study approaches on the same or on other species. Hence, we hope that in the near future, such multi-stage comparison of forest and urban morphotypes will be conducted across multiple cities.

DECLARATIONS

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CONFLICTS OF INTEREST/ COMPETING INTERESTS

We declare we have no conflicts of interest and no competing interests.

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ETHICS APPROVAL

Captures were performed under personal ringing permits delivered by the CRBPO (Centre de Recherches par le Baguage des Populations d'Oiseaux, e.g. ringing permit for Anne Charmantier and Arnaud Grégoire were number 1907 & 1904) for the Research Ringing Programme number 369. All experimental protocols were approved by the ethics committee for animal experimentation of Languedoc Roussillon (CEEA-LR, most recent approval on 05/06/2018) as well as by Regional Institutions (bylaw issued by the Prefecture n° 2012167-003).

AUTHORS CONTRIBUTIONS

AEC participated in data collection, carried out the statistical analyses, and wrote the manuscript. ML, AG and AC conceived and funded the study, collected the data and contributed to the writing of the manuscript. SP, VD and AL participated in the data collection. All the authors gave final approval for publication.

DATA AND CODE AVAILABILITY

All data and custom codes used in this study are available on Hal platform: hal.archives-ouvertes.fr/

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CHAPITRE III

DIFFÉRENCIATION PHÉNOTYPIQUE : ADAPTATION AU MILIEU URBAIN ?



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– RÉSUMÉ DU CHAPITRE III –

Les populations urbaines présentent de nombreuses divergences phénotypiques par rapport à leurs comparses rurales sur de nombreux types de traits : morphologiques, d'histoire de vie, comportementaux et physiologiques (voir Chapitre 2). Pour comprendre si et comment les espèces s'adaptent au milieu urbain, il est essentiel de déterminer si ces différences phénotypiques sont induites par des pressions de sélection différentes selon les habitats, c'est à dire si elles sont adaptatives ou non. L'objectif principal de ce chapitre est donc d'étudier la corrélation entre phénotype et valeur sélective des individus dans chaque habitat, c'est-à-dire de mesurer la sélection naturelle pour chacun des traits et d'identifier s'il existe un alignement entre la sélection mesurée et le sens de la divergence phénotypique entre habitat.

Ainsi, dans un premier temps je me suis penchée sur la mesure de sélection agissant sur les traits morphologiques et d'histoire de vie en utilisant une mesure de succès reproducteur (nombre de poussin à l'envol) comme proxy de valeur sélective (**Manuscrit 2**). Les analyses réalisées ici reposent sur la méthode d'analyse de sélection décrite par Lande & Arnold en 1983 qui modéliser la relation entre une mesure de valeur sélective et une valeur de trait. Cette méthode permet d'explorer le type (directionnelle, stabilisant ou divergente), la force et la direction de la sélection naturelle agissant directement sur chaque trait, en s'affranchissant d'une potentielle sélection indirecte liée à la corrélation entre traits. Dans un second temps, je me suis penchée sur la sélection agissant sur les traits comportementaux et physiologique en ajoutant cette fois, en plus des analyses de sélection via le succès reproducteur, des analyses utilisant la survie annuelle comme proxy nécessitant des modèles de Capture-Marquage-Recapture (**Manuscrit 3**).

Les résultats montrent peu de différences entre patrons de sélection observés en milieu urbain et forestier et la sélection naturelle ne semble pas expliquer la divergence phénotypique observée dans les populations urbaines (**Figure 1**). A l'inverse on constate que certaines particularités phénotypiques urbaines semblent aller à l'encontre de la sélection agissant dans l'habitat. En effet, les individus urbains font des pontes de petite taille alors les tailles de pontes réduites sont contre-sélectionnées en ville. De même, les femelles urbaines ont un rythme respiratoire plus rapide, traduisant un stress plus élevé lors de la contention, bien que cela confère un désavantage en succès reproducteur.

Par conséquent, les divergences de traits associées à l'urbanisation ne sont visiblement pas liées à de l'adaptation et suggèrent même parfois des cas de mal-adaptation (lorsque le déplacement de caractère va à l'encontre de la sélection). Ces résultats apportent un premier élément dans la compréhension des mécanismes évolutifs façonnant les populations urbaines. Ils suggèrent que les divergences phénotypiques observées résultent probablement de contraintes associées à la vie en milieu urbain. En effet, concernant les traits morphologiques, une diminution de la quantité et/ou de la qualité des ressources alimentaires est susceptible de réduire la croissance des individus et donc de limiter leur taille globale (Seress et al. 2020). De même, cette contrainte alimentaire peut limiter l'investissement dans la reproduction et expliquer les tailles de pontes réduites (Wawrzyniak et al. 2015). Par ailleurs, les nouvelles conditions environnementales sont susceptibles d'entrainer des modifications de traits sans que cela soit adaptatif, par exemple les températures plus élevées et la photopériode perturbée par l'éclairage artificiel peuvent induire une avancée non adaptative de la date de ponte (Dhondt & Eyckerman 1979). Enfin, les conditions stressantes peuvent agir comme un filtre dans l'établissement des individus en milieu urbain et ne laisser passer que les plus téméraires (Holtmann et al. 2017) ou induire changements comportementaux liés aux taux de stress physiologique, expliquant les

différences comportementales observées (Gormally et al. 2018).

Parmi les limites de cette approche, notons que ce type d'analyses mesure la sélection présente, et nous ne nous intéressons ici qu'à un nombre limité de traits. Pourtant, la multitude de nouvelles conditions environnementales que représente l'habitat urbain est susceptible d'impacter un myriade d'autres traits non mesurés ici. De plus, les phénomènes d'adaptation passée ont potentiellement joué un rôle important dans l'établissement/ le maintien initial des populations sauvages dans le milieu urbain et sélection passée et présente sont peut-être fort différentes. Les analyses génomiques offrent l'opportunité de chercher des traces de sélection passée dans les génomes (voir **Chapitre IV**).

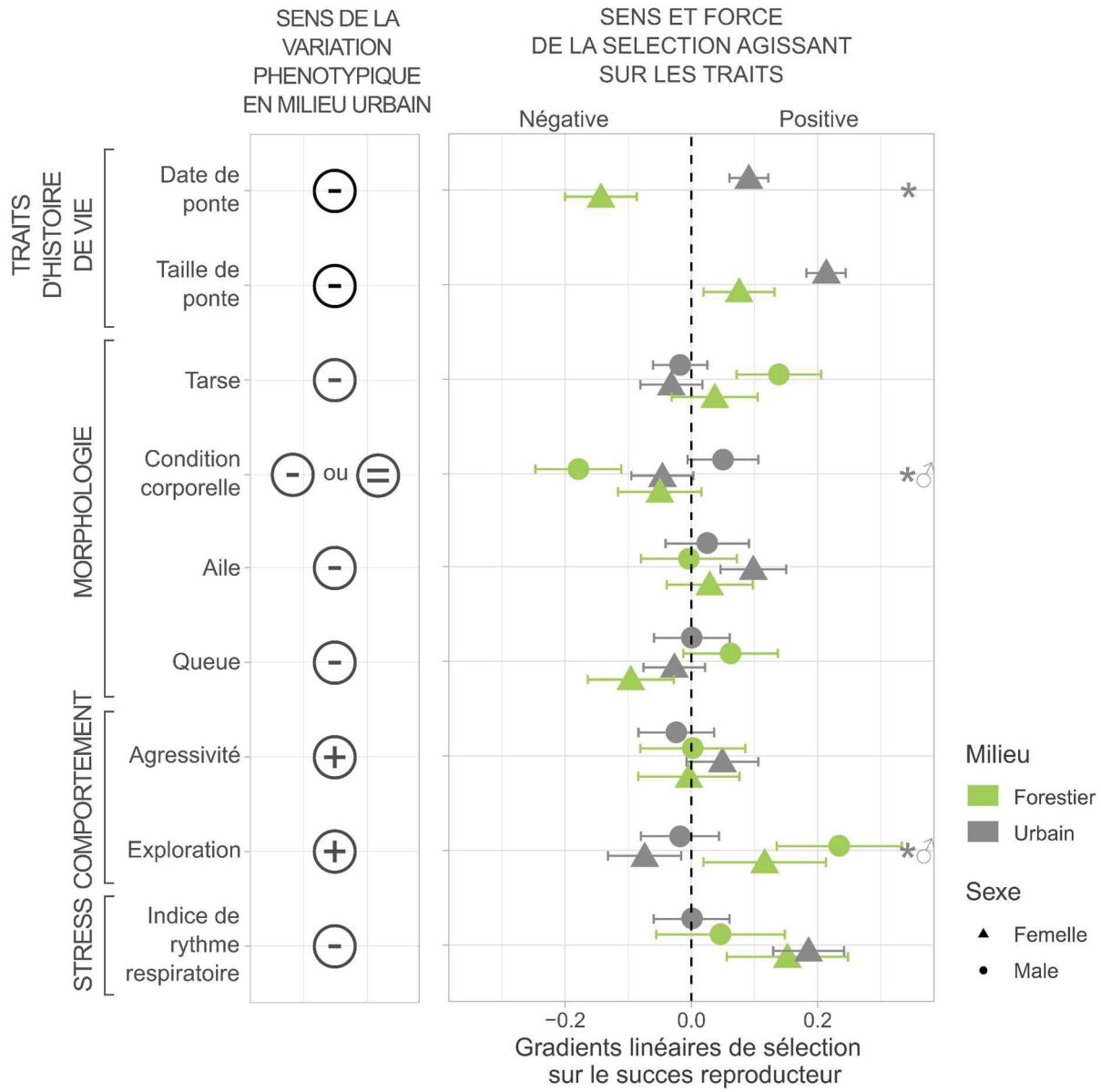

Figure 1 | Figure résumant (à gauche) le sens de variation de la différence phénotypique entre populations urbaine et rurale sur les traits étudiés ainsi que (à droite) le sens et la force de la sélection de reproduction agissant sur ces traits dans chaque habitat. A droite, les points représentent les gradients linéaires de sélection chez les femelles (triangle) et les males (ronds), et les barres d'erreur, l'erreur standard associée. Les étoiles indiquent la significativité de la différence de sélection entre habitat urbain et rural pour un trait donné.

Manuscrits associés au chapitre:

- **Manuscrit 2 :** Caizergues, A. E., Grégoire, A., & Charmantier, A. (2018). Urban versus forest ecotypes are not explained by divergent reproductive selection. *Proceedings of the Royal Society B: Biological Sciences*, 285(1882), 20180261. https://doi.org/10.1098/rspb.2018.0261
- **Manuscrit 3 :** Caizergues, A. E., Grégoire, A., Choquet R., Perret S. & Charmantier, A. Are behavioural and stress responses to urbanization adaptive? Great tits as a case study. (*in prep for Journal of Animal Ecology*)

– MANUSCRIT 2 –

Urban versus forest ecotypes are not explained by divergent reproductive selection

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– ABSTRACT – Increasing urbanization offers a unique opportunity to study adaptive responses to rapid environmental change. Numerous studies have demonstrated phenotypic divergence between urban and rural organisms. However, comparing the direction and magnitude of natural selection between these environments has rarely been attempted. Using seven years of monitoring of great tits (*Parus major*) breeding in nest-boxes across the city of Montpellier and in a nearby oak forest, we find phenotypic divergence in four morphological and two life-history traits between urban and forest birds. We then measure reproductive selection on these traits, and compare selection between the habitats. Urban birds had significantly smaller morphological features than their rural counterparts, with a shorter tarsus, lower body mass, and smaller wing and tail lengths relative to their overall body size. While urban female tarsus length was under stabilizing selection, and forest males show positive selection for tarsus length and negative selection for body mass, selection gradients were significantly divergent between habitats only for body mass. Urban great tits also had earlier laying dates and smaller clutches. Surprisingly, we found selection for earlier laying date in the forest but not in the city. Conversely, we detected no linear selection on clutch size in the forest, but positive selection on clutch size in the urban habitat. Overall, these results do not support the hypothesis that contemporary reproductive selection explains differences in morphology and life history between urban- and forest-breeding great tits. We discuss how further experimental approaches will help confirm whether the observed divergence is maladaptive while identifying the environmental drivers behind it.

– KEYWORDS – urbanization | reproductive selection | adaptation | morphology | life-history traits

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INTRODUCTION

Human activities drive tremendous environmental changes in a wide range of ecosystems around our planet. Wildlife responses to these changes provide interesting models for evolutionary biologists, as phenotypic divergence between populations is usually much more pronounced when one of the paired populations is found in a human modified habitat [1]. Among emerging human altered habitats, urban areas provide some of the most striking environmental changes, both in terms of the level of artificiality as well as in terms of the temporal scale of landscape changes. Cities offer a range of new environmental conditions, such as high levels of pollution, increased noise levels, artificial light, modified plant communities, new patterns of access to resources, and higher temperatures [2]. Research in urban ecology during the past decade has revealed many cases of phenotypic divergence between urban and native habitats. This is especially true for birds, which have been studied for over a century [3], and for whom studies have shown divergence in e.g. morphology, physiology, behaviour, song [4-6] and life history [7], highlighting the existence of urban versus rural ecotypes (i.e. populations presenting phenotypical divergence associated with particular environmental conditions).

Although some studies have found consistent patterns across species for a few traits, such as reduced body size in urban birds [8], no general pattern of morphological divergence arises for urban populations. For instance, several studies find birds have lower body condition in the city [5, 7], and suggest that these differences might be adaptive due to higher temperatures (Bergmann's rule applied to urban habitats) and the non-necessity of fat reserves because of high resource predictability [5, 9]. However, lower body mass in the city could also be maladaptive, and result from a lack of food resources (e.g. low insect abundance) [10]. Furthermore, since some studies find no difference in body mass between urban and rural birds, and others find a difference in the opposite direction [7], morphological differentiation seems to be species- and site-dependent [7, 11].

The life history ecotype of urban birds seems more generalizable than the urban morphotype (i.e. set of morphological characteristics specific to urban populations). Indeed, in the majority of species investigated, urban birds display smaller clutch sizes and advanced lay dates [reviewed in 12]. In particular, urban populations of great tits (*Parus major*) and blue tits (*Cyanistes caeruleus*) consistently have earlier and smaller clutches than those in natural habitats [13, 14]. In seasonal environments, synchronisation between a bird's phenology and the optimal timing of reproduction (e.g. peak in abundance of arthropods for insectivorous species) is controlled by environmental cues such as photoperiod, temperature, light and bud burst [15]. A mismatch between realised and optimal phenology can reduce individual fitness and affect population dynamics [16]. Modified environmental conditions in the city, in particular higher temperatures and novel plant communities, could lead to (1) incorrect information on the date of the optimal timing of reproduction and (2) modified access to resources. Hence, as with changes in morphology, changes in urban bird phenology could be maladaptive.

Surprisingly, the ecological and evolutionary processes leading to the phenotypic changes during the urbanisation process remain largely unknown [17]. In particular, whether the new phenotypes observed in cities are adaptive and whether they are the result of ongoing micro-evolutionary processes are two of the most pressing questions in urban evolutionary ecology. The assumption that rapidly evolving urban areas offer novel selection pressures is prevalent in the literature [1, 18], giving rise to a second common assumption that urban ecotypes are adaptive. In several cases, genetic studies have demonstrated that the observed phenotypic differences between urban and natural populations are genetically based [e.g. 19, 20] and have, in some cases, arisen recently despite gene flow [e.g. 21]. However, at present only a handful of studies have explicitly compared the direction and force of natural and/or sexual selection acting in urban versus natural habitats by linking phenotypes to fitness [18]. The industrial melanism in the peppered moth (*Biston betularia*) is the most famous example, demonstrating how increasing (and subsequently decreasing) air pollution induced novel selection (and subsequently reverse selection) that resulted in rapid evolution of phenotypes and genotypes associated with camouflage [22]. More recently, a study on the Northern cardinal (*Cardinalis cardinalis*) revealed

relaxed sexual selection on coloration for urban males compared to their forest conspecifics because of a disassociation between brightness of male plumage and territory attributes in urbanised areas [23].

The present study addresses the fundamental question: does selection differ in direction and magnitude between a forest and an urban avian population? Our work is divided in two parts. First, we analyse phenotypic divergence between forest and urban great tits for several morphological and life history traits, using a long-term study in and near the city of Montpellier, France. Second, we perform selection analyses on these traits and compare patterns of reproductive selection between forest and urban habitats. Based on previous studies on other populations of the same species, we predict that urban great tits will present smaller morphological characteristics than their forest counterparts. We also predict an earlier laying date in the city due to higher temperature perceived as an indicator of earlier spring and smaller clutch size linked to fewer food resources. We then test whether the divergences found in morphology and life history are aligned with differences in the direction and/or force of reproductive selection.

MATERIAL AND METHODS

Study areas, monitoring and phenotyping

Two populations of great tits (*Parus major*) were monitored in the south of France: one in a forested area and one in an urban area. The forested site, in the forest of La Rouvière, is situated 20km North-West of the city of Montpellier. At this site, between 51 and 92 great tit nest-boxes have been monitored since 1991 [24]. The urban site is within the city of Montpellier, and contains 203-223 nest-boxes that have been monitored since 2011, providing data on great tits across various levels of urbanization [13, 25]. The forest and urban sites presented largely dissimilar environmental conditions. In the city, nest-boxes were not only placed in parks, but also on trees near pavements, roads and street lamps, thus exposed to human disturbance, air and light pollution. Moreover, the vertical matrix is highly modified with numerous buildings and reduced vegetation cover composed of many ornamental flowers and trees such as platanus (*Platanus* × *acerifolia*), olive trees (*Olivacea europea*) or resinous trees. In contrast, the forest site was dominated by downy oaks (*Quercus pubescens*) and holm oaks (*Quercus ilex*), with limited human disturbance and pollution levels. Hence the rural site corresponds to a mature stage of a Mediterranean forest succession while the urban site corresponds to a Human dominated place with a higher proportion of artificial cover. On average, nest-box environment (estimated based on satellite image analysis within a 100m diameter circle around each nest-box) was covered at 61.9% by vegetation in the city versus 98.9% in the forest, the remaining percentage being impervious surfaces. Satellite pictures of both sites and details on estimation in green cover are available in Electronic Supplementary Materials (ESM, **Figure S1** & **Appendix 1**).

Nest-boxes were visited at least once a week during the breeding season to follow brood development. Parents were captured inside nest-boxes when their nestlings were between 9 and 15 days old and were individually measured for several morphological (i.e. tarsus length, body mass, wing and tail lengths) and behavioural traits, and ringed with unique metal rings. For more details about the monitoring protocol see ESM Appendix S1. At the urban site, although brood development traits have been monitored since 2011, parents have only been captured since 2013. In the analysis, only years for which both study sites were sampled are considered. Between 2011 and 2017, a total 192 forest broods and 546 urban ones were monitored for which 153 forest and 391 urban birds were captured (from once to four times between 2013 and 2017).

Statistical analyses

All data analyses were performed using the software R (version 3.4.0).

Urban versus forest phenotypic divergence

Differences between the two habitats in great tit morphology were explored using linear mixed models (packages lme4 and lmerTest) on four traits: tarsus length, body mass, wing length and tail length. Full models included year (as a categorical variable), habitat (urban vs. forest), sex (male vs. female) and age (yearling vs. adult) as fixed effects, with the following interactions: habitat×age, habitat×sex, habitat×year, sex×age, sex×year. Individual ring

number and measurer identity were fitted in all models as random effects. Since morphological traits were highly correlated (see all between-trait correlations in supplementary materials, **Table S1**), measures of body mass, wing length and tail length were scaled for the general bird size by including tarsus length as a covariate, thereby modelling body condition and relative wing and tail lengths. Avian tarsus length is a common index of structural size of the whole bird [e.g. 26]. Since great tit body mass is known to vary consistently during the day in a linear manner [e.g. 27], the hour of measure was added as a continuous explanatory variable in body mass models.

To test for differences in laying date and clutch size (hereafter referred to as LD and CS, respectively) between city and forest, we considered only first broods since second and replacement broods were rare and would require a separate analysis. As above, linear mixed models were run with habitat, year and habitat×year as fixed effects. LD was added in the CS model to account for the fact that late females usually lay smaller clutches [13, 28]. Female identity was included as a random effect to account for the non-independence of broods from the same females. Because the monitoring did not include parental captures during the first two years and because all females were not captured (when abandoning before chicks reached 9 days old, death or failure to capture), female identity was unknown for 54% of broods (342 out of 739). To avoid loss of half our dataset, we attributed a fictitious ring number to each non-captured female.

For each trait, the best model was selected using a backward stepwise procedure starting from a full model, and the significance of each fixed effect and interaction was tested using a *F*-test (R function anova()). This procedure was used to eliminate non-significant variables: at each step, the effect presenting the highest p-value was removed and the model run again without it. While we present only ANOVA of the best-fitting models below, summaries of full and best-fitting models are provided for each trait in the ESM (**Tables S2-S5**).

Measuring and comparing the force of selection

We estimated reproductive selection for the focal morphological and life history traits, and subsequently tested whether the direction and force of selection varied between the urban and forest habitats. Separate analyses were run for each sex because of sexual dimorphism (males are larger, e.g. [29] and **Table 1**).

The choice of an optimal estimation of relative fitness to adequately measure natural and sexual selection is a long standing debate [30] with no straightforward solution. Since our goal was to understand the adaptive mechanisms explaining urban/forest divergence in a set of traits with little access to complete life histories, we opted for an annual measure of fitness rather than lifetime reproductive success [31]. Also, because of the short time scale of our dataset and following arguments that natural selection is a within-generation process [32], we chose the number of fledglings as a fitness measure rather than number of recruits.

We first estimated standardised univariate selection differentials (S) on all traits individually [33, 34] in each habitat separately. The linear selection differential for a trait is estimated by the slope of the regression between a measure of relative fitness and standardised values of the phenotypic trait. Selection differentials measure the total force of selection affecting a trait, including both direct and indirect selection [32]. Since all four morphological traits were correlated with body size (**Table S1**), estimations of selection differentials were performed on measures corrected for size by using residuals of a linear regression between the trait (body mass, wing or tail length) and tarsus length run on the pooled data set with both urban and forest birds. For body mass, the time at which each measurement was taken was added as an additional explanatory variable in the linear regression, thereby providing a measure of body condition corrected for time of day.

In order to identify the target of selection when multiple, potentially correlated, traits are simultaneously studied, multivariate selection gradients β [32] estimate direct selection on each trait after removing indirect selection from other traits. These multivariate models were run for normally standardized morphological and life history traits separately. For example, linear selection gradients βi on morphological traits were estimated by the model

$$\omega = \alpha + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \varepsilon \quad (1)$$

Where ω = relative fitness, α =intercept, x_1 = tarsus length, x_2 = body mass, x_3 = wing length, x_4 = tail length and ε = error. We also estimated nonlinear quadratic (γi) and correlational (γij) selection gradients from the following model [35]:

$$\omega = \alpha+\beta_1x_1+\beta_2x_2+\beta_3x_3+\beta_4x_4+(\gamma_1/2)x_1^2+(\gamma_2/2)x_2^2 +(\gamma_3/2)x_3^2+(\gamma_4/2)x_4^2+\gamma_{12}x_1x_2+\gamma_{13}x_1x_3 +\gamma_{14}x_1x_4+\gamma_{23}x_2x_3+\gamma_{24}x_2x_4+\gamma_{34}x_3x_4+\varepsilon \quad (2)$$

When estimating selection gradients, correction for tarsus length was not needed because tarsus length was already in the model (equations (1) and (2)). Hence, only body mass was corrected for hour of the day using residuals from a linear regression between body mass and hour of measure. Selection gradients on life history traits in each habitat were estimated in the same way, with LD and CS as traits 1 and 2 in (1) and (2).

Estimation of parameters and tests of significance were conducted in two steps [e.g. 36]. First, estimates of selection differentials and gradients were obtained from linear models as described by equations (1) and (2). Second, significance (i.e. P values) of these selection differentials and gradients were estimated from linear mixed models including year and individual ring number (only female identity for life history traits) as random effects to control for variation across years and repeated measurements on the same individuals. Then, to test for a difference in selection operating in the two habitats, urban and forest datasets were combined and habitat (forest vs. urban) and habitat×trait were added as fixed effects in the models described above. Significance of the interaction terms was tested using *F*-tests (R function *anova()*).

Finally, because variance can provide insight on past selection as well as relaxed selection, a comparison of variance was made between habitats for each trait with a Fisher variance ratio test (*var.test()*, R package stats).

RESULTS

Urban versus forest differences in morphology and life history

Comparison of morphological features between urban and forest great tits revealed a strong phenotypic differentiation (**Figure 1**). Indeed, for all

Table 1 | Comparing the morphological traits tarsus length (mm), relative body mass (g), relative wing length (mm) and relative tail length (mm) between forest and urban great tits using linear mixed models. Habitat-specific means (raw data) and output of *F*-tests for significant fixed effects (in bold). N_{tarsus} = 554, N_{mass} = 550, N_{wing}= 553 and N_{tail} = 537.

	Tarsus length				**Body mass**				**Wing length**				**Tail length**			
Mean ± SD	**Forest**		**City**		**Forest**		**City**		**Forest**		**City**		**Forest**		**City**	
Females	19.54 ± 0.51		19.23 ± 0.53		16.61 ± 0.88		16.20 ± 0.89		73.39 ± 1.80		72.58 ± 1.70		61.24 ± 2.55		60.87 ± 2.43	
Males	20.01 ± 0.51		19.76 ± 0.57		17.25 ± 0.94		16.98 ± 0.97		76.43 ± 2.09		75.59 ± 2.29		65.35 ± 2.84		64.37 ± 2.93	
Fixed effects	**Sum sq.**	**d.f.**	**F**	**P**	**Sum sq.**	**d.f.**	**F**	**P**	**Sum sq.**	**d.f.**	**F**	**P**	**Sum sq.**	**d.f.**	**F**	**P**
Habitat	0,54	1	22,35	**$3x10^{-6}$**	2,88	1	9,57	**0,002**	46,86	1	17,52	**$7x10^{-5}$**	39,44	1	12,25	**0,001**
Year	ns	ns	ns	ns	24,22	4	20,15	**$4x10^{-15}$**	46,69	4	4,37	**0,002**	65,78	4	5,11	**0,001**
Sex	2,30	1	94,66	**$<2x10^{-16}$**	6,30	1	20,96	**$6x10^{-6}$**	455,90	1	170,47	**$<2x10^{-16}$**	510,34	1	158,48	**$<2x10^{-16}$**
Age	ns	ns	ns	ns	6,02	1	20,04	**$9x10^{-6}$**	251,82	1	94,16	**$<2x10^{-16}$**	236,93	1	73,58	**$<2x10^{-16}$**
Tarsus length	-	-	-	-	32,33	1	107,56	**$<2x10^{-16}$**	104,73	1	39,16	**$1x10^{-9}$**	14,10	1	4,38	**0,037**
Habitat × year	ns	ns	ns	ns	2,93	4	2,43	**0,047**	30,96	4	2,89	**0,023**	ns	ns	ns	ns
Sex × age	ns	ns	ns	ns	ns	ns	ns	ns	16,90	1	6,32	**0,012**	ns	ns	ns	ns
Hour	-	-	-	-	2,64	1	8,77	**0,003**	-	-	-	-	-	-	-	-

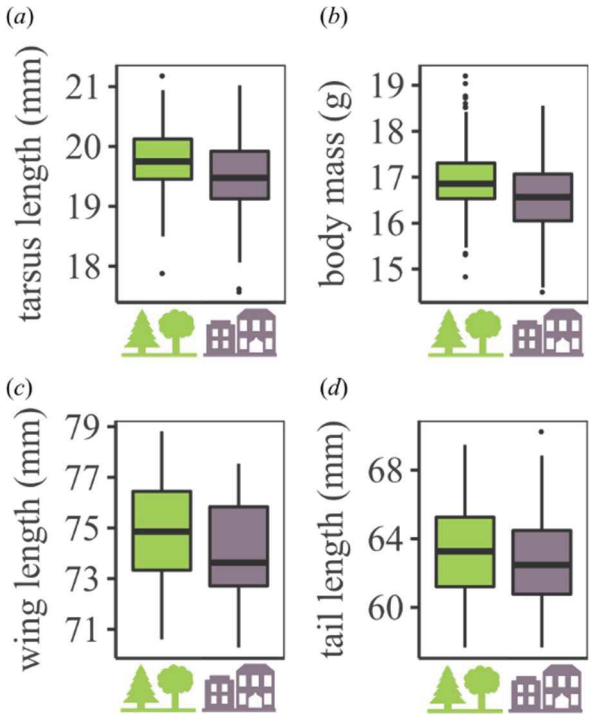

Figure 1 | Morphological divergence between forest (green) and urban (purple) great tits for (a) tarsus length, (b) body mass, (c) wing length and (d) tail length. Boxplots of predicted data from the best linear mixed models, representing minimum, first quartile, median, third quartile, maximum and outliers (points).

four traits, habitat (urban vs. forest) had a significant effect either alone or in an interaction (**Table 1**). First, urban birds were smaller than their forest counterparts, as shown by a tarsus length 1.3 to 1.6% smaller (males and females) in urban birds (P<0.001, **Table 1 & S3**). Second, urban birds had lower body mass corrected for tarsus length (interaction=0.002, **Table 1 & S3**). Third, urban birds displayed shorter wings and tails (P<0.001 & P=0.001, **Table 1 & S3**). In both populations, there was a significant sexual dimorphism, with males larger than females (**Table 1**). We also noted an effect of year, either directly or via an interaction, for all morphological traits but tarsus length (see **Figures S2-S4**). For instance, differences in wing length between the habitats varied between years (significant habitat×year interaction, P=0.023, **Table 1 & S3**). Moreover, yearlings displayed smaller features than adults (except for tarsus length which is fully grown at 15 days of age) (**Table S3**).

Concerning life history traits, urban females laid eggs on average 4.05 days before forest females (P=0.032, **Tables 2, S5 & Figure 2**) and their earliness showed annual fluctuations (significant habitat×year interaction, P=0.02 **Table 2 & S5**). Mean LD varied annually with a magnitude of 9 days across the 7 years of monitoring. Urban females laid 1.75 less eggs on average than their forest counterparts (P<0.001, **Table 2, S5 & Figure 2**). As expected, we observed a negative correlation between LD and CS when accounting for habitat and year as fixed effects and female identity as random effect (P=0.03, **Table 2 & Figure 2**).

Selection on morphology

Only selection gradients are presented in the main results (see **Table S6 & Figure S5** for selection differentials). Regarding females, no linear selection was detected on morphological traits, but we found stabilising selection on tarsus length in the city (γ=-0.166, P=0.036, **Table S7**). However, pooling the urban and forest datasets revealed that this quadratic selection was not significantly different between habitats (non-significant tarsus²×habitat interaction, P=0.632, **Table S7**). Concerning males, we detected linear selection for larger tarsi in the forest (P=0.01, **Table 3**), but once again the difference in selection between habitats was non-significant (non-significant tarsus×habitat interaction, P=0.275, **Table 3**). Moreover, we detected significant negative

Table 2 | Comparing laying date and clutch size between forest and urban great tits using linear mixed models. Habitat-specific means (raw data) and output of F-tests for significant fixed effects (in bold). N_{urban} = 546 and N_{forest} = 192.

	Laying date				Clutch size			
Mean ± SD	**Forest**		**City**		**Forest**		**City**	
Females	100.01 ± 7.24 *		97.2 ± 8.35 *		9.16 ± 1.75		7.46 ± 1.51	
Fixed effects	**Sum sq.**	**d.f.**	**F**	**P**	**Sum sq.**	**d.f.**	**F**	**P**
Habitat	1033,4	1	36,18	**$3x10^{-9}$**	261,35	1	157,63	**$<2x10^{-16}$**
Year	7521,5	6	43,88	**$<2x10^{-16}$**	34,68	6	3,49	**$2x10^{-3}$**
Habitat × year	444,9	6	2,60	**0,02**	5,97	6	0,60	0,73
Laying date	-	-	-	-	7,70	1	4,65	**0,03**

* Laying date is expressed in ordinal day, where 1 = January the 1st.

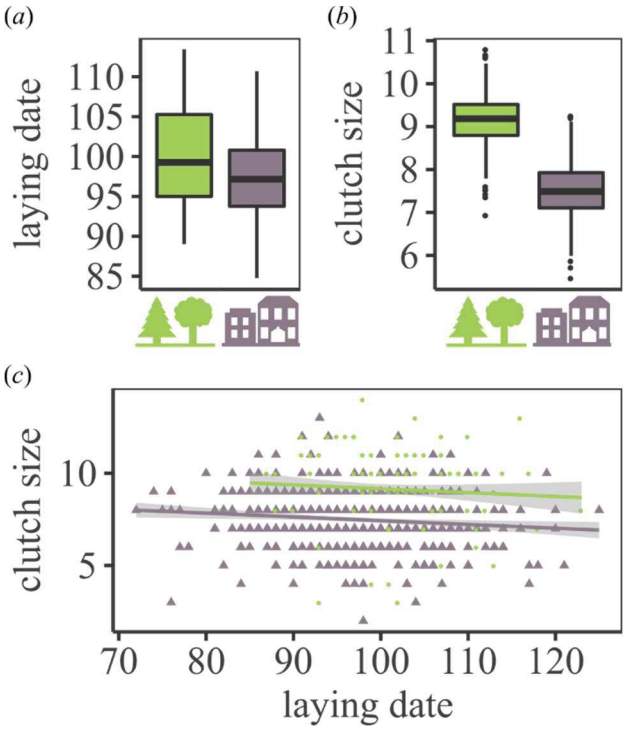

Figure 2 | Divergence in (a) laying date and (b) clutch size between forest (green) and urban (purple) great tits. Boxplots of predicted data from the best linear mixed models, representing minimum, first quartile, median, third quartile, maximum and outliers (points). (c) Correlations between laying date and clutch size in both habitats (raw data) with 95% confidence intervals in grey.

zlinear selection on body mass for forest males (**Table 3**), and a significant difference in selection between city and forest (significant interaction body mass×habitat, P=0.034, **Table 3**). This result suggests that lighter birds have higher reproductive success in the oak forest but not in the urban habitat. Finally, no difference in variance was found in any morphological trait (**Table S8**).

Selection on life-history traits

As above, the selection differentials and gradients show similar patterns, and we therefore present only the results for selection gradients (but see **Table S9 & S10** for selection differentials and quadratic selection gradients on LD and CS). There was negative linear selection favouring earlier breeding in the forest (P=0.031, **Table 4**). In contrast, LD was under no selection in the city (**Table 4 & Figure S5**). In the model combining both habitats, the LD×habitat interaction was significant (P<0.001, **Table 4**), with a difference of 0.31 between the two standardized gradients. A comparison of variances showed higher

Table 3 | Standardized linear selection gradients, estimated from multivariate analyses on tarsus length (β_{tarsus}), body mass ($\beta_{body\ mass}$), wing length (β_{wing}) and tail length (β_{tail}). Values are provided with their standard error. Bold estimates are significant ($p < 0.05$). 'Interaction' displays the value of the interaction between each trait and habitat in the merged (forest + city) model.

			Linear selection gradients											
		Sample	β_{tarsus}				$\beta_{body\ mass}$				β_{wing}			
Sex	**Habitat**	**size**	est.	±	SE	Interaction	est.	±	SE	Interaction	est.	±	SE	Interaction
Females	Forest	104	0,037	±	0,068	-0,037	-0,050	±	0,066	-0,025	0,029	±	0,068	0,025
	City	200	-0,032	±	0,049		-0,046	±	0,049		0,098	±	0,052	
Males	Forest	94	**0,138**	±	**0,067**	-0,085	**-0,179**	±	**0,068**	**0,167**	-0,004	±	0,076	0,057
	City	191	-0,018	±	0,043		0,050	±	0,056		0,025	±	0,066	

			β_{tail}			
Sex	**Habitat**	**size**	est.	±	SE	Interaction
Females	Forest	104	-0,096	±	0,068	0,062
	City	200	-0,027	±	0,049	
Males	Forest	94	0,062	±	0,075	0,016
	City	191	0,0004	±	0,060	

Table 4 | Standardized linear selection gradients estimated for laying date (β_{LD}) and clutch size (β_{CS}). Values are provided with their standard error. Bold estimates are significant ($P < 0.05$). 'Interaction' displays the value of the interaction between each trait and habitat in the merged (forest + city) model.

	Sample size	Linear selection gradients							
		β_{LD}				β_{CS}			
Habitat		est.	±	SE	Interaction	est.	±	SE	Interaction
Forest	185	**-0,143**	**±**	**0,057**	**0,308**	0,076	±	0,056	0,116
City	524	0,091	±	0,031		**0,214**	**±**	**0,031**	

variance in LD at the urban site (**Table S8**) suggesting relaxed selection on LD in the city.

The force of selection on CS also differs between the two habitats (**Figure S5**): great tits showed significant positive linear selection for CS in the city ($P<0.001$, **Table 4**) while not in the forest (**Table 4**). The pooled model revealed that this difference in selection affecting urban and forest CS was marginally significant (CS×habitat interaction, $P=0.054$, **Table 4**). Finally, variance in CS was higher in the forest than the city (**Table S8**).

DISCUSSION

In this study, we investigated whether an urban ecotype existed in great tits breeding in the city of Montpellier, and whether this ecotype could be explained by divergent selection between urban and forest habitats. We highlighted the existence of an urban morphotype in great tits, whereby urban birds showed smaller body size, reduced body mass, and smaller wing and tail lengths, compared to their forest counterparts. Moreover, urban great tits displayed earlier breeding phenology and smaller clutches. Selection analyses showed no differences in selection on morphological features between habitats, except for body mass in males, whereby lighter males had higher reproductive success in the forest but not in the city. On the contrary, life history traits displayed strong selection gradients, with an interesting contrasted selection between city and forest. Patterns of selection on laying date (LD) varied between habitats in both direction and strength: while early females were favoured in the forest, there was non-significant positive selection on LD in the city. Finally, selection on clutch size (CS) differed in strength between the city and the forest, with significant selection favouring large clutches in the city but non-significant selection in the forest. We discuss below how this direct comparison of the force of selection operating in urban and forest habitats improves our understanding of evolution in cities.

Is morphological divergence adaptive?

Our morphotype analysis is, in part, consistent with previous studies on urban birds. Most passerine studies on nestlings or reproductive individuals have reported that urban birds are smaller than their counterparts from native environments [4, 5, 37]. On the contrary, the finding that birds in the city had a lower body mass than those in the forest is consistent with some studies [e.g. 5] but not all [4, 37]. Several city features have led to predictions that being smaller could be associated with a stronger fitness advantage in an urban habitat than in a forest habitat. First, cities are 'urban heat islands' [2]. Hence, following Bergmann's rule [38], warmer city environments should translate into a smaller adaptive body size. Second, the 'credit card hypothesis' [39] postulates that higher food resource predictability in cities should allow urban birds to live without accumulating fat reserves. However, our selection analysis did not support the prediction of stronger negative directional selection in the city. If anything, the reverse could be true, since the only significant difference in selection gradient for morphological traits revealed that leaner males had higher reproductive success in the forest, but not in the city (**Table 3**). However, such a negative selection gradient on body mass is not unusual [40] and should be interpreted with care. Since body mass was measured for parents during the nestling feeding period, it is highly likely that the association between the number of fledglings and paternal body mass results from active males with large broods losing more weight during the reproductive period. It remains to be explained why urban fathers would not lose weight compared to forest fathers during the reproductive season. Overall, these results suggest

that the morphological differences between urban and forest birds are not an adaptive response to divergent selection operating presently. Note that the absence of significant selection on male and female morphology in urban birds could also be indicative of a fast evolution towards the urban morphotype following strong past selection. The hypothesis of an evolutionary stable optimum reached in the city environment is however not supported by a) a variance comparison (**Table S8**) revealing no difference between habitats for all morphological trait variances, and b) the documented population genetic structure. Indeed, a recent genomic study showed low genetic differentiation between the two focal populations (F_{ST} close to 0.01) [41], suggesting strong gene flow, and hence small potential for local adaptation [42].

Our finding of smaller relative wing and tail length in urban great tits is comparable with only one published study, on the European blackbird (*Turdus merula)*, where a comparative approach across 11 paired urban and rural sites [11] found inconsistent results across sites, but generally longer wings in urban birds. Wing and tail morphologies are directly linked to flight aerodynamics, for example shorter wings and longer tails increase maneuvrability [43]. The urban habitat being substantially different in its vertical matrix (e.g. buildings) compared to a forest, and having an increased collision risk (e.g. from numerous vehicles), it is easy to imagine that modified plumage characteristics could be advantageous. However, our selection analyses for relative wing and tail length show no evidence for reproductive selection on either trait in either habitat. Overall, while the weak selection on morphology is consistent with previous studies in passerines [40, 44], our analyses provide no support for an adaptive explanation for the morphological divergence between city and forest great tits. Thereby, morphological plasticity remains the most likely process explaining the observed phenotypic differentiation, and could be a maladaptive product of unfavourable environmental conditions [5]. This hypothesis remains to be tested using common garden or cross-fostering protocols. Such experimental approaches would help identify which environmental features (such as food abundance and predictability, predation pressure, pollution) are responsible for the divergent morphotypes. Also, while our selection analysis focuses on the breeding period, we are well aware that selection can vary among life stages thus calling for studies that consider cumulative effects of selection over the lifecycle. A complementary analysis of selection via survival is necessary to reach conclusions regarding the overall selection pressures affecting the focal traits in the two habitats. Developing a unique fitness measure that combines survival and reproduction could provide a different picture of the adaptive nature of the morphological divergence observed here [45].

Maladaptive shift in urban life-history

The observed differences in life history traits, with an earlier LD and smaller CS in the city than in the forest, are consistent with previous findings [12]. The advanced LD could be explained by modified environmental conditions in the city. Indeed, passerines follow environmental cues to time their egg laying, particularly temperature [46]. For instance, small temperature differences inside nest-boxes influence LD [47]. Also, artificial light and differences in food resource availability could advance the phenology of urban birds [12]. Smaller CS in the city could be adaptive because of the restriction in food resources for nestlings [12] or because of higher brood predation risk [48]. Once again, these arguments have, to our knowledge, not been associated with attempts to compare selection in urban and rural environments. Here, we provide the first demonstration of a difference in reproductive selection between the two habitats. While there is reproductive selection for early LD in the forest as is often observed in rural great tit populations [49], no selection is detected in the city. This difference in selection could be explained a) by traditional environmental cues for optimal LD timing becoming unreliable in the urban habitat, and/or b) by relaxed selection due to anthropogenic disturbances changing selective regimes [50]. In particular, females usually match their phenology to a peak in caterpillar abundance using environmental cues, yet there might be no such peak in the city [51], hence selection for LD might be relaxed, as suggested by higher variance in urban LD. Exploring the role of food abundance and timing would require fine-scale entomological data in our urban sites to evaluate the composition and abundance in urban arthropods and the link

between arthropod phenology and environmental cues. It has been demonstrated that more favourable pre-laying feeding conditions, induced by anthropogenic food supplementation during winter, often advance avian LD [12]. Since food supplementation has no link with natural food availability and cannot contribute to nestling diet, such human-induced early LD might not be advantageous [12]. Likewise, since we found selection favouring large clutches in urban birds, the observed smaller and less variable CS likely results from a constraint emerging from poor food resources in the urban habitat [52, 53].

Limitations and perspectives

Our analyses provide a first step towards understanding the way that natural selection shapes avian urban wildlife, but we are well aware that complementary analyses will be necessary before reaching any final conclusion about the selection patterns in the two habitats. In particular, our study focuses on a single pair of populations, and therefore we must be cautious about drawing general inferences [54]. The good news is that the abundance of cities sets an ideal opportunity to explore selection gradients in rural/urban pairs of populations in a replicated fashion, and to identify which selection drivers differ between these two types of habitats. We particularly encourage urban evolutionary scientists working on great tits to reproduce our study in their population(s) to allow a comparative approach across the species' distribution. Targeting recent colonisation of urban environments would be optimal to estimate selection in great tit populations experiencing the city as a novel breeding habitat.

The high demands of gathering long-term data on individual phenotypes and fitness probably explain why, three decades after the seminal work of Lande & Arnold [32], very few analyses have compared the force and direction of natural selection between city and native habitats. New genomic tools have offered the prospect of studying the signatures of urban adaptation without the necessity to conduct long-term studies [18], with the added advantage of exploring candidate genes for specific traits involved in urban adaptation. For instance, behavioural differences observed between urban and rural blackbirds were related to divergence in the SERT gene, involved in exploratory behaviour [55]. However, in cases where urban organisms display new phenotypes as part of a plastic response, such genomic approaches will not be useful to understand whether this plasticity is adaptive, or not. Direct estimation of selection gradients and genomic investigations are therefore best seen as complementary and should ideally be paired.

Ethics

Captures were performed under personal ringing permits delivered by the CRBPO (Centre de Recherches pour le Baguage des Populations d'Oiseaux) to Anne Charmantier (ringing permit 1907) and Arnaud Grégoire (permit 1904). All experimental protocols described were approved by the ethics committee for animal experimentation of Languedoc Roussillon (305-CEEA-LR-12066 approved in 2012) as well as by Regional Institutions (bylaw issued by the Prefecture on 15/06/2012 n° 2012167-0003).

Data accessibility

Dryad doi: 10.5061/dryad.tv45802

Author's contributions

AEC carried out the statistical analyses and participated in data collection; AG & AC conceived and funded the study and collected the data. All authors contributed to writing the manuscript and gave final approval for publication.

Competing interests

We declare we have no competing interests.

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– MANUSCRIT 3 –

Are behavioural and stress responses to urbanization adaptive? Great tits as a case study

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– ABSTRACT –

1. Urbanization is a worldwide phenomenon converting natural habitats into completely new artificial environments. Environmental conditions associated with urbanization represent great challenges for wildlife. Behaviour and stress tolerance are considered of major importance in the adaptation to novel urban habitats and numerous studies already reported shifts in behavioural and stress response traits associated with urbanization, often suggesting they represented adaptations, while rarely demonstrating it.
2. The main goal of this study was to test the adaptive nature of urban shifts in behaviour and stress response, and by adaptive we mean shifts favouring traits in the same direction as selection.
3. Using seven years of monitoring of urban and forest great tits, we first quantified patterns of variation in exploratory behaviour, aggressiveness, breath rate and survival in both habitats. Second, we performed analyses of selection on the three traits via (1) reproductive success and (2) survival, to apprehend whether shifts in behavioural and stress response traits were aligned with patterns of ongoing selection acting in the two habitats.
4. We found that urban birds displayed higher exploratory behaviour and aggressiveness, faster breath rate, but similar survival, compared to forest birds. Selection analyses overall revealed that these shifts were not adaptive and could even be maladaptive. In particular, urban birds displayed faster breath rate although it was associated with lower reproductive success in females. Higher exploration scores were correlated with higher reproductive success in forest but not urban males, suggesting relaxed selection on this trait in the city.
5. Taken together these results highlight that phenotypic shifts observed in cities do not necessarily result from new selection pressures and could constitute maladaptations. We hypothesise that divergences in behavioural traits for urban birds could result from the filtering of individuals settling in cities, i.e. a selection step that we did not analyse here. We thus encourage urban evolutionary scientists to further explore the adaptive potential of behavioural traits measured in urban habitats i) by replicating this type of study in multiple cities, ii) by implementing studies focusing on immigrant phenotypes, and iii) by measuring selection at multiple life stages (from nestlings to adults).

– KEYWORDS – adaptation | behaviour | natural selection | stress response | urbanization

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INTRODUCTION

Human induced environmental change is a worldwide phenomenon going at an unprecedented rate. These changes include habitat degradation, climate change, increased presence of invasive species, pollution, and over-exploitation of resources, and affect biodiversity in a pervasive way (Pelletier and Coltman 2018). Most species living on our planet have faced environmental changes during their past evolutionary history, yet the present extent and rate of human-induced alterations represent unprecedented novel challenges. Although a wide range of traits can be concerned, the first response of animals towards rapid environmental changes often implies behavioural shifts on a multitude of facets in response to different stimuli (reviewed in Tuomainen and Candolin 2011). For instance, behavioural shifts can affect foraging strategies, mating success, or predator avoidance behaviours (Lowry et al. 2013), hence are closely linked to individual fitness. Consequently, behaviour is pervasively considered as a crucial factor determining how animals cope with environmental changes and new selective pressures (Baldwin 1896; Mayr 1963). In addition to behaviour, traits that are related to stress response may play an equally important role in wild animal tolerance and adaptation to human-induced environmental changes. Indeed, anthropogenic environmental change often results in highly altered new habitats with high levels of perturbation (Sih et al. 2011) and living in such habitats might thus require higher stress tolerance or else induce higher levels of chronic stress that can have deleterious effects on fitness. For instance, vertebrates can cope with environmental challenges relying on the hypothalamic-pituitary-adrenal (HPA) axis (Sapolsky et al. 2000). However, if some acute stress response occurring immediately after exposure to a stress stimuli (e.g. acute release of glucocorticoid) can permit to survive towards the stimuli (Landys et al. 2006; Wingfield 2006), stress responses induced by prolonged or repeated exposure to stressors can induce chronic stress that greatly impact fitness (French et al. 2007; Lendvai et al. 2007). Hence, balance of stress response might be particularly important in order to adapt to environmental perturbations.

Urbanization is one of the most extreme human induced environmental change for biodiversity (Newbold et al. 2015). Urbanized areas combine multiple perturbations on relatively limited land surfaces including deforestation, fragmentation, artificialization, pollutions and high level of human disturbance (Grimm et al. 2008). While some species are unable to persist in these deeply modified habitats, other manage to tolerate new conditions or even to thrive on the new resources. A key predictor of the successfulness of a species in urban habitats seems to be behavioural plasticity, and in particular the capacity to innovate when facing new opportunities (Lowry et al. 2013). Furthermore, at an intra-specific level, some behavioural profiles (also referred as personalities, coping styles or temperament, Réale et al. 2007) can be better suited to cope with novel environmental challenges than others. In particular, is has been suggested that bolder, more exploratory and more aggressive individuals are particularly well suited to the urban habitat (Sloan Wilson et al. 1994; Møller 2008). In line with these hypotheses, numerous studies have found that urban individuals indeed, displayed on average bolder (Prosser et al. 2006; Evans et al. 2010; Uchida et al. 2016), less careful (Chapman et al. 2012), more exploratory (Martin and Réale 2008), and more aggressive (Evans et al. 2010; Scales et al. 2011) behaviours than their forest counterparts. Regarding stress-related traits, patterns of responses are less consistent across species and traits (Sepp et al 2017). Indeed, some studies found higher (e.g. Torné-Noguera et al. 2014), lower (e.g. Abolins-Abols et al. 2016) or equal (e.g. Senar et al. 2017) levels of acute response to an induced stress in urban individuals, and this is also the case for chronic-stress (Powell et al. 2013; Sepp et al. 2017). However, theory suggest that similar stress responses between natural and urban habitats are expected in case of adaptation to urban habitat (Sepp et al. 2017).

Despite numerous evidence for divergence in behavioural traits and stress responses between urban and rural populations, little is known about the evolutionary implications of such urban-linked shifts. In particular, to our knowledge no study ever investigated if such urban linked shifts in behaviour or stress response emerged in response to habitat specific selective pressures, even if they are largely considered as adaptive in urban habitat (e.g. Lowry et al. 2013). If theoretically, behavioural particularities observed in urban habitats can result from adaptation to new environmental conditions, such as accepting new food resources or avoiding human disturbance, they, on the opposite can also be maladaptive. The majority of urban environmental conditions are relatively recent compared to

environmental conditions experienced during a species evolutionary history. As a result, phenotypic reaction norms of individuals might not be suited to fit to these environmental conditions that were never encountered during species evolutionary histories, and shifts might result in mal-adaptation (Tuomainen and Candolin 2011; Sih 2013). Maladaptive responses might be particularly common for stress related traits in urban dwellers. For instance, in waterbirds, humans perceived as predators trigger a higher stress response resulting in temporary or permanent abandon of the nest that can heavily impact individual fitness (Carney and Sydeman 1999). In the current context of ongoing massive erosion of biodiversity, it is now more than ever necessary to understand demographic and evolutionary dynamics associated with these phenotypic shifts.

Our study takes place in a context where differences in behaviour and stress response between urban and forest populations have been repeatedly reported for the focal species, but where evolutionary implications of these differences remain speculative. Our main objective is to understand to which extent urban shifts in behavioural and stress response traits are adaptive. In this study, we focus on 3 personality traits: handling aggression, exploration behaviour and breath rate under constraint. Handling aggression reflects aggressive behaviour in response to manipulation by humans and distress behaviour (Senar et al. 2017). Exploratory behaviour measures how individuals explore a novel environment and is often used as a proxy for risk-taking behaviour (e.g. Nicolaus et al. 2012). Breath rate is a none-invasive measure of primary response to stress and rapid breath rate is an evidence of higher stress (Carere and van Oers 2004). Breath rate also reflects the activity of the sympathetic and parasympathetic nervous systems (Koolhaas et al. 1999). In individuals displaying fast exploration patterns, high handling aggression, and fast-life history strategies, the sympathetic systems is suspected to be the dominant one. On the contrary, the parasympathetic system is dominant in slow life-history individuals, displaying slower exploration and lower aggressiveness (Koolhaas et al. 1999; Réale et al. 2010; Ferrari et al. 2013).

Using a pair of forest and urban populations of great tits (*Parus major*), we first quantified divergence between urban and forest birds for the three studied traits and estimated survival in each habitat using Capture-Mark-Recapture (CMR) models. Since the focal traits are often correlated – and can form some so called "behavioural syndromes" (Sih et al. 2004) - we also investigated correlations between each pair of traits. We subsequently performed selection analyses to determine whether phenotypic divergences were aligned with patterns of ongoing selection in each habitat. Since personality traits might both affect reproduction and survival and not necessarily in the same direction (Dingemanse and Réale 2005; Smith and Blumstein 2008), we performed selection analyses using both survival (controlling for imperfect recapture rates) and reproductive success as fitness proxies. Based on previously reported results described above, we predict that urban birds will be more aggressive, more exploratory and show a higher breath rate under constraint compared to forest birds. Following mainstream theories (e.g. Lowry et al. 2013), we also predict that high aggressiveness and more exploratory behaviour will be under positive - directional - selection in the urban habitat, but not higher breath rate.

MATERIAL AND METHODS

Data collection

Population monitoring

A pair of forest and urban populations of great tits (Parus major) were monitored during the breeding period in two study sites in the south of France. The forest of La Rouvière (43° 40' N, 3° 40' E) is a Mediterranean forest dominated by downy oaks (Quercus pubescens), and with 10% of holm oaks (Quercus ilex), located ca. 20km North-West of Montpellier city. Since 1991, great tit and blue tit (Cyanistes caeruleus) breeding events in nest-boxes have been monitored in this forest. Nest-box number for great tits (entrance hole = 32mm) has fluctuated between 51 and 92 across years. In the city of Montpellier (43° 36' N, 3° 52' E) 203 to 223 nest-boxes placed both in urban parks and on streets, have been monitored since 2012 with similar protocols as in the forest. For detailed maps of nest-boxes positions in both sites see ESM **Figure S1**.

During the breeding season, nest-boxes were visited once a week to record brood development from nest building to fledging, providing records of reproductive success for each pair. Adults were captured inside nest-boxes when feeding 9 to 15 days old nestlings, measured and ringed with unique metal rings provided by the French CRBPO (Centre de Recherche sur la Biologie des Populations

d'Oiseaux). Nestlings were also measured and ringed when reaching 15 days old (Caizergues et al. 2018).

Behavioural assays

While monitoring breeding birds started in 2013 in the city, behavioural trials were performed in both study sites between 2014 and 2019. Following a parent capture in a nest-box, each bird was submitted to this sequence of events: it was scored for handling aggression, isolated in a cloth bag for 5 minutes (resting), measured for breath rate, kept in an acclimatization compartment adjacent to the main open-field cage, scored for exploration rate in the open-field during 4 minutes, extracted from the open-field cage, ringed (if not already ringed) and measured for morphological traits. Time between capture and release took on average 20 min, and in any case less than 30 min.

First, right after capture and previous to any other manipulation, each bird was tested for handling aggression (HA) by the capturer. The detailed protocol for this measure was first published by Dubuc-Messier and collaborators (2016; see **Table S4**) on blue tits. The bird was handled with one hand by the capturer; while facing outward from the manipulator it was nagged at with a finger of the other hand (see ESM **Figure S2A**) following a standard procedure. After 15s of observation the capturer attributed a handling aggression score ranging from 0 (unreactive bird) to 3 (aggressive bird striking the handler each time and spreading its wings and tail) with increments of 0.5 (see detailed scoring in electronic supplementary materials **Table S1**, Charmantier et al. 2017). Right after the HA test, the bird was put to rest in a cloth bag.

Second, after 5 minutes of resting, the bird was removed from the cloth bag, held still on its back by the handler (see ESM **Figure S2B**) who counted its breath rate. From 2013 to 2016 breath rate was estimated as the number of chest moves during 30s, while since 2017 the protocol was updated to measure the time to complete 30 chest moves. Measures from 2013-2016 were thus converted to approximate the time needed to complete 30 chests move to obtain the same scale measures. Each count was replicated twice and the handling breath rate was averaged over the two measures. The protocol had a small but significant effect on the breath rate measure (see supplementary materials **Appendix 1**) and was thus taken into account in the analyses by using residuals of the regression between breath rate and protocol, here after referred as Breath Rate Index (BRI). Breath rate is known to be positively correlated to acute stress response in Paridae (Carere and van Oers 2004): a smaller breath rate index (hence faster breath rate) is expected to reflect a stronger stress response.

Third, the bird was submitted to a "novel environment" test using an experimental open field cage built following Stuber and collaborators (2013 ; see **Figure S3**). This experiment is classically used to estimate exploration behaviour in great and blue tits (Stuber et al. 2013; Dubuc-Messier et al. 2016). After measuring of breath rate, the bird was placed in an acclimatization compartment right next to the main open-field cage (**Figure S3B, 3**) for 2 minutes before being released in the exploration room (**Figure S3B, 1**). The bird's behaviour was recorded for 4 minutes. Videos were analysed with the software BORIS (Friard and Gamba 2016) to count the number of flights and hops during the 4 minutes. The number of flights and hops r-squared transformed was used as a proxy of exploratory behaviour, hereafter called exploration score (ES), following previous studies (see for instance Dingemanse et al. 2002).

Note that for some captured individuals all three behavioural assays were not processed (for instance HA measures were almost systematically performed, but not open-field trials, which required the special arena to be available) resulting in different sample sizes across the traits.

Statistical analyses

Data analyses were performed in four steps: (1) quantifying repeatabilities, between trait correlations and phenotypic divergence between forest and urban great tits for the three traits of interest, (2) estimating overall levels of per-population survival, and then estimating strength and direction of selection on the three studied traits via (3) reproductive success and (4) survival. Analyses of phenotypic divergence between forest and urban birds, as well as selection analyses via reproductive success were performed using R software (v3.5.0, R Core Team 2018). Selection analyses via survival were performed using E-SURGE (v2.2.3, Choquet et al. 2009).

Phenotypic divergence, repeatability and correlations

Repeatability of each trait was estimated using the rpt() function from the rptR R package (Stoffel et al. 2017), with the best linear mixed model selected using the process described below. Correlation between pairs of traits were estimated for forest and

urban birds separately using Pearson correlation tests. Since our dataset included repeated measures per individuals over the years, we performed correlation tests on the first measure for each individual to avoid analyzing non-independent data.

Differences between urban and forest great tits in the three behavioural traits described above: handling aggression, breath rate index and exploration score, were explored using linear mixed models (R package lme4, Bates et al. 2015). Full models contained habitat (forest versus urban), year (as a categorical variable), sex, age (one year old (=yearling) versus 2+ years old (=adult)), rank of capture (i.e. how many times the focal bird was captured, ranges from 1 (for first capture) to 6) and air temperature as fixed effects and habitat by year and habitat by sex interactions. Individual ring number was included as random effect to correct for within individual variation and manipulator identity was also included as random effect for handling aggression and breath rate models.

Model selection was performed using the dredge() function from the MuMIn R package (v1.43.17, Barton 2020): starting from the complete model all possible models combining fixed effects were automatically run and ranked based on their quasi Akaike criterion corrected for small sample size (AICc). Model averaging of the best models (ΔAICc<2) where performed to obtain average estimate for each effect using the model.avg() function.

Analyses of survival

Based on capture-recapture data collected during the breeding seasons between 2013 and 2019 we compared individual survival and recapture probabilities across habitats (urban versus forest) and sexes following the philosophy of Lebreton et al 1992. Survival analyses were performed using a capture-mark-recapture (CMR) framework with E-SURGE software. This framework is based on individual histories of capture (i.e. a series of '1' and '0 whether a focal bird has been (re)capture or not on the corresponding year) from which it allows to estimate a survival parameter (Φ) and take into account imperfect detectability estimating a recapture probability (p). Prior to this step of modelling, we tested whether the data met the Cormack-Jolly-Seber's (CJS) assumptions, and tested for over-dispersion with the goodness-of-fit tests using U-CARE (v2.3.2, Choquet et al. 2009). In order to maximize power for testing the main effects, all models do not take time into account.

We first estimated survival (Φ) and recapture (p) probabilities including effects of habitat and sex, using the capture-recapture data set based on captures of parents between 2013 and 2019. All combinations of models including habitat×sex (interaction), habitat + sex (additive), habitat, sex, or i (=constant) effects on the two parameters Φ and p were tested (25 models), and model selection was performed using the quasi Akaike criterion (QAICc).

Selection analyses

Via reproductive success

We then estimated selection operating via reproductive success. The choice for an optimal proxy of fitness to estimate reproductive selection is a long-standing debate with no straightforward solution (Andersson 1994). Since our study system offers little access to lifetime reproductive success, we opted to focus on annual reproductive success via the annual number of fledglings. The male and the female of each pair shared the same annual reproductive success, hence to avoid replicated measures within a single model, males and females were analysed separately.

Following the classic Lande and Arnold (1983) approach, we quantified directional selection acting on each trait in each habitat by calculating linear selection differentials. To identify the target of natural selection when studying multiple, possibly correlated traits, we also estimated multivariate linear selection gradients βi after removing indirect selection from other traits. Linear selection differentials were estimated for each trait following equation (1), and linear selection gradients are estimated on all traits together following equation (2):

$$\omega = \alpha + \text{habitat} + \beta x + \beta\ \text{habitat}\times x + \varepsilon \quad (1)$$

$$\omega = \alpha + \text{habitat} + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_1\text{'habitat}\times x_1 + \beta_2\text{'habitat}\times x_2 + \beta_3\text{'habitat}\times x_3 + \varepsilon \quad (2)$$

In both equations, the strength and direction of selection is given by the slope (β or βi) of the regression between the relative fitness (ω) and the standardized value of a trait (x or x_i), α represents the intercept and ε the error. Interaction terms (habitat×x or habitat×x_i) will indicate if selection acts differentially between habitats.

Model selection was performed between all models, starting from the complete model described in equations 1 and 2 to a null model (=constant), using AICc. Once more, all models within two points of AICc were considered equivalent and estimates were obtained by a model averaging procedure as described previously. All linear mixed models included individual identity and year as random effects to control for repeated measurements on the same individuals and variation across years. To test for correlational selection (i.e. interaction term between traits), the null model of reference contained the additive effect of the three traits and habitat as fixed effect.

The aim of this study was to explore how directional, hence linear selection, was aligned with the documented phenotypic divergence between forest and city birds. However, non-linear selection (such as disruptive or stabilising selection) could also differ between the forest and the urban habitats, and thereby shape differences in trait variances. Although differences in variance are not a focal objective here, we estimated quadratic selection differentials and gradients to present a complete picture on comparative selection. Methods and results on quadratic selection can be found in Supplementary Materials **Appendix 2 and Tables S1 to S3**.

Via survival

We then estimated viability selection on the three behavioural traits separately in relation with habitat and sex. These models aimed to assess whether the traits were under selection via survival, and whether selection on these traits varied among habitats and sex.

To estimate selection acting on behavioural traits via survival we tested whether an individuals' probability of survival from one year to the next was associated with the value of the trait, based on capture-recapture data combined with behavioural data collected between 2014 and 2019. As previously described, we tested whether the data met CSJ assumptions, tested for over-dispersion with the goodness-of-fit tests using U-CARE and performed survival analyses with E-SURGE. We performed three sets of models, one on each of the traits studied in order to preserve statistical power. In our dataset, most individuals presented more than two measures for each trait. Hence, we used individual phenotypic value for each trait provided by the Best Linear Unbiased Predictor (BLUP) for each individual (see for e.g. Bergeron et al. 2013 in a similar context). BLUPs were extracted from linear mixed models on repeated measures of each trait including age, year, hour of the day, air temperature and rank of capture as fixed effects. In the model for BRI, protocol of measure was also included as fixed effect to correct for the bias induced by the change in protocol since 2017. Values of BLUPs were standardised in order to compare the strength of selection among traits and studies.

In this set of analyses, because the number of possible models highly increased with each added effect, we first selected the appropriate model structure for recapture probability (p), starting from the full model $p_{(hab\times sex+trait)}$ and sequentially removing each effect from the full model. The model with the lower AICc was selected (for a detailed list of models tested see Supp Mat **Table S2**). We then ran models with the same procedure for survival probabilities, starting from the full model $\Phi_{(hab\times sex+ hab\times sex\times trait)}$ with p following the best structure previously selected. A habitat×sex×trait interaction was interpreted as selection on the trait varying between habitats and sexes. Similarly, a habitat×trait interaction indicated that selection on the trait varied between habitats. In the end, models were ranked according to the AICc criterion and all models within a $\Delta AICc < 2$ were considered equivalent.

Comparision of variances

Finally, variance can provide insight on past directional / stabilizing selection (reduced variance) and/or on past relaxed/disruptive selection (increased variance). We thus performed a comparison of variances for each trait between forest and city habitats, using a Fisher variance ratio test (var.test(), R package stats).

Table 1 | Repeatability of handling aggression, breath rate and exploration score, estimated with rpt() R function using the best model selected (see **Table 3**), with associated standard error (SE), 95% confidence intervals (CI) and P-value.

Trait	Repeatability	SE	CI	P
Handling aggression	0.383	0.056	0.276-0.495	$7.2\text{x}10^{-10}$
Breath rate index	0.440	0.055	0.34-0.547	$2.06\text{x}10^{-14}$
Exploration score	0.523	0.050	0.428-0.627	$2.98\text{x}10^{-13}$

RESULTS

Repeatable non-correlated traits

We confirmed that all three traits were repeatable (**Table 1**). All phenotypic correlations across the three traits were below 0.1 in both habitats (**Table 2**) and only one correlation was significant, between breath rate index and explorations score in urban birds ($\rho = 0.097$, $P = 0.032$, **Table 2**).

Phenotypic divergence between urban and forest habitat

When testing for phenotypic differences between habitats, model selection revealed that a habitat effect was retained in all the best models for the three studied traits (**Table 3**). For handling aggression, two equivalent models were selected, both revealing effects of habitat, year, sex and habitat×sex. Urban individuals displayed on average higher handling aggression scores, and this difference was more pronounced in males ($mean_{urban\text{-}males}=2.62 \pm 0.23$, $mean_{forest\text{-}males}=1.74 \pm 0.11$; **Figure 1A**) than in females ($mean_{urban\text{-}females}=2.09 \pm 0.14$, $mean_{forest\text{-}females}=1.67 \pm 0.12$; **Figure 1A**).

Two equivalent models were selected when modelling the breath rate index (**Table 3**), both including a habitat effect whereby urban individuals had faster breath rate (i.e. a lower index) than forest birds ($mean_{urban}=-0.730 \pm 0.100$, $mean_{forest}=-0.32 \pm 0.27$; **Figure 1B**).

Model selection for the exploration score revealed 12 equivalent models (**Table 3**) all including a habitat effect. Urban individuals were more active explorers than forest ones (ES $mean_{urban}=5.88 \pm 0.37$, $mean_{forest}=3.54 \pm 0.72$; **Figure 1C**). Sex, temperature, capture rank and habitat×sex effects were present in numerous models, suggesting they may affect ES. Since so many models were found equally supported, we do not wish to draw any strong conclusion regarding these effects. Detailed effects sizes obtained using model averaging are presented in Supplementary Materials **Table S6**.

Table 2 | Phenotypic correlations (above the diagonals) among handling aggression, exploration score and breath rate index in A) forest and B) urban great tits, and their associated P-values (below the diagonals). Correlations are estimated on measures of 164 to 171 great tits captured in the forest of La Rouvière and 483 to 504 captured in the city of Montpellier.

A) Forest	Handling aggression	Exploration score	Breath rate index
Handling aggression		0.138	0.081
Exploration score	0.077		-0.067
Breath rate index	0.081	0.387	

B) Urban	Handling aggression	Exploration score	Breath rate index
Handling aggression		0.073	0.006
Exploration score	0.115		**0.097**
Breath rate index	0.894	**0.032**	

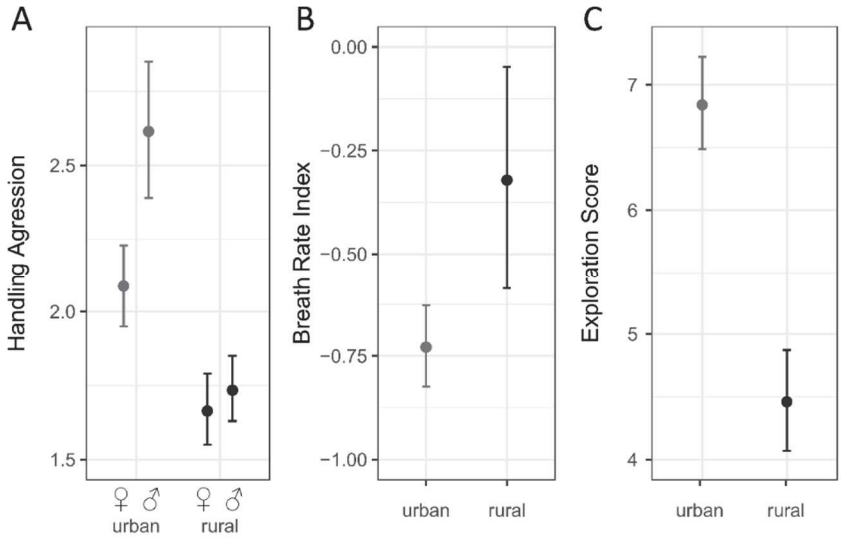

Figure 1 | Divergence between urban and forest great tits on 3 behavioural and stress related traits (A) handling aggression (presented for each sex), (B) breath rate index and (C) exploration score (for detailed estimates see Table S6). Note that lower values of breath rate index imply a faster breath rate.

Table 3 | Model selection for handling aggression, breath rate index and exploration scores as a function of habitat, year, sex, age, capture rank, temperature, habitat×year, habitat×sex and habitat×age. All models included individual as random effect and HA and BRI also included manipulator identity as random effect. Models were ranked according to their AICc values, models in black are equally supported (ΔAICc <2), detailed effects sizes are presented in **Table S6**.

Models	d.f.	Deviance	AICc	ΔAICc
Handling aggression				
habitat+year+sex+habitat×sex	**12**	**3599,54**	**3623,80**	**0,00**
habitat+year+sex+temperature+habitat×sex	**13**	**3599,46**	**3625,70**	**1,96**
habitat+year+sex+capture rank+habitat×sex	13	3599,54	3625,80	2,04
habitat+sex+habitat×sex	7	3612,68	3626,20	2,37
habitat+year+sex+temperature+capture rank+habitat×sex	14	3599,46	3627,80	4,00
Breath rate				
habitat+year+temperature+capture rank	**12**	**2797,74**	**2822,10**	**0,00**
habitat+year+temperature+capture rank+habitat×an	**17**	**2789,33**	**2824,10**	**1,97**
habitat+year+sex+temperature+capture rank	13	2797,73	2824,10	2,05
habitat+year+age+temperature+capture rank	13	2797,73	2824,20	2,05
habitat+year+sex+temperature+capture rank +habitat×sex	14	2797,06	2825,60	3,45
Exploration score				
habitat+temperature+capture rank	**6**	**4803,23**	**4815,30**	**0,00**
habitat+capture rank	**5**	**4805,64**	**4815,70**	**0,38**
habitat+sex+temperature+capture rank	**7**	**4801,96**	**4816,10**	**0,76**
habitat+temperature+capture rank	**5**	**4806,03**	**4816,10**	**0,77**
habitat+temperature+capture rank+habitat×sex	**8**	**4799,95**	**4816,10**	**0,79**
habitat+sex+capture rank	**6**	**4804,33**	**4816,40**	**1,10**
habitat+sex+capture rank+habitat×sex	**7**	**4802,34**	**4816,50**	**1,14**
habitat+sex+temperature+habitat×sex	**7**	**4802,78**	**4816,90**	**1,58**
habitat+sex+temperature	**6**	**4804,82**	**4816,90**	**1,59**
habitat+age+temperature+capture rank	**7**	**4802,85**	**4817,00**	**1,65**
habitat	**4**	**4802,97**	**4817,00**	**1,68**
habitat+age+capture rank	**6**	**4805,20**	**4817,30**	**1,96**

Survival estimations per habitat

On the overall capture-recapture dataset, a goodness-of-fit test conformed to the Cormack-Jolly-Seber's assumptions ($\chi^2 = 30.6059$, p-level = 0.246, c-hat = 1.177). Deviances and AICc were corrected for the C-hat value in all models.

In the overall analysis aiming to estimate survival and recapture for each sex in each habitat and sex, model selection revealed 5 models with substantial support (delta AICc<2, **Table 4**). In a majority of models, survival and recapture probability depended on habitat. Hence, estimates of these parameters per habitat were calculated by model averaging using the five best models. Forest great tits displayed a slightly higher survival (Φforest = 0.486) than urban ones (Φurban=0.446), however the intervals of credibility overlapped greatly (**Table 5**).

Reproductive Selection

When estimating reproductive selection on HA, BRI and ES in females using number of fledglings as a proxy of fitness, we found no selection on HA nor on ES (**Table 6**). BRI was under positive significant linear selection in urban females, meaning that females with a slower breath rate had a higher reproductive fitness. However, the difference in selection between urban and forest females for BRI was not significant, as suggested by the non-significant habitat×BRI interaction (est.=0.033, P=0.73, **Table 2**).

In males, we found no evidence for directional reproductive selection on HA and BRI (**Table 2**) but positive reproductive selection on ES in forest males. While forest males with higher ES had a higher reproductive success, this was not true for urban males (no significant selection detected). In this case, the difference in selection between forest and city

Table 4 | Model selection for survival probability in relation to habitat, sex (N=938) and handling aggression (N=881) or breath rate index (N=668) or exploration score (N=661) measured between 2013 and 2019 for the overall dataset and 2014 and 2019 for other datasets. Models are ranked according to QAICc values, and only the best models (i.e. lowest QAICc) are presented for each trait. Equally supported models (ΔQAICc<2) are represented in bold. For all models (except in the global analysis), the probability of recapture is considered constant (see Supp Mat **Table S5**).

Models	# Par.	Deviance	QAICc	ΔQAICc
Global dataset				
Φ_{hab} p.	**3**	**1698,91**	**1445,769**	**0**
Φ. p_{hab}	**3**	**1699,77**	**1446,505**	**0,735**
Φ_{hab} p_{hab}	**4**	**1697,98**	**1446,997**	**1,227**
Φ. p.	**2**	**1703,01**	**1447,235**	**1,466**
Φ_{hab} p_{sex}	**4**	**1698,88**	**1447,762**	**1,993**
Exploration score				
Φ_{hab+ES} p.	**4**	**1264,99**	**1273,034**	**0**
Φ_{ES} p.	**3**	**1267,35**	**1273,375**	**0,341**
$\Phi_{sex+ES+sex\times ES}$ p.	**6**	**1262,29**	**1274,372**	**1,339**
Φ_{hab} p.	**3**	**1268,82**	**1274,844**	**1,810**
$\Phi_{hab+sex+ES}$ p.	**5**	**1264,91**	**1274,975**	**1,941**
Breath rate index				
Φ_{hab} p.	**3**	**1272,9**	**1278,929**	**0**
Φ_{hab+BR} p.	**4**	**1272,63**	**1280,673**	**1,743**
$\Phi_{hab+sex}$ p.	**4**	**1272,88**	**1280,924**	**1,995**
Φ. p.	2	1278,003	1282,015	3,086
$\Phi_{hab+sex+BR}$ p.	5	1272,611	1282,672	3,743
Handling aggression				
Φ_{hab} p.	**3**	**1438,74**	**1170,992**	**0**
Φ. p.	**2**	**1441,29**	**1171,050**	**0,059**
Φ_{hab+HA} p.	**4**	**1437,6**	**1172,081**	**1,089**
Φ_{HA} p.	**3**	**1440,27**	**1172,229**	**1,237**
$\Phi_{hab+sex}$ p.	4	1438,723	1172,992	2,001

Table 5 | Estimates of survival (Φ) and recapture (p) probabilities in forest and urban great tits. Estimates are obtained from model averaging of the equally supported best models presented **Table 4**.

Parameter	Forest		Urban	
	est.	CI	est.	CI
Φ	0.486	0.431-0.542	0.446	0.404-0.489
p	0.747	0.651-0.822	0.712	0.628-0.784

was significant (significant habitat×ES interaction, est= -0.29, P=0.01, **Table 6**).

Finally, a Fisher variance ratio test for each trait did not reveal any difference in variance between habitats (**Table S4**).

Viability selection

Goodness-of-fit tests conformed to the Cormack-Jolly-Seber's assumptions for each trait-specific dataset (ES data-set: $\chi^2 = 29.222$, p-level = 0.084, c-hat = 1.461; BR: $\chi^2 = 28.681$, p = 0.094, c-hat = 1.434; HA data-set: $\chi^2 = 24.7$, p = 0.213, c-hat = 1.235). Deviances and AICc were corrected for the C-hat values in all sets of models.

The best supported models for the recapture probability for survival selection models on HA, BR and ES are presented in **Table S2**, in each case, the model with the lowest AICc estimated p constant. For the three traits, the survival model including habitat×trait was not among the best models selected (**Table 4**), suggesting that viability selection did not differ between forest and city. In the three sets of models, at least one of the best models included the additive trait effect (**Table 4**), indicating possible yet weak support for viability selection (see **Figure S4 / Table S8**).

DISCUSSION

In this study, we investigated differences in behavioural traits, stress response, and survival, between birds breeding in a forest versus an urban habitat, and explored if the observed divergences were aligned with patterns of reproductive and survival selection ongoing in each habitat on each trait. The three traits studied were all repeatable and not correlated, except for breath rate index and exploration score for which we found a small (<0.1) significant positive correlation in the urban habitat (**Table 2**). Overall, we found that urban individuals were on average more aggressive, had a higher breath rate and were faster explorers than their forest counterparts (**Figure 1**). In addition, estimation of survival accounting for imperfect recapture rate did not reveal any strong difference between habitats despite a slight tendency for lower annual survival of urban individuals. Regarding selection analyses, we found little evidence of selection on the three studied traits. For selection via survival, we detected no difference in selection between habitats in all three traits. Reproductive selection favoured slower breath rate in urban females, however when pooling urban

Table 6 | Linear reproductive selection gradients for handling aggression, breath rate index and exploration score in urban and forest great tits. Bold estimates are significant (P<0.05). "Int." displays the value of the habitat×trait, and P its associated P-value.

		Linear selection gradients														
		Handling aggression					**Breath rate index**					**Exploration score**				
Sex	**Habitat**	**N**	**Est.**	**SE**	**Int.**	**P**	**N**	**Est.**	**SE**	**Int.**	**P**	**N**	**Est.**	**SE**	**Int.**	**P**
Female	Forest	155	-0,004	0,080	0,067	0,955	107	0,153	0,096	0,033	0,730	107	0,116	0,097	-0,170	0,147
	City	309	0,049	0,057			297	**0,186**	**0,056**			299	-0,074	0,058		
Male	Forest	146	0,002	0,083	-0,064	0,508	97	0,046	0,102	-0,052	0,618	98	**0,234**	**0,099**	**-0,290**	**0,010**
	City	282	-0,024	0,060			270	0,001	0,060			257	-0,018	0,062		

and forest data together, the habitat×trait interaction was not significant, i.e. there was no evidence for a difference in selection between habitats. Finally, we detected diverging patterns of selection for exploratory behaviour between the two habitats in males. Indeed, while positive selection was found in the forest habitat, none was detected in the city, suggesting that more exploratory behaviour is advantageous in the forest but not in the city.

As predicted, we found phenotypic divergence between urban and forest habitats for the three studied traits. Higher aggressiveness and exploratory behaviour are in line with previous studies in urban populations (e.g. Martin and Réale 2008; Evans et al. 2010). Regarding breath rate, previously reported results were more contrasted. Our finding that urban individuals displayed faster breath rate is similar to some studies (e.g. in the Great tit, Torné-Noguera et al. 2014) but contrasts with others that did not found any difference (e.g. in the Great tit: Senar et al. 2017) or reported slower breath rate in the city (e.g. in the Dark-eyed junco *Junco hyemalis*: Abolins-Abols et al. 2016). Shifts in behavioural and stress response traits in urban habitats are often regarded as evolutionary adaptations to new urban environmental conditions (Lambert et al. 2020). In fact, a genetic determinism underlying some of the reported phenotypic differences is supported by genetic analyses revealing genetic urban-rural divergences associated with genes involved in aggressive and exploratory behaviours (in particular SERT and DRD4, see e.g. in the Black swan *Cygnus atratus*: Van Dongen et al. 2015; in the Great tit: Riyahi et al. 2017; in the Black bird *Turdus merula*: Mueller et al. 2020). Recently, Lambert et al (2020) claimed that only six urban studies convincingly demonstrated an evolutionary adaptation to urbanisation, and none of these six examples concerned animal behaviour. Here we provide one of the first studies investigating the link between individual behaviour and fitness in an urban context, to test for the adaptive nature of the urban-specific behaviours (see also Halfwerk et al. 2019). Our selection analyses performed here revealed that shifts in behaviour and stress response traits in the urban habitat were not aligned with patterns of directional selection. For instance, selection acting on exploratory behaviour in males was relaxed in the urban habitat compared to strong selection favouring faster forest explorers, as there was no evidence that higher exploratory behaviour displayed by urban birds conferred an advantage in reproductive success. Alternatively, it is possible that spatio-temporal variation in natural selection within the city masks the complexity of selection on these characters. First, higher exploratory behaviour might be advantageous in some parts of the city (for instance in highly urbanized areas) but not in others. If there is such spatial variation in selection, estimating selection at a fine scale within the city habitat might be necessary to understand if high exploratory behaviour could indeed be adaptive at least in some areas of the city. Second, recent work by Corsini and collaborators (2020) suggests that selection on great tit morphology might act mainly during the early stages of life. If strong early-life selection also impacts behavioural traits, our analysis focused on adult behaviour could potentially miss an important phase of selection occurring before individuals start to reproduce.

Breath rate under constraint is linked to stress physiology and is used as a measure of acute stress response, meaning it reflects short-term response to a stressful event, in contrast with chronic-stress referring to long term response (Dantzer et al. 2014). Faster breath rate shows higher short-term stress response to a stressor. In opposition to what is expected in case of adaptation to urban habitat, we found that urban individuals displayed faster breath rate in response to handling and thus higher stress

response than forest individuals, and that, even if higher breath rate was associated with a reduction of reproductive success in urban females. Taken together these results suggest that faster breath rates observed in the city are maladaptive. Note that previous studies found that acute stress responses associated with urbanization could differ depending on population, species and traits studied (Powell et al. 2013). In particular, it might be interesting to explore difference in chronic-stress levels between habitats to understand if individuals also display higher basal levels of stress in response to city life. Higher chronic-stress might have particularly deleterious repercussions on quality of life of individuals and ultimately fitness, and thus might be more constrained by natural selection than acute-stress response, thereby reflect another dimension of adaptation to urban habitat.

Correlations of behavioural traits are often observed in animals and referred as "behavioural syndromes" or even "pace-of-life syndromes" (POLS) if life history traits are involved (Réale et al. 2010). Syndromes represent variation of personality and life history strategies between individuals. At two ends of a POLS continuum, fast individuals display low survival, high reproductive outputs, precocious reproduction, and bold behaviours, while slow individuals show high survival, low reproduction and shy personalities. In urban habitats, slower pace-of-life is expected to evolve in response to new selective pressures (Sepp et al. 2017, but note this meta-analysis did not consider behavioural traits). In contrast with these expectations we found that urban birds displayed fast life features: higher exploratory behaviour, higher aggressiveness, higher breath rate and (slightly) lower survival (contrasting with a similar study on great tits Hõrak and Lebreton 1998). Also, no correlation between the three studied traits was found, except a small ($\rho<0.1$, **Table 2**) but significant correlation between breath rate and exploration score, in line with findings of Carere and van Oers (2004). The three focal traits can thus be considered as independent dimensions in a POLS approach. Taken together these results suggest that (1) city dwelling species can display faster pace-of-life compared to forest ones and (2) structure of syndromes (i.e. between traits correlation) might be disrupted by urban environmental conditions (Sol et al. 2018). Indeed, some environmental stressors can alter relationships between physiological and behavioural traits, and thus could be responsible for the non-correlation between measured traits (Killen et al. 2013).

The result of the non-adaptive nature of behavioural and stress response shifts linked with urbanization raises numerous questions regarding the origins of such shifts and the implications for populations. First, despite the fact that behavioural shifts in urban habitats do not confer better fitness outputs in this case study, they could be necessary to colonise urban habitats or tolerate the new environmental conditions. Indeed, bolder great tits are known to disperse more and on longer distances than shyer ones (Dingemanse et al. 2003) and could thus be more prone to colonise urban habitats. Second, the phenotype-environment covariance could be explained by the hypothesis of "personality matching habitat choice" following which individuals could preferentially settle in habitats that best suits their personality in order to reduce stress and costly behaviour (Edelaar et al. 2008; Jacob et al. 2015). In line with this hypothesis, Holtmann and collaborators (2017) demonstrated that bolder individuals settled in areas with high human disturbance in a population of dunnocks (Prunella modularis). Third, behavioural shifts can result from plasticity in response to the environmental conditions experienced by individuals. For example, some personality traits can emerge from endocrine stress physiology (Baugh et al. 2017) and experiments demonstrated that stress stimuli inflicted to individuals could induce a reduction of their neophobic behaviours in house sparrows (Gormally et al. 2018). Hence, some behavioural shifts can emerge from a habituation process, in contrast with other traits such as aggressive behaviour which are consistent across lifetime (Cavalli et al. 2018), showing that phenotypic flexibility can be a major component of the capacity of individuals to tolerate urban environments.

The plastic versus genetic origin of the behavioural and stress response differences between urban and rural environments remains largely unknown/debated, with some studies suggesting plastic origins while others demonstrate the existence of genetic bases (Mueller et al. 2013; Riyahi et al. 2015; Riyahi et al. 2017; Minias et al. 2018). While we have shown here that the three focal traits are repeatable, repeatabilities of 38 to 52% leave an important margin for plastic responses in these traits. A challenge to explore this plasticity will be i) to obtain repeated individual phenotype measures across a range of different environments,

and ii) to identify environment drivers of plasticity within the multitude of urban-specific environmental features (e.g. light, air and sound pollution, vegetation cover, human disturbance..). This plastic response could be initiated during early life stages. For instance, stress and hormone levels experienced in mothers and transmitted to eggs can affect offspring personality (Bertin et al. 2009; Rokka et al. 2014). Determining the contribution of genetics and plasticity in behavioural and stress responses shifts observed in urban habitat would greatly benefit from experimental designs such as cross-fostering or common gardens.

This study is we believe, the first to formally test the link between avian behaviour as well as stress response traits, and fitness components, in a context of urbanization. Our results suggest that, in opposition with expectations formulated in the literature, urban great tits display a faster pace-of-life while more exploratory behaviour and enhanced aggressiveness may not be adaptive. In addition, urban individuals displayed faster breath rates even if this phenotype was counter-selected in females, revealing maladaptation. However, it is important to keep in mind that further investigation and complementary analyses will be necessary to draw any general pattern of selection ongoing in urban habitat. In particular, our study is based on a single pair of urban and forest population, and replicating this on other populations will be a crucial step to understand whether the result found here are generalizable. In addition, the fact that we do no detect selection acting on the trait studied does not necessarily mean that other aspects of behaviour and stress responses might not be under selection. In particular, management of chronic stress might be of capital importance in the urban habitat to avoid health degradation. Hence, we suggest to explore urban linked shifts in chronic stress and perform similar analyses as used here to test for the existence of physiological adaptation. Finally, selection analyses as performed here are data hungry and require numerous years of population monitoring. Alternatively, molecular tools can offer great opportunities to detect molecular adaptation to urban habitat and directly identify involved genes, as previously mentioned DRD4 and SERT gene (Garroway and Sheldon 2013; Riyahi et al. 2017). We thus suggest urban evolutionary biologist to combine both quantitative genetic and genomic approaches to fully understand evolutionary processes ongoing in urban habitats (Perrier et al. 2020).

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AUTHORS CONTRIBUTIONS

AEC collected the data, carried out the statistical analyses and wrote the manuscript. AG and AC conceived and funded the study, collected the data and contributed to the writing of the manuscript. SP collected the data and RC participated in data analyses. All authors gave approval for publication.

ETHICS

All experimental protocols were approved by the local ethics committee for animal experimentation of Languedoc Roussillon (CEEA-LR, 05/06/2018) and regional institutions (bylaw issued by the Prefecture n°2012167-003). Captures were performed under personal ringing permits delivered by the CRBPO (Centre de Recherche par le Baguage des Populations d'Oiseaux) for the Research Ringing Program number 369.

DATA AVAILABILITY STATEMENT

Data will be placed on the open repository Zenodo upon acceptance of the paper.

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CHAPITRE IV

TRACES GÉNOMIQUES D'ADAPTATION ET RÔLE POTENTIEL DE LA MÉTHYLATION DANS UN CONTEXTE URBAIN



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– RÉSUMÉ DU CHAPITRE IV –

Dans le contexte des changements globaux, l'étude de l'adaptation moléculaire aux nouvelles conditions environnementales se concentre en général sur les mécanismes génétiques (McNew et al. 2017). En effet, les processus d'adaptation moléculaires agissent principalement au niveau des séquences d'ADN puisque des modifications de celles-ci entrainent de la variation phénotypique héritable ayant des répercussions sur la valeur sélective des individus. Ainsi, la recherche de traces de sélection dans les génomes peut renseigner sur des processus de sélection passée subis par les populations. Récemment, de nouvelles études suggèrent que des changements d'expression ou de fonction de gènes, indépendant des changements des séquence d'ADN (= mécanismes épigénétiques), initialement considéré comme étant réinitialisés à chaque génération, peuvent parfois être transmis à la descendance et donc être aussi impliqués dans l'évolution des phénotypes (Bossdorf et al. 2007). En particulier, la méthylation de l'ADN, *i.e.* l'ajout d'un groupe méthyle à une cytosine, peut être induite par l'environnement et affecter l'expression des gènes, et donc potentiellement jouer un rôle dans l'adaptation à des changements rapides d'environnement (Jaenisch & Bird 2003). A l'inverse des changements génétiques, souvent longs à se mettre en place et à augmenter en fréquence dans la population, les changements de méthylation peuvent se produire au cours de la vie d'un individu en lien avec des conditions environnementales vécues, et moduler le phénotype exprimé plus rapidement (Waterland & Jirtle 2003; Weaver et al. 2004). Ainsi, dans le contexte rapide d'urbanisation, la méthylation de l'ADN est susceptible de jouer un rôle majeur dans les différences phénotypiques observées et potentiellement dans l'adaptation (si elle implique des traits héritables) ou du moins l'acclimatation (si elle est réinitialisée à chaque génération) aux nouvelles conditions environnementales.

L'objectif principal de ce chapitre est d'explorer les réponses génomiques et épigénomiques associées au milieu urbain et de comprendre dans quelle mesure elles sont répétées entre villes, en utilisant des données issues de trois couples de populations de mésanges charbonnières urbaines et forestières en Europe (Barcelone, Montpellier, Varsovie). A l'aide de séquençage à l'échelle du génome de données génomiques (RAD-seq) et épigénomiques (RRBS) nous avons ainsi exploré l'existence de traces de sélection dans les génomes et de potentielles régions différentiellement méthylées pouvant contribuer aux divergences phénotypiques et à l'adaptation en milieu urbain.

Nos résultats indiquent l'existence de peu de marques de forte sélection associée au niveau urbain au niveau génomique mais une multitude de loci sous sélection d'intensité plus faible, marqueurs d'une adaptation polygénique. De plus, nous détectons de nombreuses régions différentiellement méthylées en ville, majoritairement situées dans des promoteurs, des sites de démarrage de transcription (TSS) ou directement dans le corps des gènes, soulignant leur potentielle importance dans la modulation de l'expression et de l'épissage des gènes. Par ailleurs, des analyses d'enrichissement d'ontologie de gènes révèlent que les marques de sélection génomiques chez les oiseaux urbains sont enrichies sur des gènes associés au système nerveux, au système sanguin, aux réponses hormonales ainsi qu'au stress. Les gènes associés aux régions différentiellement méthylées sont quant à eux représentatifs du système nerveux, de l'immunité, des activités métaboliques, du comportement et des processus endocrines. Enfin, nos résultats indiquent un faible taux de parallélisme entre les différentes villes, suggérant un potentiel impact spécifique de chaque ville sur les génomes et/ou la possibilité de répondre de manière différente à des conditions environnementales similaires.

Ces travaux constituent un premier pas dans la compréhension des mécanismes génomiques et épigénomiques associés au milieu urbain. Ils montrent que la vie en ville peut induire des réponses génétiques et épigénétiques associées à des gènes ayant un rôle potentiellement majeur dans l'adaptation aux nouvelles conditions urbaines. Par ailleurs, ils apportent un élément de réponse quant à la fréquence des processus évolutifs se produisant de façon parallèle dans différentes villes, et suggèrent qu'il existe peu de réponses moléculaires similaires acquises indépendamment entre villes soulignant la possibilité d'arriver aux mêmes résultats adaptatifs en empruntant différents chemins (Losos 2011). Enfin, le faible taux de parallélisme soulève le fait que les conditions environnementales urbaines peuvent potentiellement grandement varier d'une ville à l'autre (Szulkin et al. 2020a).

Manuscrit associé au chapitre :

- **Manuscrit 4:** Caizergues, A.E., Le Luyer, J., Arnaud Grégoire, A., Szulkin, M., Señar, J.C., Charmantier, A. & Perrier, C. Testing for parallel genomic and epigenomic footprints of adaptation to urban life in a passerine bird (*in prep for PNAS*)

– MANUSCRIT 4 –

Testing for parallel genomic and epigenomic footprints of adaptation to urban life in a passerine bird

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– ABSTRACT – In the current context of rapid, human induced global change, identifying the molecular mechanisms involved in rapid adaptation to new environmental conditions is a pressing question. Beyond genetic adaptation, usually requiring long evolutionary time scales, epigenomic modifications may play a substantial role in rapid adaptation. In particular, DNA methylation, involved in regulation of gene expression and thus phenotypic expression, can be modified within a generation and even transmitted to the next generation. Here, we investigated potential footprints of genomic selection and differentially methylated regions (DMR) linked with city life, by comparing three pairs of urban and forest populations of great tits (*Parus major*) across Europe. We found little evidence for strong selective sweeps associated with urbanization in genomes, but multiple traces of weaker, polygenic selection. Furthermore, we found multiple DMR between forest and city great tits, largely overlapping TSS or promotor regions, suggesting their importance in gene expression modulation. (Epi)genomic marks associated with urbanisation showed enrichment for biological functions associated with the nervous system, immunity, behaviour, hormonal response and stress, suggesting the importance of such functions in avian adaptation to urban habitats. Interestingly, we found little evidence of parallel genetic and epi-genetic responses among replicates, supporting the hypothesis of largely non-parallel rapid de novo adaptation to similar environments. Finally, our results are consistent with accumulating evidence that polygenic adaptation and methylation processes might be involved in rapid phenotypic shifts in response to rapidly changing environments such as urbanized areas.

– KEYWORDS – adaptation | DNA methylation | epigenomics | genomics | urbanization

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INTRODUCTION

Identifying mechanisms involved in rapid adaptation to changing environmental conditions is a pressing evolutionary question of the Anthropocene (Malhi 2017). A vast majority of studies investigating mechanisms involved in rapid adaptation have focused on phenotypic plasticity on the one hand, and genetic evolutionary mechanisms on the other. At their crossroad, recent work underlines the potential role of epigenetics in rapid adaptation to environmental changes. In particular, variation in DNA methylation induced by environmental changes can modulate the phenotypic expression of genes (Jaenisch and Bird 2003; Duncan et al. 2014). Such methylation-linked phenotypic variations can occur during an individual's lifetime, especially early on during the organism's development (Waterland and Jirtle 2003; Weaver et al. 2004). Although methylation changes acquired a lifetime may most often be non-inheritable (Reik, Dean, & Walter, 2001; but see Crews et al., 2007; Janowitz Koch et al., 2016), epigenetically induced phenotypic shifts may nevertheless confer the capacity to cope with rapidly changing environments. Moreover, during the course of evolution, divergent genetic variants regulating epigenetic modifications may also be selected for, hence promoting the evolution of divergent epigenotypes and epigenetically-linked phenotypic variation (Richards et al. 2010). While epigenetic studies focused on human diseases and medical topics are now abundant, studies in an ecological context are still rare (Derks et al. 2016). However, a small number of epigenetic studies on natural populations revealed that DNA methylation shifts might play a determinant role in adaptation to environmental variation (Foust et al. 2016). There is hence an urgent need for empirical investigations of simultaneously rapid genetic and epigenetic evolution in response to environmental changes (Danchin et al. 2011).

Amongst global changes, urbanization rapidly and irreversibly converts natural habitats to man-made environments and is hence considered as a major threat to biodiversity (Brondizio et al. 2019). For species who appear to cope with urbanisation, urban habitats present a myriad of novel environmental conditions compared to the habitat where they evolved, including high levels of chemical, light and sound pollution, high proportion of impervious surfaces, high habitat fragmentation, low vegetation cover and high human densities (Grimm et al. 2008; Szulkin, Garroway, et al. 2020). Such extreme environmental changes are expected to result in numerous new selection pressures on city-dwelling species (Szulkin, Munshi-South, et al. 2020). Accordingly, rates of recent phenotypic change, concerning multiple types of traits related to behaviour, morphology, phenology and physiology, seem to be greater in urban areas than in any other habitat types, including non-urban anthropogenic contexts (Alberti et al. 2017; Thompson et al. 2018). While potential genetic bases of such urban-driven changes remain largely unknown (Mueller et al. 2013; Perrier et al. 2018; Salmón et al. 2020), the exploration of epigenetic variation has only begun and at relatively low resolution (Riyahi et al. 2015; McNew et al. 2017; Watson et al. 2020). Of particular importance, very little is known about the level of parallelism and hence of the predictability of genetic and epigenetic evolution in response to urbanisation in different cities (Santangelo et al. 2018; Rivkin et al. 2018). Comparing genomic and epigenomic responses in several independent cities offers a unique opportunity to study the parallelism and predictability in evolutionary mechanisms implicated in rapid local adaptation to urbanization and more generally to novel environments (Perrier et al. 2020; Santangelo et al. 2020).

In this study, we used genome-wide and epigenome-wide sequencing approaches to compare genetic and epigenetic responses among three pairs of great tit *Parus major* populations in urban and forest habitats across Europe. We investigated both average genomic and methylation differentiation and we searched for particular genomic footprints of divergent selection as well as differentially methylated regions, between forest and urban populations. Our results show that despite limited genetic differentiation and few genomic footprints of divergent selection between forest and urban populations, urban life was associated with numerous differentially methylated regions associated with neural development, behaviour and immunity. Hence, this study shows the potential role of epigenetic response in rapid adaptation to changing environments in urban areas. Importantly, we found

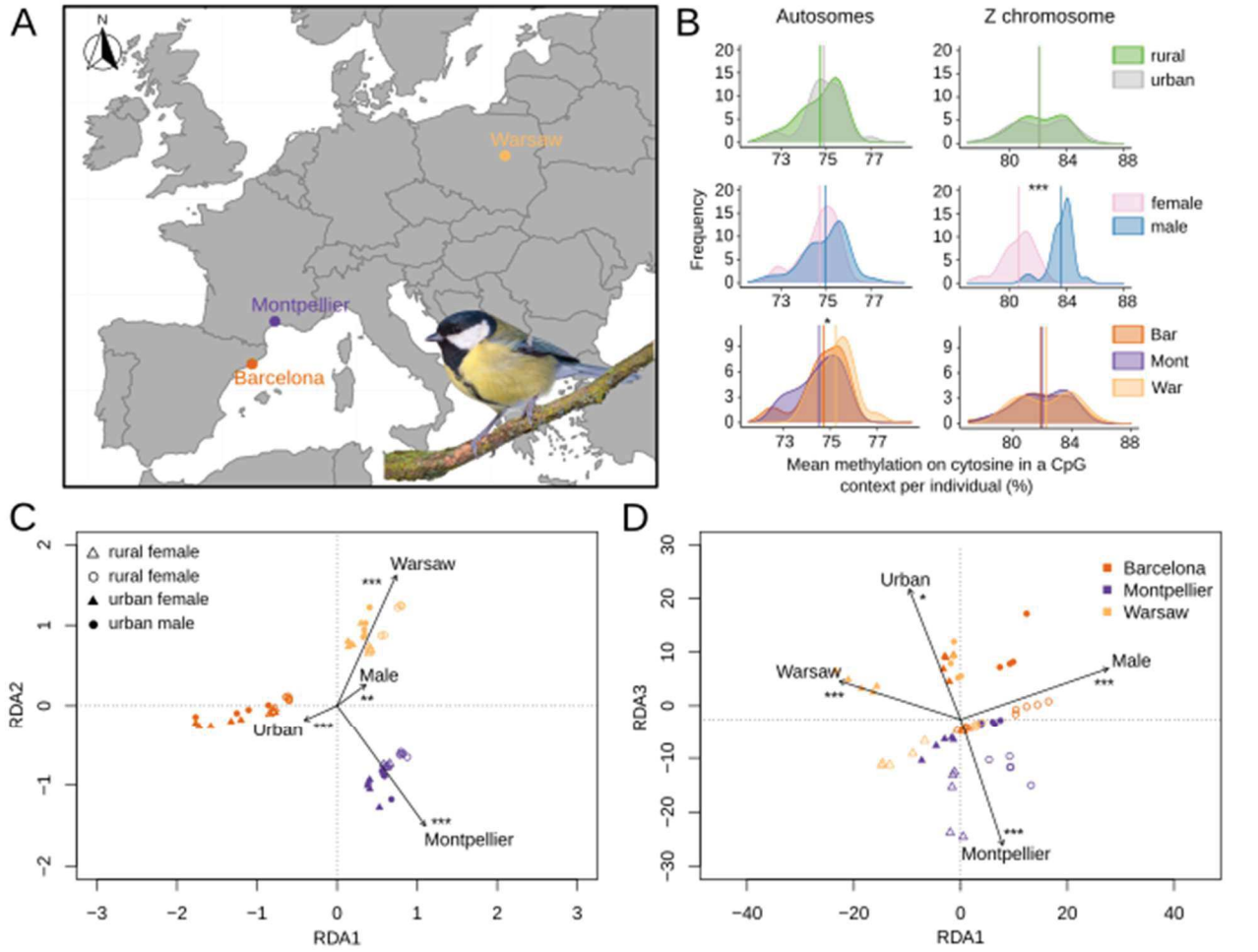

Figure 1 | (A) Great tit blood sample locations in Europe (in urban and forest sites in and near Barcelona, Montpellier & Warsaw). (B) Distribution of mean percent of methylation on autosomes and on the Z chromosome, compared between habitats (forest versus urban), sexes and locations. (C) Redundancy analysis (RDA) on genomic data (74,137 filtered SNPs). (D) RDA on methylation levels (based on methylation levels observed at 157741 positions). In (C) and (D), triangles represent rural habitats, circles represent urban habitats, empty and solid symbols represent females and males respectively. *** P-value < 0.001, ** P-value < 0.01 and * P-value < 0.05, related to the explanatory factors.

little parallelism between cities in both genomic and epigenomic responses to urbanization, possibly confirming the hypothesis that multiple evolutionary ways exist to independently cope with similar novel environmental conditions.

RESULTS

Little genetic and epigenetic average differentiation between urban and rural populations

Genetic. We used a redundancy analysis (RDA) on 74,137 SNPs obtained by RAD-sequencing to document genetic variation among the studied great tits populations, with location (Barcelona, Montpellier or Warsaw), habitat (urban vs. forest), and sex as explanatory variables. The model was highly significant ($P < 0.001$) but explained only a small fraction (*i.e.* less than 2%) of the total variance: $R^2 = 0.018$ **(Figure 1C**, Supp. Mat. **Table S1**). All three variables were significant (location: $P = 0.001$; habitat: $P = 0.004$; sex: $P = 0.001$). Partial RDA revealed that the net variation explained by the habitat ($R^2 = 0.004$, $P = 0.001$) was inferior to the net variation explained by location ($R^2 = 0.012$, $P = 0.001$) but higher than sex ($R^2 = 0.002$, $P = 0.004$, **Table S1**). As expected, when removing the Z chromosome from the data, sex became non-

significant (P = 0.260), whereas the effects of other variables remained significant and of similar magnitude (**Table S2**).

Genome-wide differentiation between populations, measured with Weir and Cockerham's F_{ST} (Weir and Cockerham 1984) was relatively low on average (mean F_{ST} = 0.019), in the order of 1 to 2% between habitats for each location ($F_{STBarcelone(rur-urb)}$ = 0.018+-0.001 ; $F_{STMontpellier(rur-urb)}$ = 0.012+-0.001 ; $F_{STWarsaw(rur-urb)}$ = 0.018+-0.001) (see Supplementary Information **Table S3**), suggesting relatively high gene flow and limited genetic drift among populations. Mean F_{ST} on allosomes was lower (mean F_{ST} = 0.014, **Table S5**) than on the Z chromosome (mean F_{ST} = 0.022, **Tables S6**).

Methylation. Similarly to genetic data, we performed an RDA on methylation level of 157741 CpG sites to describe epigenetic variation among individuals in relation to location, habitat and sex. The model was significant but explained less than 1% of the total variance (R^2 = 0.007, P = 0.001). All variables contributed significantly (location : P = 0.001, habitat: P = 0.03, sex: P = 0.001, **Table S7**). Partial RDA revealed that location and sex explained a similar proportion of the total variance which was higher than habitat (location: R^2 = 0.003, P = 0.001; sex: R^2 = 0.003, P = 0.001; habitat: R^2 = 0.001, P = 0.3). When removing the Z chromosome from analyses, results remained similar (**Table S8**), showing that difference in methylation was not entirely driven by potential diverging patterns associated with sexual chromosomes.

We then investigated more finely whether individual methylation on CpG cytosines varied between location, habitat (urban vs forest), sex, and location×habitat interaction, using an ANOVA, run on autosomes and Z chromosome separately (**Figure 1B**, **Table S9**). For autosomes, we detected a significant effect of location (F = 3.319, P = 0.044), with Montpellier individuals showing lower methylation levels that Warsaw ones (**Figure 1B**, Tukey test: P = 0.04) and no other difference between pairs of cities. Also, no significant effect of sex (P = 0.263) or habitat (P = 0.478) was found, suggesting that urbanization did not have an important overall effect on global methylation levels. For the Z chromosome, we found a strong difference between sexes, with homogametic males showing 2.98% more methylated Z than females (**Figure 1B**; P = 1.45x10-15), while no significant difference between location (P = 0.577) or habitat (P = 0.915) was found.

Non parallel strong genomic footprints of divergent selection between urban and forest populations and evidence for polygenic adaptation

We used two methods to investigate outlier SNPs potentially under divergent selection between forest and urban populations: an F_{ST}-outlier based method (v2.1, Foll and Gaggiotti 2008, with default parameters), and a multivariate method (using an RDA, Forester et al. 2016) aiming respectively at identifying strong outliers indicating footprints of differentiation for each population pair and weaker outliers across the several population pairs studied at once. First, Bayescan identified 15 outliers for Barcelona, 11 for Montpellier and 10 for Warsaw, overall distributed on 15 chromosomes and associated to 13 genes in 5kb upstream or downstream regions (**Figure 2**)(q-value < 0.1, see **Figure S1**). None of these outliers was shared between the three population pairs, revealing no convergence between cities. Second, using the multivariate approach based on a RDA (following the procedure for constrained RDA described earlier), we investigated the habitat effect and extracted a list of 1163 loci with outlier loading score following Forester et al 2018 method (**Figure 2**, see **Figure S2** for threshold), suggesting a higher number of loci undergoing weaker and polygenic selection. These 1163 loci were associated with 561 genes.

To investigate the potentially statistically enriched gene ontologies (GO) for the set of genes associated to RDA outliers, we used topGO R package (Alexa and Rahnenführer 2009) with GO referenced for the chicken *Gallus gallus* (ggallus_gene_ensembl). GO analyses revealed the existence of overrepresented ontologies (P < 0.05 and at least 3 genes per GO). Among the most promising GO terms we found functions related to the nervous system (GO:0048846, axon extension involved in axon guidance; GO:0035418 protein localization to synapse; GO:0021987 cerebral cortex development; GO:0007274, neuromuscular synaptic transmission), the blood system (GO:0045777 positive regulation of blood pressure; GO:0042311, vasodilatation),

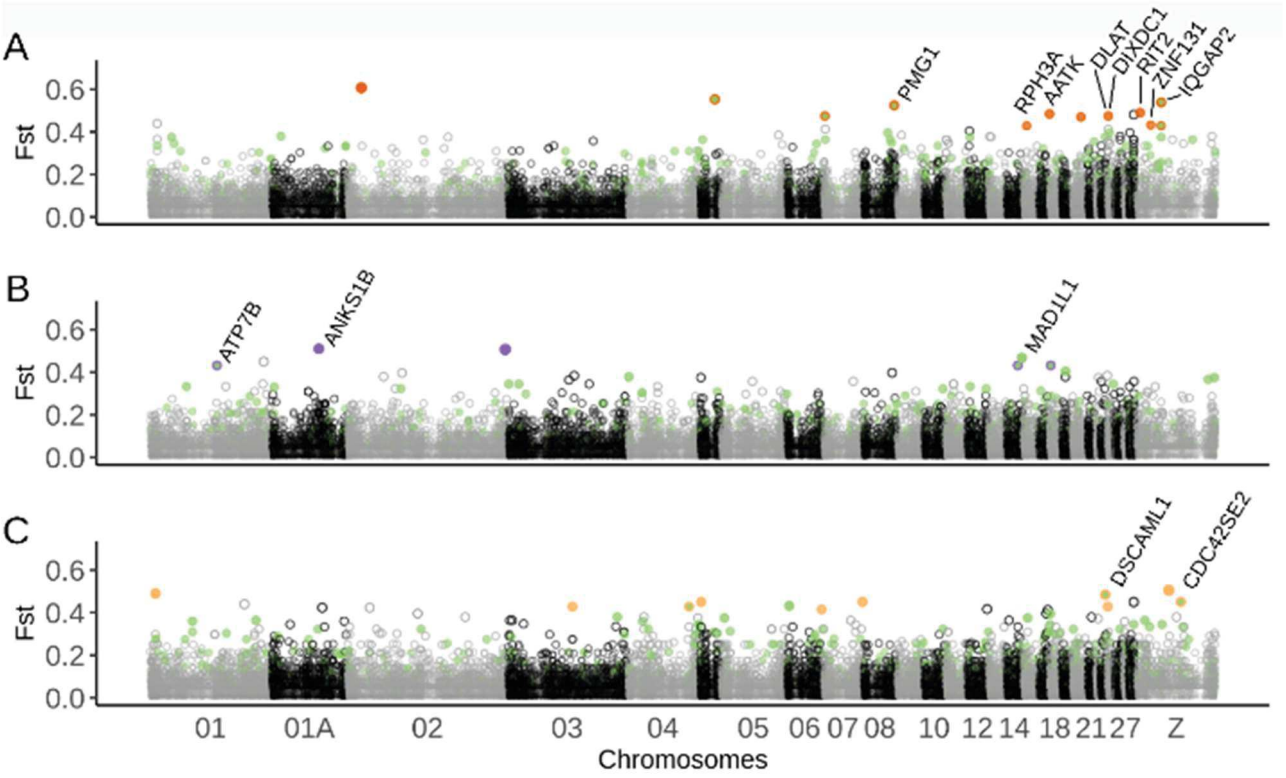

Figure 2 | Manhattan plot of mean F_{ST} between urban and forest populations along the Great tit genome for (A) Barcelona, (B) Montpellier and (C) Warsaw. Dark orange (A), purple (B) and light orange points (C) represent significant outlier SNPs identified by the F_{ST}-outlier test Bayescan for each population pair, given with their associated genes in 5 kb. Green points represent outliers found with the multivariate RDA approach. A few SNPs were identified by both the bayescan and the RDA method and signaled as a green point circled with the colour used for the considered pair.

hormonal response (GO:0071277, cellular response to oestrogen stimuli) and stress (GO:0033555, multicellular response to stress), revealing potential important functions involved in adaptation to urban habitats. Detailed GO results are presented in Supp. Mat. **Table S10** and **Figure S3**.

Evidence for differentially methylated regions in urban environment

We identified a total of 224 distinct DMRs between urban and forest great tits: 80 for Barcelona, 68 for Montpellier and 93 for Warsaw. Only 14 DMRs (6.25%) were found repeatedly in at least two comparisons, among which only 3 were found in the three comparisons. 7 (3.1%) of these 14 parallel DMRs were in the same direction of methylation in urban compared to forest areas (**Figure 3**, **Figure S4A**). Barcelona urban birds presented significantly more hypomethylated than hypermethylated DMRs ($\chi^2 = 11.25$, $P < 0.001$), but no difference was found for Montpellier ($\chi^2 = 0.941$, $P = 0.332$) nor for Warsaw ($\chi^2 = 0.011$, $P = 0.917$). DMR were distributed across all the 32 chromosomes and on 37 unplaced scaffolds. 203 on the 224 different DMR (91%) overlapped genes or 5kb flanking regions, among which 52% were directly located in gene bodies, promoter or TSS sequences (35.3% in gene bodies and 47.3% in promoters/TSS) suggesting their putative functional role in gene expression and regulation and/or splicing events.

Following the procedure previously described, GO analyses on the pooled genes list revealed an overrepresentation of modules associated to nervous system (GO:2000300, regulation of synaptic vesicle exocytosis; GO:0050804 modulation of synaptic transmission), immunity (GO:005728, negative regulation of inflammatory response; GO:0050852, T cell receptor signaling pathway), metabolic activity (GO:006816 calcium ion transport; GO:0055072, iron ion homeostasis, GO:0043087, regulation of GTPase activity), behaviour (GO:0007626, locomotory behavior) and endocrine process (GO:0044060: regulation of



Figure 3 | Circos plot of differentially methylated regions (DMRs) identified between populations of forest and urban great tits in and near Barcelona, Montpellier and Warsaw (from inner to outer circles). Red points show hypermethylated regions in urban great tits relatively to forest birds, and blue points show hypomethylated regions. For graphical clarity, only a subset of genes are represented: genes associated with the 10% most extreme DMR (triangles) and genes found associated with DMR in at least two cities (stars). Names of the genes found within 5 kb of the represented DMRs are given.

endocrine process). All enriched GO are presented in Supp. Mat. **Figure S5** and **Table S11**.

We also searched for DMR between sexes, following the same procedure. We identified 206 DMRs associated with sex, of which 58 for Barcelona, 81 for Montpellier and 99 for Warsaw. On a total of 206 DMR, 181 (57.3%) were on genes or in a 5kb upstream/downstream region around genes. GO analyses revealed enrichment of genes involved in development, growth and morphogenesis, among others (see detailed enriched GO **Table S11** & **Figure S5**).

Almost twice more sex DMRs were shared between locations (11,7%) than between habitats (6.25%, see Figure S4 A & B ; z-test: χ^2-squared = 3.885, P = 0.049). When taking into account the direction of methylation difference, 7 sex DMRs were shared between at least two cities (9.7%)

which was three times more than for habitat DMRs (3.1%; z-test: X-squared = 7.904, P = 0.005).

DISCUSSION

The urban sprawl is a worldwide phenomenon deeply affecting the environment and thus requiring fast adaptive responses in city dwellers. While a large body of literature already describes a myriad of phenotypic shifts in urban populations of numerous species (Chamberlain et al. 2009; Lowry et al. 2013; Sepp et al. 2017), among which great tits, the molecular bases of these shifts and their evolutionary implications remain yet to be documented and understood, which requires further genomic and epigenomic analyses. This study uses for the first time genomic and epigenomic analyses to decipher the potential molecular bases implicated in phenotypic shifts and adaptation in several urban populations of a passerine bird. Genomic analyses revealed weak yet significant average differentiation between urban and forest populations, suggesting ongoing gene flow and limited drift in urban populations. These analyses also identified a limited number of loci putatively under strong selection, non-repeated between pairs, and numerous loci supposedly under weaker selection, compatible with a polygenic model of evolution. From the epigenomic side, we found weak average differentiation of the methylome between urban and forest birds, suggesting an absence of genome-wide epigenetic deregulations, which is notably in line with an absence of strong genetic differentiation. In turn, we identified several strongly differentially methylated regions between urban and forest birds, mostly non-repeated between pairs and hence potentially implicated with local evolution of urban populations. Genes associated to either genomic footprints of divergent selection or differentially methylated regions had relatively similar functions, related to the nervous system, metabolism, immunity and behaviour, that have been repeatedly convicted in other studies. Hence, by identifying non-repeated genetic and epi-genetic responses among replicated forest-urban population pairs, our findings support the hypothesis of mostly non-parallel rapid de novo adaptation to similar environments via both genetic and epigenetic mechanisms. Our results are in line with accumulating evidence that polygenic adaptation and epigenetic reprogramming may be involved in quick phenotypic shifts in response to rapidly emerging constraints such as urbanization.

Overall genetic differentiation between populations was relatively low (F_{ST} ranging from 0.9% to 3.4%), although higher than what has been found at a much larger scale across the species distribution (e.g. around 1% of F_{ST} between UK and Spanish or French and Spanish populations, Laine et al 2016). Low but significant differentiation levels are in line with previously documented divergences between city and forest great tit populations (Perrier et al. 2018; Senar and Björklund 2020), which altogether suggest important gene flow, large effective population size and limited genetic drift at multiple spatial scales (Kvist et al. 2003). This overall genetic context is particularly suitable to search for genomic footprints of divergent selection between urban and forest populations, which would easily be identifiable above the neutral level of genetic differentiation.

We found a limited number of strong footprints of divergent selection, which is in line with previous results of Perrier et al (2018) in Montpellier, and of Salmon et al (2020) across nine European cities. Similarly to low level of parallelism between cities observed by previous studies (Reid et al. 2016; Salmón et al. 2020), none of these outliers were shared between cities, suggesting limited parallel evolution and supporting a scenario of independent *de novo* evolution between cities and/or very different selection pressures between cities. Indeed, there may be multiple evolutionary solutions to the same environmental challenges (Losos 2011) and multiple traits are linked to the same functional outcome (Thompson et al. 2017). Besides, the identification of numerous outliers by the multivariate framework applied at the scale of all six sampling sites supports a model of polygenic urban adaptation implicating multiple genes, biological pathways and phenotypic traits (Boyle et al. 2017). Polygenic adaptation may be expected in urban habitats since the multiple new environmental conditions in cities most probably result in many novel selective pressures acting on a multitude of functional traits (Shochat et al. 2006), and because many of these traits may be quantitative, and genetically correlated (Lande 1979). Further

polygenic analyses on more samples and more markers (i.e. whole genome data) are however required to increase our capacity to estimate more precisely the potential effect of each genetic variant implicated in the adaptation to life in the city (Zhou and Stephens 2012; Robinson et al. 2014).

Several genomic footprints of divergent selection between urban and forest environments were in, or in the vicinity of, several genes that have already been described as playing a role in neuronal development, behaviour or cognitive abilities. In particular, the NR4A2 gene plays an important role in recognition of novel objects and memory in mice (McNulty et al. 2012). Reaction to novel objects and novel food is known as one of the main factors determining the capacity of a species to thrive in an urban environment (Lowry et al. 2013). The DCX gene is related to neuronal plasticity (Kim et al. 2006) and experimental approaches revealed that artificial light at night induces an overexpression of this gene linked to a change in behaviour and expression of depressive-like behaviour in crows (Taufique et al. 2018). Finally, the CHRNA1 gene is associated with aggressive behaviour in chicken (Buitenhuis et al. 2009), and higher aggressiveness is commonly observed and hypothesized as adaptive in urban bird populations (Sprau and Dingemanse 2017). Besides, the gene ontology enrichment analysis, performed on the entire set of genes identified via the outlier genome scan, reinforced these findings since multiple enriched GO terms were associated with the nervous system and stress response as well as hormonal response. These results are informative on the type of traits involved in avian urban adaptation in cities and corroborate previous results from (Sih and Del Giudice 2012; Sol et al. 2013; Salmón et al. 2020) suggesting that natural selection repeatedly acted on neuronal, behavioural and cognitive traits that could contribute to the phenotypic shifts described in urban great tits..

No genome wide pattern of differentiation in methylation between urban and forest great tits was detected. Yet we observed a difference in mean methylation level between birds from Warsaw and Barcelona on their autosomes, as well as between males and females on the Z chromosome, showing that methylation differences were identifiable. In addition, we found no mean difference in methylation level between habitats, revealing that urbanization did not strongly affect overall methylation levels in a specific direction. This overall low differentiated methylation context is perfectly suitable to investigate more localized zones that could differ in their levels of methylation. Note that the strong methylation contrast between males and females, particularly on sex chromosomes (Figure 1B), is in line with previous reports in vertebrates (e.g. Teranishi et al. 2001; Waters et al. 2018) showing that methylation plays a major role in sex differentiation via regulation of gene expression and genetic imprinting.

Despite the non-significant effect of habitat on overall methylation levels, we found a large number of DMRs between pairs of forest and urban populations, suggesting that urbanization did affect particular regions of the genome. DMR were less likely to occur within a gene body than by chance, but it was not the case for promoter or TSS regions. This latter result contrasts with Watson and collaborators (2020) who recently found an under-representation of DMR in both gene body and regulatory regions in urban great tits from Malmö (Sweden). Across the three cities, 35.3% of DMR were directly localized in gene bodies, and 47.3% in TSS or promoter regions, suggesting a potential role in gene expression modulation. Direction of methylation patterns did not follow any absolute pattern (no over-representation of hypo- or hypermethylated DMR in urban birds, Figure 3), in line with Watson and collaborators' analyses on blood sample. However, birds in Barcelona presented significantly more hypo methylated DMR than hypermethylated ones, a result that warrants further investigations.

Only a limited number of urbanization-linked DMR were shared between two or more locations. In contrast, three times more sex linked DMRs we found in two locations or more. Such result suggests that urbanization-linked epigenetic modifications most probably do not occur in a parallel way across cities, but rather that each city might have its particular epigenetic response. Indeed, in the study field of urban evolutionary biology, cities are often regarded as valuable replicates of human induced habitats (Donihue and Lambert 2015; Santangelo et al. 2020), and it is often expected that

parallel adaptive responses to similar selective pressures will occur. This expectation is particularly strong when phenotypes show parallel changes, as is the case for the Great tit, which is consistently smaller and lays earlier and smaller broods in the various cities where it has been studied, compared to forest habitats (e.g. Caizergues et al. 2018; Seress et al. 2020). However, as discussed above, parallel adaptation to similar environmental conditions is not particularly expected in the case of independent evolution, especially for multilocus traits. Additionally, cities are different from each other because of a wide array of climatic, cultural, historical and socioeconomic factors (Szulkin, Garroway, et al. 2020). In fact, besides the obvious differences in cities' climatic conditions depending on their position on the globe, land use, fragmentation and pollutants levels can largely vary across cities (Cárdenas Rodríguez et al. 2016). In a general way, pollutants are known to affect DNA methylation and result in both hypo and hypermethylation, but the pattern of change observed largely rely on the pollutant involved (Head 2014). In this case, differences in cohorts of pollutants present in cities could be responsible for differences in patterns of methylations. Taken together, these results highlight the importance of questioning the assumption that cities are replicated environments that must be considered similar.

Furthemore, no DMR was overlapping with a strong genetic outlier, hence, differences in methylations seem to not directly be linked to an SNP under selection. As mentioned earlier, increasing evidence suggests that DNA methylation can be associated with environmental and stress factors (env: Foust et al. 2016, stress: Sun et al. 2018) especially during early development (Meaney and Szyf 2005). For instance, here we found 4 genes (POMC, ADAMTS3, PAPD4 et GCC1), associated with DMR that were previously described in great tits as undergoing major changes in methylation levels in case of exposure to higher levels of pollutants (Mäkinen et al. 2019). However, the functions of these genes remain to be determined, and they could thus be interesting to target in future studies. In addition, the past literature has repeatedly found SERT and DRD4 as two major genes involved in urban specific avian human avoidance (or wariness) behaviours (see for e.g. Riyahi et al. 2015 in the Great tit, Garroway and Sheldon 2013 in the blackbird, Van Dongen et al. 2015 in the black swan & Mueller et al. 2020 in the burrowing owl) (see Supp Mat Figure S8 to S12 to see patterns of methylation associated with 6 classical great tit linked candidate genes). In this study, we found no DMR associated with these two genes in either of the three forest-city pairs. However, we found a significant urban-related change in methylation linked to the DRD3 gene, belonging to the same gene family as DRD4 and known to be similarly involved in chicken aggressive behaviour (Li et al. 2016). In line with these results, GO analyses revealed enrichment in genes associated with neuronal functions, behaviour but also blood, immune and endocrine systems (Table S10, Figure S3), revealing the potential need of physiological adjustments in urban habitat. Surprisingly, a recent study on great tit differences of methylation between city and forest habitats in another European city found no GO enrichment in blood, but some in liver tissue (Watson et al. 2020, note that they investigated DMSs, Differentially Methylated Sites, which differs from DMRs identified here). These contrasted results highlight the fact that methylation patterns highly depend on the analysed tissues (Derks et al. 2016), and show, once more, that urban linked methylation might not be similar from one city to another. In addition, it has been demonstrated that DNA methylation can undergo seasonal variation (Viitaniemi et al. 2019). Hence, analyses on multiple tissues and life-stages replicated in multiple pairs of urban and forest populations will allow to draw a broader view of the impact of urbanization on global methylation patterns and to understand replicated parallel occurrence across cities. However, tissue-specific and age-specific analyses in multiple individuals across several pairs of urban and forest environments poses major technical, budget and ethical limitations and should be coordinated very carefully. Additionally, specific drivers of shifts in methylation remain to be disentangled to understand which environmental factors are responsible for which change in methylation. To do so, experimental frames manipulating environmental factors such as performed by Mäkinen and collaborators (2019) would be particularly useful. More integratively, information on how shifts in methylation patterns

affect phenotype, fitness, and adaptation, often remain elusive (Sepers et al. 2019). To our knowledge, a limited number of studies attempted to link methylation and levels of expression in natural population contexts (Derks et al. 2016; Laine et al. 2016), and even fewer in urban habitats (but see e.g. McNew et al. 2017; Watson et al. 2020). Hence, future work might need to tackle the question of the origin and adaptive significance of these variations in a controlled framework.

CONCLUSION

In this study, we found genomic footprints of selection and differentially methylated regions associated with urbanization, suggesting that genomic as well as epigenetic processes could play an important role in the rapid adaptation to the urban habitat. To our knowledge, our study is the first to use replicated pairs of cities and forest habitats when studying urban linked methylation, offering a great opportunity to investigate convergent responses to anthropogenic environmental conditions. Taken together, our results revealed limited parallelism between cities regarding selection as well as methylation patterns, suggesting that cities might not present exactly similar environmental conditions or that different genetic pathways are involved in adaptation to urban environmental conditions, while still associated with similar biological functions. Furthermore, we highlight the need to unravel both environmental origins and evolutionary implications of methylation shifts, to understand to which extent environmental induced methylation can contribute to adaptation.

MATERIAL AND METHODS

Study sites and sampling

Three pairs of great tit populations in urban and forest environments were sampled in the three European cities of Barcelona (Spain), Montpellier (France) and Warsaw (Poland). For each location, 10 individuals were sampled within the city and 10 individuals were sampled from nearby forest. Blood samples were collected from breeding individuals during spring between 2016 and 2018 (except 2 individuals for Barcelona city collected in 2014 and 2015) and kept in alcohol or queen's buffer. Samples had balanced sex ratio (5 males and 5 females for each population) except for the forest population of Barcelona where 6 females and 4 males were sampled.

DNA extraction, RAD-seq and Reduced-Representation Bisulfite Sequencing

We used QIAGEN DNeasy blood and tissue kit to extract genomic DNA from blood samples following the provided instructions for nucleated blood samples. DNA was quantified using a NanoDrop ND8000 spectrophotometer and a Qubit 2.0 fluorometer with the DNA HS assay kit (Life Technologies). DNA quality was examined on agarose gels. We then performed RAD-sequencing and RRBS-sequencing using standard protocols. For RAD-sequencing (restriction-site-associated DNA sequencing; Baird et al. 2008), the library preparation was done by the Montpellier GenomiX (MGX) platform (CNRS, Montpellier), using the enzyme SbfI. Each individual was identified using a unique six nucleotides tag, individuals were randomly multiplexed in equimolar proportions by libraries of 37 individuals. Each library was sequenced on a lane of an Illumina HiSeq 2500. Paired-end sequencing was used to produce 150 bp reads. This generated an average of 4.9M reads per individual. The DNA of 60 individuals were processed twice to test for reliability of the genotyping process. The RRBS-sequencing started with DNA digestion using MspI restriction enzymes, which cuts CCGG sites and targets regions that are CG rich, permitting to have a high proportion sequences in promoter regions. Each individual was identified using a unique six nucleotides tag. Individuals were randomly multiplexed in equimolar proportions by libraries of 10 individuals. Bisulfite treatment hence converted unmethylated cytosines into uracil, then converted to thymine after PCR amplification. Each library was then sequenced on a lane of an Illumina HiSeq 2500. Paired-end sequencing was used to produce 50 bp reads. This generated an average of 19.3M reads per individual.

Genomic analyses

Fastp v.0.19.7 (Chen et al. 2018) was used to trim the RAD-seq reads, keeping reads with a minimum quality of 15 before mapping individual sequences

against the reference genome of the Great tit (Laine et al. 2016, GenBank assembly accession: GCA_001522545.3) with BWA v0.7.17 (Li and Durbin 2010). Genotyping was conducted with stacks v2.41 (Rochette et al. 2019) "*gstacks*" and "*population*" functions, using "*snp*" model, filtering for mapping quality > 10, alpha = 0.05, minor allele frequency > 0.05 and observed heterozygosity < 0.65. Loci were retained if present in at least 90% of individuals in each population. Loci with extreme low or high coverage were removed (5% extremes filtered out using vcftools v0.1.15, Danecek et al. 2011). After filtering, 74,137 SNPs were retained for subsequent population genomic analyses.

To document genomic variation among urban and rural great tits from the three locations we used a redundancy analyses (RDA), with location (Barcelona, Montpellier and Warsaw), environment (urban or rural) and sex as explanatory variables. Partial RDA was also produced to test for each variable effect (environment, location or sex) alone after controlling for all other variables. The effect of a given factor was considered significant with a P < 0.05.

To estimate genome-wide differentiation between populations we used Weir and Cockerham's F_{ST} (Weir and Cockerham 1984) computed using the StAMPP R package (Pembleton et al. 2013). Average F_{ST} was estimated using all SNPs, and confidence intervals were assessed using 1000 bootstrap replicates.

To identify SNPs potentially under divergent selection between urban and forest habitats we first used Bayescan v2.1 (Foll and Gaggiotti 2008). Analyses were run for each pair of populations (Barcelona, Montpellier & Warsaw) separately, with default parameter option. As recommended by Foll and Gaggiotti (2008) we considered SNPs as outliers when they displayed a q-value above the 0.1 threshold.

To detect weaker footprints of divergent selection typically expected in polygenic adaptations in response to complex environmental heterogeneity, a multivariate method was used (Forester et al. 2016). Following a similar procedure as described above for the RDA analysis, we used a constrained RDA to investigate the effect of habitat (forest versus city) and to identify outliers SNPs displaying more than3 times SD from the mean score on the constrained axis.

Methylation calling and analyses

The RRBS reads were first trimmed using fastp software v0.19.7 (Chen et al. 2018), and quality filtered keeping only reads with a quality > 15. The Great tit reference genome used for mapping RRBS reads was masked for C-T polymorphism to avoid the possibility of misinterpreting this pre-existing polymorphism as methylation. BISMARK software v0.20.0 (Krueger and Andrews 2011) was used for mapping reads on the masked reference genome with default parameters and 1 maximum allowed mismatches (-N 1). Methylation information for cytosine in a CpG context with sufficient coverage (≥10x) was extracted. An anova on mean individual methylation was run to test for location, habitat and sex effect on overall methylation levels.

Similarly, to genetic differentiation, an RDA was performed to describe epigenomic variation in function of location, habitat and sex. A partial RDA was also conducted to test for the habitat effect alone.

To identify differentially methylated regions (DMR) logistic regression including sex as covariate was performed after tiling regions of 1000pb for each location using MethylKit R package *"calculateDiffMeth"* function. Only DMR having > 10% overall difference between forest and urban habitat and q-value < 0.001 were kept. We then investigated if DMRs overlapped genes in 5 kb upstream and downstream regions using bedtools.

Genes associated to genomic outliers and DMRs, and gene ontology analyses

We investigated if genomic outliers and DMR overlapped genes in 5 kb upstream and downstream regions using BEDTools v2.28.0 (Quinlan and Hall 2010). Gene ontology analyses were performed with the R package topGO (Alexa and Rahnenführer 2009) to identify potential statistically enriched to investigate if on the list of all genes associated to RDA genomic outliers and the list of all genes associated to DMR.

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CHAPITRE V

UNE FEUILLE DE ROUTE POUR L'ÉTUDE DE L'ADAPTATION AU MILIEU URBAIN



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– RÉSUMÉ DU CHAPITRE V –

Ce dernier chapitre de thèse est constitué d'un chapitre de livre sur l'évolution biologique en milieu urbain : Urban Evolutionary Biology, édité par Marta Szulkin, Jason Munshi South et Anne Charmantier et publié par Oxford University Press en 2020, auquel j'ai contribué pendant ma thèse. Il propose une feuille de route pour les biologistes voulant étudier l'évolution en milieu urbain et décrit une liste détaillée des principales étapes (dont plusieurs ont été réalisées dans ma thèse) nécessaires à la compréhension des mécanismes évolutifs associées à l'urbanisation. Ce chapitre développe particulièrement les différentes approches génomiques qui peuvent être mises en œuvre, en focalisant soit sur des gènes candidats soupçonnés d'être impliqués dans les traits importants pour l'adaptation en ville, soit sur des marqueurs génomiques nucléaires (tels que le RADseq utilisé dans le **chapitre IV**), soit enfin sur une approche de séquençage complet du génome.

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– MANUSCRIT 5 –

Adaptation genomics in urban environments

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– ABSTRACT – The field of urban ecology has provided many fascinating examples of organisms that display novel biological features in urban environments compared to natural habitats. Quantitative genetics provides a framework that can be used to investigate whether this phenotypic differentiation between urban and natural habitats is adaptive and is the result of heritable changes in response to divergent selection. New generation sequencing tools offer unique opportunities to expand our understanding of the genes and genetic mechanisms implicated in evolution in urban environments. In this chapter, we first review quantitative genetics studies investigating the mechanisms of evolution in the city. We then review pioneering genomic studies that have shed light on the genes and genetic mechanisms implicated in urban microevolution. We discuss how further use of cost effective high resolution genomic approaches may improve the comprehension of both genomic and epigenomic mechanisms implicated in such evolution. Finally, we provide an overview of how the integrated use of quantitative genetics, field experiments and genomics could expand our knowledge of the processes leading to urban evolution.

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1. INTRODUCTION

Urbanisation has been escalating for a thousand years and is predicted to continue rising, with up to two thirds of the global human population expected to live in cities by 2050 (UN 2014). Urbanisation is the process whereby natural and rural areas are converted to cities. It drives rapid and tremendous environmental changes, including air, light, noise and chemical pollution, altered climate, high human and vehicle densities and modifications of the distribution of organisms' living resources. The wildlife living in the city has to cope with these novel assortments of environmental conditions that may be often stressful, but also sometimes beneficial in terms of increased survival or reproductive success. For the past four decades, urban ecology has provided ample and pervasive evidence that organisms in cities often differ from those in natural habitats in several key phenotypic characteristics, including both morphological and behavioural traits. The field of urban evolutionary ecology builds on its heritage from the field of urban ecology and aims to document and understand the evolutionary mechanisms underlying micro-evolution in urban populations (Donihue & Lambert 2015; Johnson & Munshi-South 2017). Studies have revealed striking phenotypic changes in organisms facing human-modified habitats that are much more significant than those seen in populations in natural habitats (Alberti et al 2015, 2017). Quantitative genetics and population genetics are of growing importance in this field, respectively deciphering genetic and environmental effects on phenotypic variance and decoding the DNA variation behind heritable variation (**Figure 1**), which may ultimately lead to speciation (Thompson et al 2018).

Many of the emerging phenotypic differences found between populations from natural and urban environments have been attributed to phenotypic plasticity (Hendry et al 2008). Indeed, evidence that these trait changes are of genetic origin and are adaptive is still scarce (Pelletier & Coltman 2018), possibly due to a lack of appropriate data. Despite this shortage of data, there is a clear expectation that city populations are exposed to, and might genetically respond to, novel and divergent selection pressures (Alberti et al 2015). However, maybe counter-intuitively, there is also evidence that anthropogenic disturbances often weaken rather than increase the strength of natural selection (Fugère & Hendry 2018), and therefore the nature and strength of selection in cities may be difficult to predict. Similarly, our overall assessment of the frequency and magnitude of adaptation and maladaptation in cities remains very poor, and the presence of one or the other is often hypothesized without being properly tested (Donihue & Lambert 2015; Johnson & Munshi-South 2017). Hence how often, and by which mechanisms, urbanisation results in rapid evolutionary changes, and the nature of these changes, remain open questions. Quantitative genetics can provide answers to these questions, as we review in section 2 of this chapter.

Similar to investigations in other ecological contexts, evolution in cities will depend on the strength of natural selection, on the extent of genetic diversity available for selection to act on, on the strength of genetic drift, and on the extent of gene flow (Johnson & Munshi-South 2017). Population genetic studies of urban organisms have begun documenting genetic drift, gene flow, mutations and genomic footprints of selection in urban populations (Johnson & Munshi-South 2017). Nevertheless, while genetic drift and gene flow have been repeatedly investigated using a wide variety of population genetics methods (see Chapter 4 by Munshi-South & Richardson in this book), an understanding of the molecular mechanisms underlying urban adaptive evolution is only beginning to materialize (Johnson & Munshi-South 2017; Schell 2018). As we review in section 3, genomic tools made available by recent sequencing developments are instrumental in decoding these molecular mechanisms.

It is known that adaptations result from selection for beneficial DNA variants in a population. However, there is growing evidence that epigenetic variation, this partly-heritable variation that does not arise from variation in DNA sequences, can also be implicated in rapid adaptation (Jablonka & Raz 2009). Such epigenetic variation could favour adaptive plasticity and adaptation in new environments. This could happen for example by triggering plastic responses towards the local fitness peak or by increasing mutation rates. In section 4 we discuss the ways that researchers are taking advantage of new sequencing technologies to

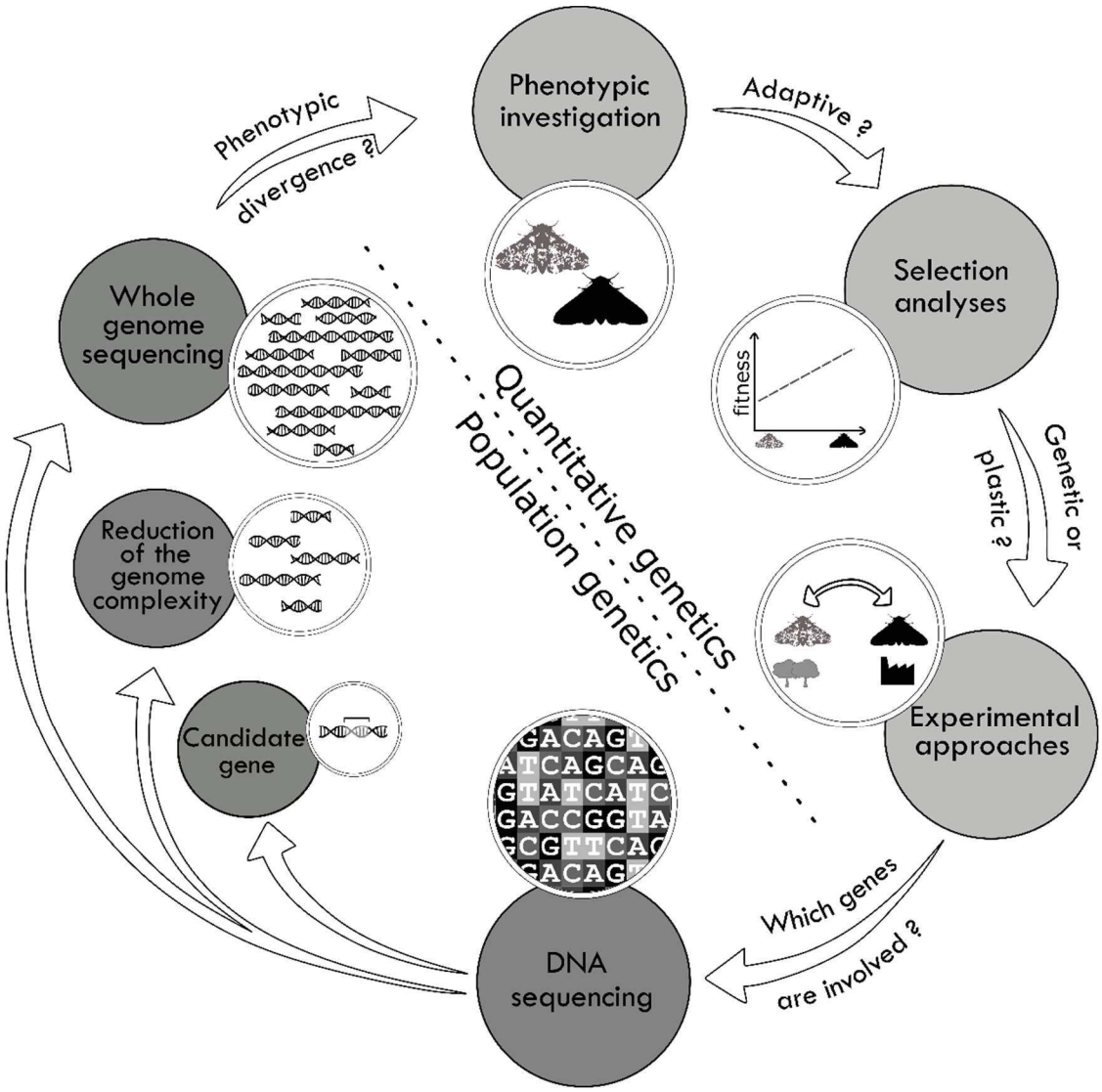

Figure 1 | Inference of evolutionary processes in urban environments. The genetic or plastic nature of phenotypic and fitness responses in urban environments are investigated using quantitative genetic approaches and experiments. DNA sequencing of candidate genes, of reduced genome complexity or of the entire genome, investigates the genes implicated in phenotypic variation, response to selection and local adaptation in urban environments. Note that other possible arrows can be drawn between the different learning steps.

investigate the role of epigenetic variation in urban evolution.

In this chapter, we review how quantitative genetics, population genomics, and epigenomics offer the potential for unprecedented insights into evolution in urban populations. Given the rapidly growing productivity of the Urban Evolutionary Ecology field, this chapter is not exhaustive, but rather presents a diversity of examples illustrating recent advances (see **Table 1**).. We first review how quantitative genetics successfully investigates whether the observed phenotypic variation in urban environments is heritable, adaptive and results from selection. We then review how the population genomics framework offers unique opportunities to pinpoint the genetic variants implicated in evolution in urban environments. We discuss how further use of high-resolution genomic approaches, as well as epigenomics, may improve our understanding of such evolution. We finally conclude on the necessity of integrative studies of quantitative genetics and population genomics for a deeper comprehension of evolution in urban environments.

2. EVOLUTIONARY SIGNIFICANCE OF TRAIT VARIATION IN AN URBAN CONTEXT: EVIDENCE FOR GENETIC ADAPTATION

Evidence of phenotypic divergence between rural and urban populations abounds in the literature

Table 1 | Case studies of adaptive evolution in urban environments. For each type of inference illustrated in Figure 1, we highlight some emblematic studies that are discussed in this chapter.

Field	Method	Results in several emblematic studies
Quantitative genetics	Phenotypic investigation	Melanism is associated with industrial pollution in the peppered moth (*Biston bettularia*) (Ketterwell 1958)
		Differences in morphology, life history and behaviour are found in urban populations of great tits (*Parus major*) (Charmantier *et al* 2017)
	Selection analysis	Non-dispersing seeds in urban sidewalks are selected for in the weed *Crepis sancta* (Cheptou *et al* 2008)
		Divergence in morphology and life history traits are not adaptive but probably result from reduced access to resources in the city in great tits (Caizergues *et al* 2018)
	Experimentation	Resistance to urban water pollution in the Atlantic killifish (*Fundulus heteroclitus*) (Whitehead et al 2017)
		A genetic origin of increased heat tolerance is reported in urban acorn ants (*Temnothorax curvispinosus*) (Diamond *et al* 2018)
Population genetics	Candidate gene	Candidate genes for personality traits (SERT & DRD4) show SNP variations between urban and rural populations in the blackbird (*Turdus merula*) (Mueller *et al* 2013) and the black swan (*Cygnus atratus*) (Dongen *et al* 2015)
		The SERT gene shows genetic and epigenetic variation associated to urbanisation in the great tit (Riyahi *et al* 2015)
	Reduced genomic complexity	Multiple SNPs are associated to urbanisation in great tits (Perrier *et al* 2018), bumblebee (*Bombus lapidarius*) (Theodorou *et al* 2018) and white-footed mice (*Peromyscus leucopus*) (Harris & Munshi-South 2017)
		Multiple differentially expressed genes in the city are found in great tits (Watson et al 2017)
		Epigenetic variations is found in urban populations of Darwin's finches (*Geospiza fortis* and *Geospiza fuliginosa*) (McNew *et al* 2017)
	Whole genome sequencing	A transposable element is identified as responsible for the industrial melanism in British peppered moths (Hof *et al* 2011 & 2016)
		A few structural variants are associated with resistance to freshwater pollutants in the Atlantic killifish (Reid *et al* 2016)

across a wide variety of taxa. However, the evolutionary causes and consequences of such divergence in traits remain largely unknown. Urbanisation is a growing process driving fast and monumental environmental changes that may require adaptation in order to maintain population persistence (e.g. Donihue & Lambert 2014; Alberti 2015; Pelletier & Coltman 2018). Thus, there is a pressing need to understand how wildlife copes with these changes. Is phenotypic variation across urban and natural populations a response to new environmental pressures induced by the city, *i.e.* are urban phenotypes adaptive? Are these responses of a plastic or genetic origin? To address these questions, various methods exist that encompass different subdisciplines of evolutionary biology (**Figure 1**). In this section of the chapter, we briefly outline and discuss some of the methods that do not require the analysis of molecular sequences. In particular, we highlight lessons and results from evolutionary quantitative genetic studies on urban adaptation, *i.e.* studies of the genetic basis of complex traits and of selection acting on these traits, based on individual repeated measures of these traits and the associated fitness within populations.

2.1. Providing quantitative genetic empirical tests of urban-specific selection

Measuring selection in action requires estimating the effects of focal characters on individual fitness. The evolutionary trajectory of these traits in a given population will depend jointly on the genetic basis of (co)variation for these characters and on the direction and amplitude of selection. The form taken by natural

selection and the way it varies across environmental conditions can be quantified using the basic Lande and Arnold (1983) analytical framework to estimate linear or quadratic selection gradients, or via more sophisticated methods that account for statistical complications such as non-normal fitness distributions (e.g. Morrissey & Sakrejda 2013). Although the claim that urbanisation triggers new selection pressures has long been pervasive in the urban evolutionary literature, empirical evidence comparing selection gradients between urban and rural populations is very recent (Fugère & Hendry 2018 PNAS, see e.g. Gorton et al 2018 and Caizergues et al 2018). We believe that this delay is due to the scarcity of individual monitoring programs, and hence individual fitness estimations, in urban environments. Following seven years of monitoring of urban and forest great tits (*Parus major*), Caizergues et al (2018) compared the selection gradients in the two habitats, revealing that the observed divergence in the bird morphology and life history was not adaptive, *i.e.* not aligned with divergence in on-going selection. For instance, while birds laid their brood on average three days earlier in the city, selection for early breeding phenology was found only in the forest and not in the urban habitat. Measuring fitness in the laboratory under different environmental conditions provides a means to bypass long-term programs while assessing the adaptive nature of urban phenotypes. For instance, a shift towards higher heat tolerance in urban acorn ants (*Temnothorax curvispinosus*) was consistent with results showing positive selection for heat tolerance under laboratory rearing conditions, providing evidence for local adaptation of these ants to the urban heat island (Diamond et al 2018; see also Chapter 6 of this book).

2.2. Testing for plastic versus genetic basis of adaptation

Assessing the potential for an organism to adapt to rapidly changing environmental conditions requires a deep understanding of i) the standing additive genetic (co)variation in key adaptive traits, and ii) the potential for phenotypic plastic responses in these traits. If selection analyses reveal that an urban phenotypic shift is adaptive, a plastic origin in the shift can be distinguished from a genetic basis by examining whether quantitative trait differences among populations disappear or are maintained in a common garden experiment. This experimental approach has been successfully applied to show that fragmentation and urbanisation have driven local adaptation in the annual weed *Crepis sancta* (Asteraceae), following its recent colonisation of the city of Montpellier (France). Using a combination of *in situ* and laboratory quantitative genetic experiments, including common garden greenhouse experiments, Cheptou et al. (2008) demonstrated that reduced dispersal observed in the urban weed was the result of rapid contemporary evolution in the proportion of dispersing *versus* non-dispersing seeds produced, and that this was a response to novel selection (see more details on this exemplary case study in Chapter 8 of this book by Cheptou and Lambrecht).

In the previously mentioned study on acorn-dwelling ant species, where selection on thermal tolerance acted in opposite directions between the warmest and coldest rearing conditions (Diamond et al 2018), a common garden laboratory set-up revealed both plastic and genetic processes underlying the higher heat tolerance found in city acorn ants. When raising several colonies of acorn ants sampled in urban and rural sites across three cities, offspring heat tolerance increased with laboratory rearing temperature in all populations, indicative of a plastic response. However, for two of the cities, urban populations displayed greater heat tolerance in warm rearing temperatures, indicative of a genetically based local adaptation.

Compared to common garden experiments in controlled laboratory conditions, reciprocal transplants in field conditions can discriminate between plastic and genetic origins of differentiation, while at the same time assessing the adaptive nature of the differentiation using individual lifetime fitness estimated in the wild. This approach was applied in a recent study on common ragweed (*Ambrosia artemisiifolia*), where seeds collected from multiple urban and rural sites in the Minneapolis-Saint Paul metropolitan area were planted and monitored in two urban and two rural areas (Gorton et al 2018, and see chapter 9 for more details on this study). Results from this experimental field study provide evidence for a genetic divergence between urban and rural populations in multiple phenological traits (including

flowering time) since strong differences were maintained between plants of urban and rural origin. In terms of adaptive divergence, selection was stronger on the foreign genotype in each habitat, rending support for local adaptation. Additionally, the study reported high spatial heterogeneity for flowering time and fitness across the urban areas, suggesting that local urban adaptation should be investigated at a microgeographical scale in this species (approaches to capturing habitat heterogeneity are discussed in Chapter 2 by Szulkin et al.).

Note that while **Figure 1** presents experimental approaches as the classical first step to differentiate between genetic and plastic origins for urban/rural phenotypic differentiation before implementing genomic approaches, this step can be bypassed. Indeed, field or laboratory projects such as common garden experiments are costly in time and effort, and their feasibility largely relies on the type of study species under investigation. In situations where a common garden experiment is a near-impossible task, quantitative genetic approaches based on individual data collected through long-term monitoring programs can test whether a trait is heritable and whether phenotypic differentiation is an adaptive response to selection (Charmantier et al 2014). In such a quantitative genetic framework, multivariate models integrate the (genetic) covariation between measured traits involved in adaptation. Building the genetic variance-covariance matrix (G) for different traits allows taking into account multivariate constraints to evolution (Blows 2007). Since evolution in a trait depends on its genetic covariance with other traits as well as on selection acting on these traits, genetic correlations have been identified as one of the main explanation for evolutionary stasis in studies investigating responses to climate change (Merilä 2012). We believe that the diversity and complexity of traits involved in urban adaptation will make it necessary to go beyond a single trait approach in this context as well.

As illustrated in **Figure 1**, once the adaptive and genetic basis of a phenotypic difference have been demonstrated, DNA sequencing offers a range of methods to investigate which genes are involved (see section 3). Indeed, conclusive support for local adaptation in a heritable and selected trait requires proof that the trait differentiation is genetically determined. Fortuitously, technological genomic advances in recent years are now providing a collection of tools to identify the genetic variation underlying trait variation, and answer the fundamental questions regarding the nature of adaptation in such cases. These tools, described below, can provide evidence for genetic signatures of novel selection in cities and unravel the genetic architecture of urban adaptation (Johnson & Munshi-South 2017; Schell 2018).

3. PINPOINTING GENES IMPLICATED IN ADAPTATION TO URBAN ENVIRONMENTS

Population genomics and quantitative genomics offer conceptual and technical frameworks to identify between-individual variation in the genetic code and address how this variation affects phenotype, fitness and ultimately the evolutionary trajectories of populations in different environments. Many other questions derive from this general topic, particularly those related to the genomic architecture of traits and of local adaptation, such as: does the appearance of novel variation in traits and the local adaptation of populations to novel environmental conditions, here specifically discussed in the context of urban space, rely on standing genetic variation, *de novo* mutations, or both (Barrett & Schluter 2008)? Do such adaptive processes and trait variation involve a few loci with large effects (Mendelian traits), many loci with small effects (quantitative traits), or both? Among different spatial replicates, is evolution parallel (*i.e.* repeated) at the level of individual loci or biological functions or does it follow dissimilar paths?

There are two main reasons why urban environments offer an ideal framework to ask these questions and test the associated hypotheses (Donihue & Lambert 2014; Johnson & Munshi-South 2017). First, urban environments often apply new selection pressures compared to more natural habitats (see previous section), providing a perfect setting to investigate the genetic loci and mechanisms of rapid and genuine adaptation to environmental change. Second, since cities are distributed in a repeated fashion with often similar characteristics, they are ideal to test for the extent of genetic parallelism and its level (*i.e*. gene level *versus* functional level)

during repeated adaptation to similar new selection pressures in geographically distant locations (see Chapter 3 of this book).

3.1. Pioneering use of low-resolution anonymous markers in urban evolution

Before genomic tools applicable to non-model species became widely available, the investigation of the genetic loci underlying trait variation and adaptation in urban environments relied largely on variable anonymous DNA markers. Anonymous markers, such as microsatellites, amplified fragment length polymorphisms and sequence-specific amplified polymorphisms, have mainly been used to infer diversity, differentiation, effective population size and gene flow among urban and natural populations (*e.g.* Wandeler et al 2003; Munshi-South et al 2013; see Chapter 4 of this book). However, they offer relatively little power to identify genes implicated in adaptation to urban environments since the chance that they are linked to adaptive variants is very small. Nevertheless, a few studies have indirectly used these types of markers to link population genetic parameters to fitness estimates, thereby providing information relative to the putative adaptation or adaptive potential of urban populations compared to rural ones. For instance, Hitchings & Beebee (1997) genotyped common frogs *(Rana temporaria)* from urban and rural ponds using allozyme electrophoresis, showing lower genetic diversity, higher inbreeding and lower fitness in urban populations than in rural ones. This decrease in fitness could originate from genetic drift in urban habitats, which are generally less connected and smaller than rural ones. Since anonymous markers can help delineate patterns of gene flow, effective population size and genetic diversity, they can also help test whether urban and rural populations are genetically isolated due to reduced dispersal and / or reduced gene flow (see Chapter 4 of this book). Theoretically, such genetic isolation would facilitate the establishment of local adaptation (Lenormand 2002) in urban environments, if the effective population size were large enough to retain genetic diversity and allow selection to be effective in the face of genetic drift. Other studies have used similar types of anonymous markers to infer mutagenic effects of chemical pollutions in urban areas (Somers et al 2002). Air, water and soil pollution in urban contexts have been related to higher mutation rates in both humans and mice that may be partly heritable (Yauk et al 2018; Somers & Cooper 2009). While new mutations are mostly deleterious or neutral (Eyre-Walker & Keightley 2007), it has been suggested that increased mutation rates in urban environments, and more generally in stressful conditions, could play a role in adaptive evolution (e.g. Taddei et al 1997).

3.2. Candidate genes

The early documentation of the functional roles of several candidate genes in model species (Zhu & Zhao 2007) allowed ecological studies testing hypotheses relative to the roles of such genes on some phenotypic variations in both natural and urban environments. Among the most famous candidate genes studied in the context of urban adaptation are DRD4 (dopamine receptor D4 gene) and SERT (serotonin transporter), both of which are implicated in behavioural variations in humans (McGeary 2009) and other animals (including birds, detailed below). These candidate genes have been extensively screened in urban genetic studies in order to investigate their potential effects on large behavioural variation documented in several organisms living in both cities and natural environments. For example, in an inspiring study, Müller et al (2013) genotyped either microsatellite or Single Nucleotide Polymorphisms (SNP) at seven candidate genes, including DRD4 and SERT, in 792 individuals from 12 paired populations of urban and rural blackbirds (*Turdus merula*) across the Western Palaearctic. The authors found significant and repeated differentiation at the SERT gene between urban and rural populations and a marginally significant pattern for DRD4. They therefore proposed that behavioural traits related to aggression and associated with the SERT and DRD4 polymorphisms in blackbirds might have experienced parallel selection pressures in urban environments. Further studies in other avian species later provided evidence for similar variation at SERT and DRD4 genes underlying behavioural variation between urban and rural environments. DRD4 variation was associated with wariness and urbanisation in black swans (*Cygnus atratus*), with

urban birds being less wary (Dongen et al 2015). In great tits, urban and forest birds showed differentiation at SERT (Riyahi et al 2015), at DRD4 and in behavior (Riyahi et al 2016). Many other candidate gene studies in urban environments involve resistance to chemical substances (reviewed by Belfiore & Anderson 2001). Collectively, these studies demonstrate that adaptation in an urban environment can operate via parallel response to selection on important traits controlled by shared standing genetic variation at a few large effect genes.

Although genotyping candidate genes identified beforehand can be an entry point to studying adaptation in urban environments, the knowledge gained will remain narrow, it will be difficult to generalize and have little statistical significance. Indeed, such candidate gene approaches deliver very little to no knowledge about variation in the rest of the genome, offering virtually no chance to detect any other large effect genes implicated in adaptation. In addition, without such wider information about genetic variation within and between populations, it is difficult to measure the significance of these results obtained for candidate genes. Finally, such candidate gene approach is well suited for a simple trait with a Mendelian basis, but it is a poor choice for studying complex polygenic traits for which many genetic variants may influence phenotypic variation and adaptation (see http://jon-slate.staff.shef.ac.uk/why-im-wary-of-candidate-gene-studies/ for a detailed discussion of candidate gene studies and the specific case of DRD4). Fortunately, genomic tools facilitate the screening of a genome to identify large effect genes responsible for simple trait variations and oligogenic local adaptation. They also allow the identification of the numerous genes and biological functions implicated in complex trait variations and polygenic adaptation, while providing a statistically powerful context using many neutral genetic variants.

3.3. Urban evolution entering the genomic era: methods used so far

With the rapid development of sequencing technologies and the decrease in their cost, it has become possible to considerably increase the resolution with which one could screen genomes of non-model species to pinpoint evolutionarily relevant genomic variation (Stapley et al 2010). A large range of methods has been developed, while trying to optimize trade-offs between genome coverage, number of individuals, and sequencing cost (Schötterer et al. 2014). As the most expensive method, individual whole genome sequencing gives exhaustive information along the entire genome. Moreover, not only SNPs are called but also structural variations (e.g. transposable elements, inversions, copy-number variations, insertions and deletions). For instance, Reid et al generated 20 million biallelic variant sites in the genome of the Atlantic killifish (*Fundulus heteroclitus*) using full genome sequencing and identifying genetic determinants of adaptation in urban polluted sites (see later in this section for a detailed description of this study). Conveniently, it is possible to lower the cost of such genomic investigations, for example by using RAD-sequencing and other Genotyping By Sequencing methods, which enable researchers to call fewer SNPs and do not require strong prior genomic resources or knowledge of the species' genetic resources (Narum et al 2013; Andrew et al 2016). These latter methods rely on the reduction of the complexity of the genome via enzymatic digestions, multiplexing of barcoded individuals' DNA, amplification of fragments targeted by digestion, sequencing of the entire library of fragments, and downstream bioinformatics (Davey et al 2011). For example, Theodorou et al (2018) used a RADseq protocol on a sample of 181 red-tailed bumblebees (*Bombus lapidarius*), calling 110,000 SNPs (discussed later on). While genomic methods – using the entire genomic sequence or taking a reduced genomic complexity approach - have been effective in looking for evolutionarily relevant genomic variants in non-model species sampled in natural and experimental setups, they are only beginning to be implemented in the context of urban evolution (Johnson & Munshi-South 2017). Overall, although genomic tools have been applied in several urban evolutionary contexts oriented towards 'non-adaptive' questions, particularly regarding gene flow and genetic diversity (*e.g.* Combs et al 2018; Miles et al 2018; Mueller et al 2018; See this book's Chapter 4 for further examples), only a few studies have used these tools in order to investigate footprints of urban adaptation. We will review these studies in the next sections.

3.4. Genome wide sequencing pinpointing oligogenic adaptations in urban environments

In a pioneering genomic study of industrial melanism in the British peppered moth (*Biston betularia*), Van't Hof et al (2011 & 2016) identified the genetic origin of the melanic *carbonaria* morph selected for in industrial areas using whole genome sequencing. This colour polymorphism has long been recognized as a simple trait regulated by a single-locus dominant allele. However, the location and the nature of this locus were unknown. After unrewarding candidate gene approaches using 16 genes previously implicated in melanisation pattern differences in other insects (Van't Hof & Saccheri, 2010), Van't Hof et al (2011) constructed a linkage map of the polymorphism, leading to the discovery of a 200kb region on chromosome 17. Several years later, using a full genome assembly and fine mapping of the associated region, Van't Hof et al (2016) showed that the insertion of a large, tandemly repeated, transposable element into the first intron of the gene *cortex* on chromosome 17 was responsible for the *carbonaria* morph. Furthermore, by sequencing a larger set of individuals for this genomic region, the authors inferred that the transposition event occurred around 1819, consistent with the history of the melanic morph records in this species. This textbook example shows how a unique new mutation can control trait variation and adaptive response in a new environment driven by industrial activities.

Reid et al. (2016) studied the genes and genetic mechanisms of resistance to urban/industrial water pollution (polychlorinated biphenyl - PCB and Polycyclic aromatic hydrocarbon - PAH) in the Atlantic killifish (*Fundulus heteroclitus*). Given the dramatic differences in pollution resistance between individuals from polluted and unpolluted waters, it was known that pollution resistance in this species was likely based on a small number of genes (Whitehead et al 2017). Sequencing the entire genomes of 384 individuals with a clever design that implicated both medium and low coverage to lighten the cost, Reid et al. (2016) discovered that several signalling pathways and toxicity-mediating genes were targeted by selection across polluted sites, illustrating parallel evolution at the biological pathway level. They also showed that the populations harboured different footprints of selection at the molecular level (variable deletions and gene copy number), indicating that adaptation took a variety of non-parallel molecular routes, although few genes and similar biological pathways were involved. The authors argued that standing genetic variation was likely the predominant substrate of such adaptation. This study therefore illustrates how a repeated selective pressure in the urban environment triggered divergent molecular evolution underlying a convergent adaptive response at the biological pathway level (see also Chapter 3 for a discussion on parallelism and convergence). Later on, Oziolor et al (2019) used similar genome-wide data to investigate adaptive toxicant resistance that recently evolved in the Gulf killifish (*F. grandis*). The authors found that the resistance in the Gulf killifish was due to a few loci that introgressed very recently from the Atlantic killifish. This second study therefore suggests that interspecific gene flow may be an important source of adaptive genetic variation and of adaptation in such case of rapid and extreme change in selection regime.

These studies illustrate the benefits to be gained from harnessing genomic techniques to identify the genes and genetic mechanisms of adaptation in urban environments. In particular, they show the need for using high-resolution tools that are powerful enough to pinpoint the narrow, and sometimes abundant, footprints of selection associated with adaptation. Whole genome sequencing applied on a few individuals is extremely powerful, and possibly indispensable, for locating narrowly distributed adaptive variants (e.g. the 200kb region for the peppered moth *carbonaria* large effect locus).

3.5. Polygenic adaptation in urban environments

Although strong selective agents in urban environments sometimes promote rapid evolution of monogenic or oligogenic variation associated with simple trait variation (as seen above for melanism in the peppered moth or the resistance to pollution in the killifish), selection in urban environments may also trigger much more complex adaptive responses. First, the differences in selection pressures between urban and natural environments (e.g. Fugère & Hendry 2018) may target many different traits that are, in turn, influenced by different genes. Second, many

traits affected by urban environments might be quantitative, and hence have a polygenic basis (i.e. influenced by many genetic variants of small to intermediate effect size). In this context, genome scans of individuals in urban and natural environments and gene-urbanisation level associations can be used to detect as many of the putative loci implicated in adaptation as is possible. In contrast to studying oligogenic adaptation, studying polygenic adaptation requires genotyping hundreds or even thousands of individuals so that the many variants with small to medium effects expected to contribute to the adaptive response can be identified. A few studies, exemplified in the next paragraph, have tried to identify the potential footprints of divergent selection between urban and rural sites by including relatively large numbers of individuals genotyped at a reduced number of SNPs using RAD-seq, RNAseq or SNP arrays in order to decrease sequencing cost.

Harris et al. (2013) and later Harris & Munshi-South (2017) collected genome scans on white-footed mice (*Peromyscus leucopus*) from New York City that were genotyped first at 31,000 and then at 155,000 SNPs typed using RNA-sequencing. They identified hundreds of genetic variants involving metabolic processes that were associated with urbanisation. Theodorou et al (2018) performed genome scans and gene-urbanisation associations based on 110,000 SNPs genotyped by RAD-seq in red-tailed bumblebees (*Bombus lapidarius*). The authors identified 287 loci potentially under directional selection, some of which were associated with urban land use, and were found in loci most likely implicated in metabolic processes, heat stress and oxidative stress more often than expected by chance. Perrier et al. (2018) used 50,000 SNPs identified with RAD-sequencing to perform gene-urbanisation associations and genome scans in great tits sampled across an urbanisation gradient. The authors identified several genetic variants that were associated with urbanisation. The strengths of these gene-urbanisation associations were however relatively low, suggesting a polygenic answer to selection. Perrier et al. (2018) hence used these loci additively to explain differences in response to urbanisation between populations or individuals, a method that was conceptually inspired by polygenic risk scores.

These studies illustrate the potential benefits from using these cost-effective genomic approaches on subsampled genomes to identify various genes and biological functions implicated in adaptation in urban environments. However, we must recognize that these studies probably did not identify all of the genes implicated in urban adaptation. Indeed, while methods based on a genome with reduced complexity (like RAD-seq) can be appropriate for species displaying high linkage disequilibrium, they might very well result in insufficient coverage of the genome for species displaying low linkage disequilibrium (Lowry et al 2016 & 2017; but see Mckinney et al 2017 & Catchen et al 2017). In the latter case, methods based on whole genome sequencing (as used by Reid et al (2016) and Van't Hof et al (2016)) should probably be favoured. Nevertheless, identifying the loci implicated in polygenic adaptation may be challenging, even with full genome data, since this task requires both to localize the many loci implicated and many individuals to successfully estimate the small effect size of these loci. It is thus likely that future studies aiming at deciphering polygenic adaptation in urban environments will need to genotype both more loci and more individuals.

3.6. Further use of genomics in the field of urban evolution: methodological and taxonomic perspectives

Since sequencing whole genomes requires a large budget, researchers develop methods to decrease the cost while nevertheless sequencing numerous populations or individuals. One approach is to sequence full genomes at low coverage. For instance, Reid et al (2016) first sequenced one pair of natural and urbanised-polluted populations at medium coverage (average read depth: 7x) and 3 additional pairs at low coverage (0.7x). Another way to decrease the cost of sequencing, which has not yet been used in the urban context, is to sequence pools of 50 to 200 individuals ("pool-seq" or "pooled sequencing"; **Figure 2**) to capture average allele frequencies (Schlötterer et al 2014). This method produces cost effective estimates of differentiation between populations based on large sample sizes (i.e. numerous individuals are generally needed to estimate allele frequency changes between large

connected populations). A strategy at the crossroads of pool-seq and individual genome sequencing is to sequence individual genomes at low coverage and to then pool sequences in a flexible way (*e.g.* by sex, location, or type of environment; Micheletti & Narum 2018). This integration of low coverage whole genome sequencing and pool-seq seems very well suited for pinpointing adaptive loci in urban contexts, which typically requires genotyping many individuals but also comparing pairs of several urban and rural sites. However, pool-seq and low coverage sequencing also have limitations, notably the difficulty to estimate linkage disequilibrium and to distinguish sequencing errors from low-frequency polymorphisms (Schlötterer et al 2014).

Several other methodological considerations might very well be important for deciphering the genomic footprints of adaptation in urban environments. First, the mutations fuelling adaptation in the city appear to have diverse origins since the few studies to date have revealed the roles of single nucleotide polymorphisms, transposable elements, copy number variations, and insertions/deletions. Therefore, statistical analyses of short reads that have mainly focused on SNP variation could dedicate more effort to detecting the structural variation implicated in adaptation, taking advantage of both specialized software and online databases (Albers et al 2011; Duan et al 2013). Additionally, while genomic studies often use short read sequencing, long reads may improve the detection of structural variants (Disdero et Filée 2017).

There are other genomic aspects of adaptation in urban environments that can also be screened using recent genomic advances. Particularly, metagenomics has the power to pinpoint taxonomic diversity and adaptive variation in microbial communities in urban environments. Indeed, while the studies of adaptation in urban environments have yet primarily focused on relatively large organisms, microbial species are probably the organisms responding most rapidly to selection in urban environments and could be studied relatively easily using genomics (e.g. Su et al 2017; Gouliouris et al 2019). Furthermore, microbiomes

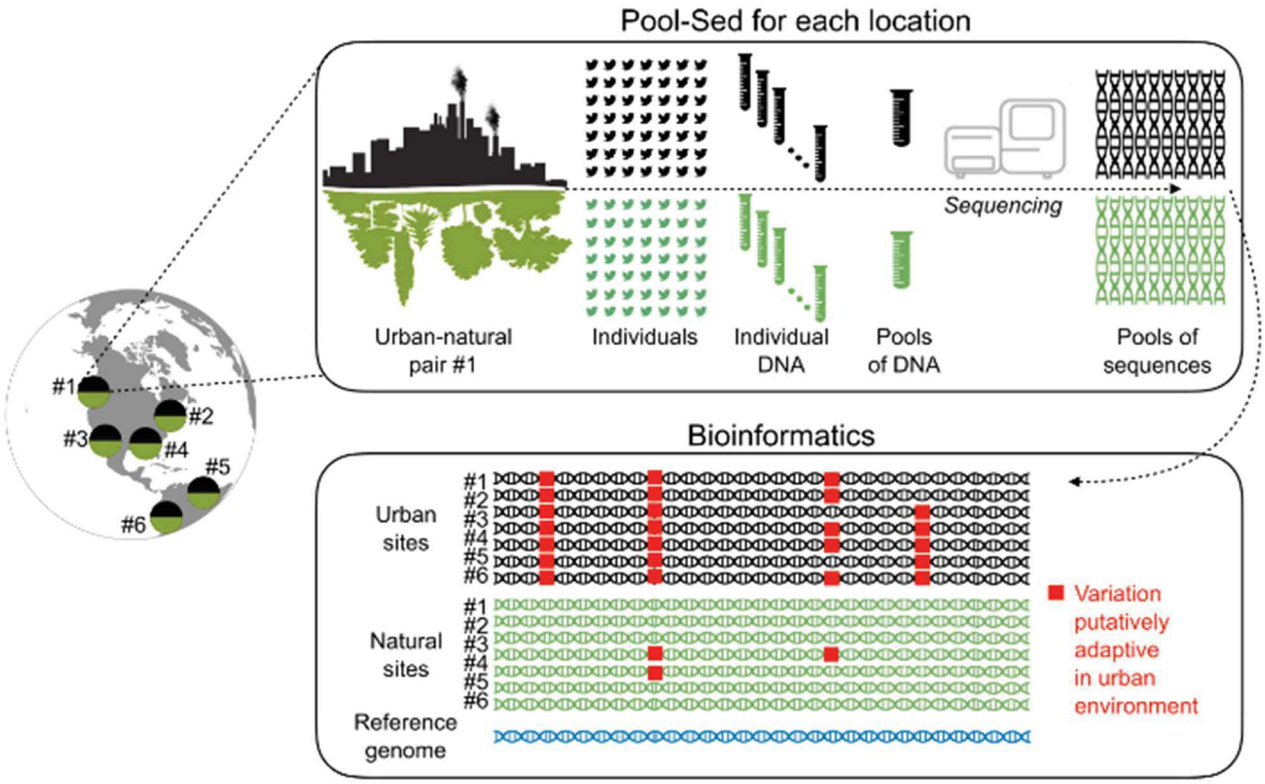

Figure 2 | Illustration of the potential implementation of Pool-Seq for population genomics in urban environments. DNA extracts from several individuals are pooled and sequenced for each urban and natural environment replicated across space. Bioinformatic analyses: align reads to a reference genome, genotyped SNPs and other structural variants, and reveal putatively adaptive variations in urban environments.

(e.g. gut & skin microbiomes) diversity and adaptation may be involved in the acclimation and adaptation of their host organisms (Alberdi et al 2016) notably in urban environments (e.g. Saxena et al 2018; Teyssier et al 2018). Therefore, it makes no doubt that metagenomics will play an important role in ecological studies aiming at deciphering diverse cases of adaptation in urban environments.

4. EPIGENETICS AND THE CITY

Although adaptation is achieved by response to selection via standing additive genetic variation or new genetic mutations, epigenetic variation may also be implicated (Verhoeven et al 2016). Epigenetics can be defined in several ways, all aiming to understand the heritable molecular variations giving rise to different phenotypes from a similar genetic sequence (Felsenfeld 2014). In the context of studying urban adaptation, the probably most appropriate definition of epigenetics is the study of the sources of heritable (or partly heritable) phenotypic variations that do not arise from nucleotide variation and that are induced by environmental variation, sometimes referred to as transgenerational epigenetic inheritance. In particular, epigenetics examines features and mechanisms contributing to the regulation of gene expression and hereby affecting phenotypes. These features and mechanisms include histone modification, DNA methylation, and non-coding RNAs. The transgenerational stability of epigenetic features induced by transitory environmental variation, in particular DNA methylation, varies greatly from no heritability to stable transmissions over hundreds of generations (reviewed by Jablonka & Raz 2009). This transient epigenetic response may favour adaptation in new environments, because the plasticity resulting from epigenetic regulation may on the short term allow gaining time for adaptation to happen, and because some epigenetic processes like methylation may on the long term increase mutation rates that could fuel adaptation (Danchin et al 2018)). Taking advantage of the development of sequencing technologies, epigenetic features and mechanisms have been successfully investigated in various contexts, although mainly in humans and other model species (reviewed by Allis & Jenuwein 2016).

In the context of ecological studies, including urban environments, epigenetic studies have focused mainly on the level and distribution of methylation. Indeed, several protocols have been developed to investigate methylation patterns using bisulfite treatment, immunoprecipitation, or methylation sensitive enzymes, followed by sequencing of a candidate gene, a genome that has been reduced in complexity (*i.e.* a given proportion of the genome) or the entire genome (reviewed by Jones 2012, Jeremias et al 2018). We describe here two recent studies of methylation patterns in urban environments in birds.

In our first example, Riyahi et al (2015) estimated methylation patterns of two famous candidate genes for personality traits, DRD4 and SERT, in great tits from urban and forest environments. They applied bisulphite treatments of the DNA (the treatment of DNA with bisulfite converts cytosine to uracil and leaves 5-methylcytosine unaffected) and strand-specific sequencing to perform the methylation profiling of the CpG dinucleotides in the DRD4 and SERT promoters and in the CpG island overlapping DRD4 exon 3. They also used pyrosequencing to quantify the methylation levels at each CpG. Riyahi et al found 1% to 4% higher methylation in urban birds than in forest birds. One CpG dinucleotide located 288 bp from the transcription start site of SERT was related to the exploration score in urban birds. The analyses also revealed that the methylation difference was repeatable in DNA extracted from blood and from brain. Indeed, methylation pattern often differ between different types of cells, notably between brain and blood cells in great tits (Laine et al 2016). On the genetic side, one SNP in the minimal promoter of SERT was associated with novelty seeking behaviour in captivity and the allele favouring novelty seeking was found at higher frequency in urban birds. Riyahi et al (2015) therefore concluded that both genetic and epigenetic variability in the SERT gene have an important role in shaping potentially adaptive variation in personality traits in great tits from urban and forest environments.

In our second example, McNew et al (2017) investigated epigenetic variation in Darwin's finches (*Geospiza fortis* and *G. fuliginosa*) in rural and urban environments. Instead of using a candidate gene approach like in the first example, they used

methylated DNA immunoprecipitation (MeDIP) in two different tissues to survey genome-wide methylation differences (or differentially methylated regions – DMRs) between urban and rural birds of both species. They identified thousands of DMRs between urban and rural populations, enriched for loci implicated metabolic pathways, and MAPK and TGFß/BMP signalling. However, very few of these DMRs were shared between the two types of tissues or between the two species, suggesting that such analyses may require more power, gained via higher coverage of the genome and/or more individuals.

Several studies have also documented how urban parameters can alter methylation patterns in lab mice. Yauk et al (2008) investigated DNA damage, mutation, and DNA methylation in the gametes of male mice experimentally exposed to particulate air pollution in an urban environment. In mice exposed to ambient air, the study showed a 1.6-fold increase in sperm mutation frequency at a particular locus and their DNA methylation was higher than that for mice breathing filtered air. Hypermethylation remained significantly elevated in mature sperm after removal from the environmental exposure. The authors stressed that mutations and hypermethylation of the germline have the potential to affect the descendants of exposed individuals, particularly in terms of genome stability and regulation of gene expression.

Other epigenetic features can be studied using recently developed protocols (Harris et al 2010; Schield et al 2015; Allis & Jenuwein 2016; Verhoeven et al 2016), and might be of particular importance in the context of ecological studies of rapid adaptation in urban environments. These protocols, like the genomic ones we have discussed, are flexible and the costs can be alleviated by using pooling strategies or by restricting sequencing to reduced parts of the genome, such the GC reach ones. Indeed, it remains very costly to get access to the entire methylome of many individuals, and the characterisation of methylation patterns requires relatively well-annotated genomes. Furthermore, it will be necessary to investigate the functional link between DNA methylation (as measured in e.g. McNew et al 2017) and gene expression (as measured in e.g. Watson et al 2017), as well as to assess the heritability of epigenomic marks.

5. CONCLUSIONS AND SUMMARY OF THE PERSPECTIVES

Urban Evolutionary Ecology is a young and dynamic field, with more questions than answers (Johnson & Munshi-South 2017). Opportunistically, this field builds on urban ecology, efficiently using the theory and tools developed in the broader field of evolutionary biology. As we highlighted in section 2, quantitative genetics uses long term or experimental data to test whether urban phenotypes are adaptive and whether they are the result of plastic or genetic responses. We have cited some of the numerous success stories in diverse taxa (see **Table 1**), showing either plastic or genetic responses to city life that allow the species to thrive in the urban setting. Now that evolutionary ecologists are firmly considering urbanisation as a unique opportunity to study evolution in action, many more case studies with correlative or experimental quantitative genetics are anticipated in the near future. However, limitations to the applications of quantitative genetics in the city will be similar to those for studies in natural environments, notably the difficulty to measure fitness, to obtain pedigrees and to conduct in situ experiments. In particular, the increasingly short-term nature of funding and contracts threaten the sustainability of long term monitoring projects even though they have proved highly productive in the past (Clutton-Brock & Sheldon 2010). A valuable feature of research projects led in urban environments is that they are often well-suited for launching new Citizen Science projects. Involving city-dwellers in data collection can reduce the research cost, and also has many benefits in terms of Science dissemination and reconnecting people with Nature (Schuttler et al 2018). While the general public's view of biodiversity is most often inspired by ecological studies and conservation efforts on iconic wildlife, urban evolutionary ecology will most likely involve many good model species that are not often thought about by non-scientists, such as *Daphnia magna* or the weeds *Crepis sancta* and *Trifolium repens.* Citizen science on these models will provide a wider perspective for citizen scientists on the ecology, evolution and conservation of biodiversity.

As highlighted in section 3 of this chapter, genomics has started to allow the identification of genes implicated in evolution in urban populations.

Evolutionary responses to selection most likely involve a highly variable number of loci, from the monogenic or oligogenic variation implicated in resistance to pollutants or in the diversification of colour morphs (*e.g.* Reid et al 2016; Van't Hof et al 2016), to the polygenic variation potentially underlying many traits under selection (*e.g.* Harris & Munshi-South 2017; Perrier et al 2018; Theodorou et al 2018). The simultaneous growth of genomics and urban evolutionary ecology will most likely result in many new exciting insights into the genetic mechanisms and genes implicated in urban evolution. Genomic studies implementing whole genome re-sequencing will likely identify the narrow and potentially numerous footprints of selection (Lowry et al 2016 & 2017; but see Mckinney et al 2017 & Catchen et al 2017). However, although sequencing costs have consistently decreased, whole genome re-sequencing is still expensive and therefore alternative strategies using Pool-seq (Schlötterer et al 2014; Micheletti & Narum 2018) may be necessary to allow the application of high-resolution tools without prohibitive costs. These methods will also probably be more suitable for designs with replicated urban versus natural comparisons. Other genomics developments, such as measuring selection gradients on individual SNPs (e.g. an example from a non-urban context: Mullen & Hoekstra 2008) or considering the diverse nature of variants implicated in evolution more systematically (e.g. structural variants) and using metagenomics will also strengthen the understanding of evolution in cities.

As highlighted in section 4, epigenetics might be involved in rapid evolution in the city. Epigenomics is offering a growing diversity of tools that can be used to detect the assortment of epigenetic marks and mechanisms that may be involved and to elucidate their potential influence in urban adaptation. These developments will coincide with investigations in the broader field of Evolutionary Biology aimed at understanding the role of epigenetic mechanisms in Evolution in general, and in novel environments more specifically. Pioneering studies have already started to investigate potential epigenomic marks implicated in evolution in urban environments, and there is no doubt that this field will grow quickly in the next decades.

For a comprehensive view of urban evolution, these genetic, genomic and epigenomic tools will need to be used in an integrative way, along with detailed studies of population demography and of the spatio-temporal structure and intensity of urbanisation. Indeed, the age and size of urbanised patches, in combination with the species demography, will probably determine the likelihood of adaptation (as discussed in Perrier et al 2018). For example, older urbanised environments and species with short generation time are more likely to provide case studies on adaptive evolution than are long-lived species in recently urbanised areas. Also, species with small foraging and dispersal distances in large urbanised areas will be more likely to display adaptations than species with long dispersal distances or in small urbanised areas. Finally, more intense urbanisation should result in stronger selection on adaptive traits, and hence provide more opportunities to witness footprints of urban selection. Overall, the temporal and spatial heterogeneity of the urban environment both within and across cities offers unique possibilities to approach eco-evolutionary processes in a comparative manner (across species and cities), well beyond a single species urban *versus* rural comparison.

GLOSSARY

Selection gradients: Partial regression coefficients of relative fitness on a given trait.

Local adaptation: Evolution of a higher mean fitness of a local population in its native environment compared to other environments, and compared to other populations in this environment.

Standing genetic variation: Allelic variation that has been segregating within a population.

***De novo* mutation:** Allele appearing in a population by new a mutation event.

Sequencing: Determining the succession of nucleotides along DNA segments.

Whole Genome Sequencing (WGS): Determining the complete DNA sequence of an organism's genome.

Single Nucleotide Polymorphism (SNP): Recurrent variation of a single nucleotide at a specific position in the genome.

Structural variants: Variation in the structure of the genome, within chromosomes, such as deletions, insertions, duplications, copy-number variations, inversions and translocations.

Genotyping By Sequencing (*sensu lato*): Methods that reduce the complexity of multiple DNA samples before sequencing them and eventually calling SNP.

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DISCUSSION



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L'urbanisation croissante représente un défi pour la biodiversité. Alors que de nombreuses espèces évitent les zones urbanisées, on y retrouve tout de même des populations sauvages bien établies qui sont confrontées à une myriade de nouvelles conditions environnementales dont une partie correspond à des variants possibles observés dans la nature (ex : fragmentation), d'autres à des conditions 'originales' liées à l'artificialisation humaine (ex : nuisance sonore, lumineuse). L'objectif principal de ce présent travail était d'explorer les impacts de la vie en milieu urbain sur le phénotype des populations urbaines de mésanges charbonnières (**Chapitre II**), d'en identifier les implications évolutives (**Chapitre III**), ainsi que d'explorer les potentielles traces d'adaptation génomique et les variations épigénomiques spécifiques aux populations urbaines (**Chapitre IV**). A travers un suivi à long terme d'une paire de populations de mésanges charbonnières urbaines et forestières ainsi qu'à l'aide de collaborations européennes (**Chapitre IV**), j'ai pu, dans un premier temps, confirmer l'existence de variations de traits phénotypiques particulières aux individus urbains. En effet, les mésanges urbaines présentent des différences morphologiques, de traits d'histoire de vie et comportementales. Elles ont en moyenne une taille réduite et des ailes et une queue plus courtes, mais pas nécessairement une condition corporelle plus faible (résultat dépendant du proxy de condition corporelle utilisé voir **Manuscrit 1** et **Manuscrit 2**). De même chez les poussins urbains on constate une taille plus petite, mais pas de réduction de la condition corporelle. Certaines variations morphologiques sont aussi détectées au sein de l'environnement urbain, le long d'un gradient d'urbanisation ; de façon intéressante, ces variations dépendent souvent de l'année, du sexe ou de l'âge des individus. Concernant la reproduction, on constate des dates de ponte plus précoces et un nombre d'œufs réduit en milieu urbain. Enfin, les mésanges urbaines ont des comportements plus explorateurs et sont plus agressives, et elles ont une plus forte réponse au stress induit par la manipulation par l'Homme.

Les analyses de sélection réalisées dans le **Chapitre III** révèlent peu de sélection sur les traits étudiés. Dans les rares cas où l'on détecte de la sélection dans un habitat ou l'autre, celle-ci ne va pas dans le même sens que la divergence phénotypique observée, et se produit parfois dans le sens opposé. Par exemple, la sélection naturelle favorise en ville les individus reproducteurs qui pondent des couvées plus grandes et qui ont des rythmes respiratoires plus lents alors que les oiseaux urbains pondent en moyenne deux œufs de moins qu'en forêt et montrent un rythme respiratoire accéléré. De plus, on détecte parfois de la sélection en milieu forestier (sélection positive pour le tarse des mâles forestiers, pontes précoces ou encore des comportements plus explorateurs en forêt) qui disparait en milieu urbain.

Au niveau génomique, nous détectons peu de loci sous forte sélection associés au milieu urbain dans les génomes, mais une multitude de traces de sélection faible et polygénique. Par ailleurs, plusieurs régions du génome sont différentiellement méthylées entre populations urbaines et rurales, et ces zones se situent majoritairement sur des promoteurs, des TSS ou des corps de gènes, indiquant leur potentielle importance dans la régulation de l'expression génétique. Des analyses d'ontologie de gènes révèlent que la liste de gènes associés aux loci sous sélection est significativement enrichie en gènes participant à des catégories associées au système nerveux, au système sanguin, à la réponse hormonale et au stress. De même, parmi les gènes associés au régions différentiellement méthylées on trouve un enrichissement de gènes dans des fonctions liées au système nerveux, à l'immunité, à l'activité métabolique, au comportement et aux processus endocrines. Ces résultats suggèrent que ces catégories fonctionnelles sont particulièrement importantes dans l'adaptation des oiseaux au milieu urbain.

I. LA SELECTION NATURELLE EN MILIEU URBAIN

I.1. Un phénotype urbain particulier, mais non-adaptatif

Les différences phénotypiques observées entre habitat urbain et habitat forestier concordent majoritairement avec des résultats précédemment rapportés dans la littérature, bien que peu d'études aient eu des approches multivariées et multi-âges. En effet, bon nombre d'études rapportent des individus

urbains globalement plus petits (Liker et al. 2008; Meillère et al. 2015), ayant une reproduction plus précoce (Chamberlain et al. 2009), pondant moins d'œufs par ponte (Chamberlain et al. 2009), et présentant des comportements plus agressifs et explorateurs (Evans et al. 2010; Atwell et al. 2012) ainsi qu'une réponse au stress accrue (Torné-Noguera et al. 2014 mais voir Abolins-Abols et al. 2016; Senar et al. 2017). A l'inverse, nos résultats de survie légèrement réduite en milieu urbain contraste avec ce d'autres études (Hõrak & Lebreton 1998). Et de manière générale, l'ensemble de nos résultats est contraire aux prédictions formulées dans le cadre des syndromes de rythme de vie (**Introduction**, **Figure 4**), montrant que la vie en milieu urbain n'est pas nécessairement associée à des rythmes de vie lents.

La survie égale ou légèrement diminuée observée dans notre cas d'étude peut être due aux particularités environnementales liées au climat méditerranéen. En particulier, l'hypothèse d'une meilleure survie en milieu urbain découle de l'impact métabolique important que représente l'hiver dans les zones tempérées, et l'effet îlot de chaleur dans les villes réduit ce coût métabolique et la forte mortalité qui lui est associée. Or, en milieu méditerranéen l'hiver est moins froid que dans des régions au climat continental, ainsi, le bénéfice apporté par l'effet îlot de chaleur en ville y est potentiellement réduit et au contraire peut entrainer un stress thermique important en été en induisant des températures caniculaires.

Par ailleurs, un résultat équivoque concerne la différence de condition corporelle observée entre les deux habitats qui semble dépendre du proxy utilisé. En effet, en utilisant le « Scaled Mass Index[7] » comme décrit par (Peig & Green 2009) aucune différence significative n'est détectée (**Manuscrit 1**) alors qu'en utilisant le proxy qui consiste en corriger la masse individuelle par la longueur du tarse dans un modèle linéaire (masse ~ tarse + autres effets, **Manuscrit 2**) on détecte une condition corporelle significativement réduite chez les individus urbains. Ces résultats contrastés soulignent l'importance cruciale du choix de proxy à utiliser dans les études comparatives. Dans ce cas précis, d'après Peig et Green (2009), le « Scale Mass Index » représente l'estimateur le plus fiable de la condition corporelle, alors que le proxy « lm(masse ~ taille) » sous estimerait la relation entre masse et taille. Ainsi, les résultats les plus fiables ici semblent être l'absence de différence de condition corporelle rapportée dans le **Manuscrit 1**. Les individus urbains présentent donc une condition corporelle équivalente aux individus forestiers, laissant penser que leur taille réduite leur permettrait de maintenir un rapport taille/poids similaire, malgré une disponibilité en ressources plus faible.

Les analyses de sélection réalisées dans les **Manuscrits 2** et **3** révèlent que les variations de phénotypes entre habitats ne sont pas alignées avec les patrons de sélection mesurés. Ces résultats constituent une première étape dans l'explication de la variation phénotypique associée au milieu urbain et vont encore une fois à l'encontre des prédictions jusqu'alors énoncées dans la littérature et des attendus entrant dans le cadre de la théorie des syndromes de rythme de vie. En effet, la majorité des études observant de la divergence phénotypique associée à l'urbanisation suggèrent une probable origine adaptative de cette divergence (*e.g.* Atwell et al. 2012), guidés par l'hypothèse largement répandue que la vie en milieu urbain est associée à de nouvelles pressions de sélections, bien que ces nouvelles pressions aient rarement été quantifiées (sauf notamment par Ibañez-Alamo & Soler 2010; Senar et al. 2014). Pourtant, tout au long de nos travaux présentés ici, nous ne trouvons aucune preuve que la divergence phénotypique observée en milieu urbain ne confère de manière contemporaine un avantage en terme de reproduction et/ou de survie. Au contraire même, il semblerait que certains phénotypes émergent malgré le fait qu'ils soient contre sélectionnés. Par exemple, le fait d'avoir une réponse accrue au stress de manipulation (rythme respiratoire plus rapide, **Manuscrit 3**) est corrélé à un succès reproducteur plus faible des femelles en milieu urbain, pourtant les individus urbains présentent malgré tout un rythme respiratoire plus rapide que les forestiers.

Par ailleurs, la méthode de mesure de sélection développée par Lande & Arnold (1983) n'estime que la sélection en cours sur les traits. Or, la sélection ayant agi par le passé peut aussi avoir joué un rôle important dans l'établissement et l'adaptation des populations urbaines. Les sélections stabilisante ou

[7] Le « Scale Mass Index » (ou Index de masse : masse attendue des individus si tous les individus faisaient la même taille

directionnelle ont tendance à réduire la variance, il est donc possible d'avoir une indication de la sélection passée en en étudiant la variance des traits. De plus, les analyses génomiques permettent aussi de rechercher des traces d'adaptation dans les génomes. Encore une fois, les analyses de variances réalisées dans cette thèse ainsi que la recherche de traces de sélections sont en accord avec le fait que les phénotypes urbains ne soient pas adaptatifs. En effet, la variance des traits n'est pas réduite en milieu urbain (**Chapitre III**) et les analyses génomiques ne révèlent qu'un nombre réduit de traces de sélection forte dans les génomes (**Chapitre IV**), trop peu nombreuses pour expliquer le grand nombre de traits phénotypiques variant.

Concernant les changements de patrons de méthylation, on en sait très peu sur l'impact qu'ils ont réellement sur le phénotype, bien qu'ils touchent des catégories de gènes qui peuvent potentiellement avoir un rôle majeur dans l'adaptation face aux conditions urbaines. Pour mieux comprendre le rôle de ces différences de méthylations, de futures analyses sont nécessaires pour (1) comprendre dans quelle mesure les patrons observés peuvent entrainer des différences d'expressions de gènes et (2) faire le lien entre patrons de méthylation et valeur sélective des individus en fonction de l'habitat (voir **Figure bilan**).

I.2. Pourquoi peu de sélection en ville ?

II.2.a Pas ou peu de sélection

L'absence de détection de sélection en milieu urbain peut être expliquée par le fait que la sélection naturelle y soit relaxée. En effet, les conditions environnementales nouvelles peuvent tamponner certaines contraintes initialement présentes dans les habitats naturels (Rodewald & Arcese 2017). Par exemple, cet effet tampon peut résulter d'une augmentation de la prédictibilité ou une homogénéisation des ressources et ainsi découpler les individus urbains des cycles naturels de fluctuation de la nourriture (Shochat et al. 2006; Buyantuyev & Wu 2012). En particulier, une telle homogénéisation des ressources peut avoir un impact sur la sélection agissant sur la condition corporelle ; la « credit card hypothesis » énoncée par Shochat (2004, "hypothèse de la carte de crédit" en français), prédit par exemple qu'en cas de ressources prédictibles, il ne serait plus nécessaire de stocker du gras et de maintenir une forte condition corporelle. Cependant, nos résultats ne semblent pas en accord avec ces prédictions puisque la condition corporelle n'est pas plus faible en ville qu'en milieu urbain en moyenne (**Manuscrit 1**). Cette prédictibilité des ressources peut aussi avoir un fort impact sur les pressions agissant sur la date de ponte. En effet, chez les passereaux insectivores, et particulièrement les mésanges, la date de ponte est un caractère fortement relié à la valeur sélective car elle détermine la synchronie entre la période de besoin maximal en nourriture des poussins pour leur croissance et la période d'abondance des chenilles dans le milieu. En milieu urbain, on ne constate pas de sélection sur la date de ponte, ce qui est potentiellement dû à une disponibilité, moindre certes, mais continue, des ressources alimentaires et l'absence d'un pic d'abondance (Seress et al. 2018), entrainant un relâchement de la sélection sur ce trait. Par ailleurs, les densités de prédateurs sauvages peuvent aussi être réduites en milieu urbain, et ainsi alléger certaines pressions de sélection, notamment celle agissant contre les comportements « prise de risque ». Et la température hivernale plus douce en ville peut aussi permettre de diminuer les contraintes sur plusieurs traits (plus besoin d'être grand pour maintenir une température corporelle élevée) et aussi directement sur la survie annuelle. Cependant, nos résultats ne sont pas alignés avec ces deux dernières hypothèses. En effet, concernant les traits comportementaux, l'exploration accrue confère un avantage en termes de succès reproducteur chez les mâles forestiers mais pas chez les mâles urbains, bien que ceux-ci soient en moyenne plus explorateurs. Ainsi, l'origine des comportements plus « téméraires » en milieu urbain reste à démontrer, et des pistes de réflexions à ce sujet sont abordées par la suite (notamment section 2.b).

Ces résultats concordent de plus avec les analyses génomiques réalisées dans le **Manuscrit 4** qui révèlent peu de marques de forte sélection et une multitude de sélection plus faible. De manière générale, l'évolution des séquences d'ADN par sélection est un processus long, impliquant des échelles de temps bien plus grandes que celle de l'urbanisation (Johnson & Munshi-South 2017). Ainsi, le nombre de traces de sélection présentes dans les génomes peut être limité par le temps. S'ajoute à l'échelle de temps court, les flux de gènes importants détectés entre ville et forêt (indiqués par les F_{ST}

faibles, **Manuscrit 4**) pouvant considérablement freiner l'adaptation locale (Lenormand 2002).

De façon intéressante tout de même, la multitude de traces de faible sélection détectée concerne majoritairement des gènes aux fonctions biologiques liées au développement nerveux, au comportement ou encore à l'immunité, indiquant une importance potentiellement majeure de ces fonctions dans l'adaptation à l'habitat urbain.

II.2.b Sélection mal détectée ?

Bien que nous ne détections que très peu de traces de sélection sur les traits que nous étudions, il est important de prendre en compte le fait que de la sélection puisse tout de même exister, mais que pour diverses raisons nous ne parvenions pas à la détecter. En particulier, nos analyses de sélection et nos recherches de traces de sélection dans les génomes se fondent sur une comparaison binaire entre habitat urbain et habitat forestier. Or, l'habitat urbain est un habitat très hétérogène représentant en réalité une mosaïque de micro-habitats caractérisés par des niveaux d'artificialité ou de dérangement humain pouvant varier sur un large gradient (voir par exemple **Chapitre I, Figure 2** et Szulkin et al. 2020a). Ainsi, les pressions de sélection subies par un individu vivant dans une zone très urbanisée peuvent être différentes de celle subies par un individu occupant un parc urbain. En effet, comme démontré par Corsini et collaborateurs (2020) la sélection agissant sur la masse des poussins semble s'intensifier avec le niveau d'artificialité de l'habitat. Il serait donc intéressant d'étudier les patrons de sélection observés à fine échelle pour prendre en compte l'hétérogénéité environnementale. Par ailleurs, au-delà de leur variation spatiale, les conditions environnementales sont aussi susceptibles de varier au cours du temps, pouvant induire une fluctuation temporelle des patrons de sélection (Kingsolver et al. 2012). Ainsi, il serait particulièrement informatif de mesurer la sélection au sein de chaque année pour identifier des potentielles fluctuations et pour réduire le bruit associé aux études compilant plusieurs années simultanément. Cependant, de telles études requièrent une puissance statistique conséquente et nécessiteraient d'augmenter considérablement les tailles d'échantillonnage.

Par ailleurs, les mesures de valeur sélective ici n'ont été faites que sur des phénotypes d'individus adultes, or la sélection est susceptible de se produire à des stades plus précoces de la vie des individus. En effet, une récente étude de Corsini et collaborateurs (2020) met en évidence une sélection via la survie sur la masse chez des poussins de mésanges charbonnières, démontrant un avantage net des gros poussins à survivre jusqu'à l'âge de l'envol. D'ailleurs, la masse des poussins à 15j semble aussi avoir une importance capitale dans la survie de ceux-ci après l'envol (Tinbergen & Boerlijst 1990; Naef-Daenzer et al. 2001). Ainsi, ces résultats suscitent des questions quant à la pertinence des proxys de valeur sélective utilisés, en particulier concernant les analyses de sélection sur le succès reproducteur.

En effet, utiliser un mauvais proxy de valeur sélective pourrait biaiser les résultats et empêcher de détecter de la sélection. En particulier lors de nos analyses de sélection sur le succès reproducteur, nous avons utilisé le nombre de poussins à l'envol. Or d'après les arguments énoncés dans le paragraphe précédent, cette mesure pourrait surestimer le nombre de poussins « viables » produits (*i.e.* le nombre de poussins ayant une condition corporelle suffisante pour survivre après l'envol) et particulièrement dans le milieu urbain où les poussins semblent être sous forte contrainte nutritive (Biard et al. 2017; Seress et al. 2020). Ainsi, si les mésanges urbaines réussissent à amener à l'envol un nombre raisonnable de poussins, aucune étude n'a été menée quant à la survie de ces poussins juste après leur envol. Une autre mesure classique de succès reproducteur est le nombre de poussins recrutés dans la population d'étude. A l'inverse du nombre de poussins à l'envol, cette mesure ci est souvent contestée car au-delà de représenter la valeur sélective des parents, elle reflète aussi une bonne partie de la capacité à survivre des descendants seuls et donc implique la valeur sélective de deux générations, et est biaisée par la dispersion. De façon intermédiaire, il pourrait être intéressant de réitérer les analyses de sélection en ne considérant plus le nombre total de poussins à l'envol mais le nombre de poussins de 15 jours passant au-dessus du seuil de masse critique connu pour leur permettre de survivre après l'envol (15.69g, Naef-Daenzer et al. 2001). Dans une autre dimension, il pourrait être intéressant de se pencher, non pas sur des paramètres annuels de

la valeur sélective mais sur des paramètres résumant la vie totale des individus, par exemple le succès reproducteur total (« lifetime reproductive succès » en anglais). Cette mesure a déjà permis de détecter de la sélection sur des traits morphologiques chez d'autres espèces de passereaux (*e.g.* Jensen et al. 2004).

Enfin, concernant nos analyses génomiques, la détection de traces de sélection peut être limitée par l'approche utilisée qui ne couvre qu'une partie du génome (RAD-seq) et ne donne donc accès qu'à une partie de l'information. Il serait intéressant dans le futur de réaliser le même type d'analyses avec une meilleure résolution, notamment en séquençant des génomes complets (très couteux cependant) pour avoir accès à la totalité de l'information génétique ou d'étudier plus précisément certains gènes candidats (**Figure bilan**).

II. ROLE POTENTIEL DE L'ENVIRONNEMENT DANS LES DIFFERENCES PHENOTYPIQUES

II.1. Rôle des contraintes environnementales

Les différences phénotypiques observées peuvent potentiellement être expliquées par des différences de conditions environnementales. Premièrement, les différences de morphologie résultent probablement de fortes contraintes nutritives subies par les individus urbains. En effet, la taille réduite est observée dès le stade poussin et des études récentes montrent que les poussins de mésanges charbonnières urbaines subissent un retard de croissance important (Biard et al. 2017) qui disparait en cas de supplémentation alimentaire (Seress et al. 2020, mais voir les resultats de Demeyrier et al. 2017 qui ne trouve pas le même résultat dans nos sites d'étude). Ainsi, des individus ayant souffert de manque de nourriture lors de leurs premiers stades de développement peuvent présenter une taille réduite par rapport à des individus mieux nourris. Un tel manque de nourriture peut aussi affecter la morphologie au stade adulte et notamment la qualité des plumes (discuté par Meillère et al. 2017). En effet, le plumage est couteux à produire et des carences en certains nutriments ainsi que des stress peuvent contraindre la pousse des plumes (Romero et al. 2005) et aboutir à des plumes de moins bonne qualité s'usant plus vite.

Deuxièmement, les tailles de pontes peuvent aussi être lourdement impactées par un manque de ressources nutritives. En effet, la production d'œufs par les femelles représente un coût considérable nécessitant un apport important en de nombreux nutriments pouvant se faire rare en milieu urbain comme par exemple les caroténoïdes qui entrent dans la composition du jaune d'œuf (Isaksson & Andersson 2007). Ainsi, en cas de ressources limitées une femelle peut être contrainte à pondre un nombre réduit d'œufs (Wawrzyniak et al. 2015). Par ailleurs, la date de ponte est un trait dépendant grandement d'indices environnementaux tels que la hausse des températures ou l'augmentation de la durée des jours annonçant le printemps. Ainsi, la température plus élevée en ville ainsi que l'éclairage artificiels jouent potentiellement un rôle important dans la précocité des dates de pontes observées en ville (Schlaepfer et al. 2002; Kempenaers et al. 2010).

L'origine plastique ou génétique de ces variations de traits restent tout de même majoritairement mal connues. Alors que certains articles démontrent une origine plastique (Seress et al. 2020), d'autres trouvent des indices d'une base génétique (Riyahi et al. 2017). Ici, des études complémentaires seront nécessaires pour démêler le rôle que jouent génétique et plasticité dans la variation observée des traits en milieu urbain. Pour se faire, les expériences de jardin commun ou de transplantation croisés pourraient permettre de comprendre si les différences ont une origine génétique ou non (voir **Figure bilan**). Puis, si la divergence est au moins en partie plastique, des approches expérimentales plus poussées et spécifiques pourront permettre de tester quels facteurs sont susceptibles d'en être la cause.

II.2. Colonisation de l'habitat par des phénotypes particuliers

La covariance phénotype-environnement peut être expliquée par l'hypothèse de « choix d'habitat adapté » (en anglais : « matching-habitat-choice ») suggérant une dispersion non aléatoire des individus, résultant d'un choix préférentiel de certains individus pour un habitat qui correspond le mieux à leur phénotype (Edelaar et al. 2008; Jacob et

al. 2015). Le principe du « choix d'habitat adapté » est particulièrement étudié en relation avec les traits du comportement. En particulier, plusieurs études font cas de colonisation préférentielles de certains types d'habitat par des individus possédant un certain type de personnalité, réduisant ainsi le stress encouru et évitant les coûts comportementaux trop importants. Ainsi, il est supposé qu'en milieu urbain les personnalités « téméraires » seraient plus adaptées que les personnalités « timides », on s'attend donc à une colonisation préférentielle des zones urbanisées par des individus téméraires (Holtmann et al. 2017). Par ailleurs, les capacités de dispersion des individus peuvent aussi directement dépendre de leur phénotype. Par exemple, chez les mésanges charbonnières, les individus les plus téméraires et les plus explorateurs dispersent sur de plus longues distances que les individus plus « timides » (Dingemanse et al. 2003).

Pour explorer cette hypothèse plusieurs types d'études complémentaires peuvent être menées (**Figure bilan**). Premièrement, des modèles de Capture-Marquage-Recapture entre plusieurs types d'habitat pourraient permettre d'identifier si certains phénotypes colonisent préférentiellement certains habitats. Ce type de modèles nécessite cependant une puissance statistique considérable, et n'est pour le moment pas réalisable avec le jeu de données dont nous disposons actuellement. Alternativement, équiper de jeunes individus de phénotype connu avec des balises GLS pourrait nous informer sur les évènements de dispersion et tester le lien entre phénotype (par exemple l'agressivité en main ou le rythme respiratoire) et dispersion natale et/ou dispersion de reproduction. A l'heure actuelle, les dispositifs existants sont à la limite du poids maximum recommandé pour être placé sur des oiseaux de petite taille (autour de 8g > 3% de la masse de l'oiseau) mais les évolutions technologiques rapides permettront peut-être de développer, dans un future proche, des dispositifs suffisamment miniaturisés pour être embarqués sur des mésanges charbonnières.

III. SELECTION SUR DES TRAITS NON ETUDIES ICI

III.1. Traits potentiellement important dans l'adaptation au milieu urbain

Dans ce projet, nous avons exploré l'adaptation en milieu urbain à travers plusieurs types de traits : morphologiques, d'histoire de vie, comportementaux et physiologique. Cependant, l'adaptation au milieu urbain est susceptible d'impliquer de nombreux autres types de traits. En particulier, les analyses génomiques réalisées ici montrent que les traces de sélection dans les génomes tombent statistiquement plus souvent sur des zones associées à des fonctions immunitaires, du déplacement ou encore du système nerveux. Les traits liés aux capacités cognitives semblent en particulier pouvoir jouer un rôle majeur dans l'adaptation au milieu urbain (Sol et al. 2005). En effet, plusieurs études suggèrent que les individus/espèces aux capacités d'innovations plus élevées sont susceptibles de mieux appréhender la vie en milieu urbain (*e.g.* Ducatez et al. 2017). Du plus, les interactions inter et intra-spécifiques étant chamboulées (Ruiz et al. 2002; Faeth et al. 2005) on s'attend aussi à une forte importance des traits liés à la tolérance des interactions rapprochées, ou aux traits impliqués dans la sélection sexuelle (Senar et al. 2014). Enfin, de façon générale, de nombreux traits phénotypiques sont corrélés entre eux (syndromes comportementaux, syndromes de traits d'histoire de vie, **Introduction, Figure 4**). Dans une population, un trait peut donc augmenter en fréquence par sélection indirecte sur un trait corrélé, résultant en des différences phénotypiques n'étant pas nécessairement adaptatives. Ainsi, bien que nous ne détections pas ou peu de sélection sur les traits étudiés, il est très probable que les populations urbaines subissent malgré tout de la sélection sur d'autres traits.

III.2. Importance de la plasticité phénotypique

La plasticité phénotypique peut avoir une importance majeure dans la réponse aux changements environnementaux rapides. En particulier, il est souvent suggéré dans la littérature que les espèces et individus les plus plastiques ont une meilleure tolérance aux nouveaux habitats présentant de

nouvelles contraintes fortes (Sih et al. 2011). En effet, les nouveaux environnements sortant de la gamme de conditions typiquement connues par les populations (comme c'est le cas du milieu urbain) peuvent induire des stress aigus avec des conséquences importantes pour la valeur sélective des individus (Bradshaw & Hardwick 1989; Badyaev 2005). Ainsi, suite à la colonisation d'un habitat ou à la transformation drastique d'un environnement, la première étape avant l'adaptation est de tamponner les stress liés à ces nouvelles conditions pour maintenir l'homéostasie et garantir la survie (Waddington 1953). Ce n'est que si les individus survivent grâce à une plasticité immédiate en réponse au changement de l'environnement que la sélection pourra résulter, au fil des générations, en une réponse évolutive des traits héritables permettant une adaptation génétique (effet Baldwin : Simpson 1953; Lande 2009). La plasticité phénotypique a donc une importance cruciale au moins dans un premier temps, pour éviter l'extinction des populations dans un nouvel habitat auquel elles ne sont pas adaptées (Chevin & Lande 2010). A l'inverse, des prédictions théoriques postulent que la variation phénotypique d'origine plastique peut contraindre ou du moins ralentir le taux d'évolution adaptative en « protégeant » le génotype des effets de la sélection (Falconer 1981).

Par ailleurs, dans les environnements particulièrement fluctuants, où les pressions de sélection varient fréquemment au cours du temps, avoir la capacité de réagir rapidement face à ces variations (parfois même au cours d'une génération) peut constituer un avantage considérable en terme de valeur sélective (de Jong 1995). Dans ce cas, la sélection peut favoriser la plasticité si celle-ci est héritable (Hallsson & Björklund 2012). Ainsi, plutôt que d'agir directement sur la valeur d'un trait, la sélection naturelle peut au contraire agir sur la capacité des individus à avoir des réponses plastiques, malléables en fonction de l'environnement. Il est donc nécessaire de mesurer le rôle de la plasticité phénotypique dans la variation des phénotypes observés et de comprendre le lien entre les phénotypes et la valeur sélective dans un contexte de fluctuation temporelle et spatiale de la sélection. A première vue, bien que nous n'ayons pas mesuré la variation annuelle de sélection sur les traits observés, nous constatons une forte fluctuation annuelle et spatiale des phénotypes appuyant l'intérêt d'étudier la sélection naturelle, la plasticité phénotypique et lien entre les deux.

Dans le futur, comprendre le rôle de la plasticité dans l'adaptation au milieu urbain et tester si cette plasticité favorise ou au contraire freine l'action de la sélection naturelle est un enjeu majeur pour estimer l'impact évolutif à long terme de l'urbanisation sur les populations sauvages. L'étude de la plasticité et de son lien avec l'environnement passe par l'estimation de normes de réactions individuelles (Nussey et al. 2007) nécessitant d'avoir accès à une grande quantité de données phénotypiques individuelles répliquées dans l'espace et dans le temps. Pour obtenir ces données répétées, il est essentiel de poursuivre et de généraliser les suivis de populations à long terme sur de nombreuses années. De même, il est important d'utiliser une approche multidimensionnelle portant à la fois sur plusieurs traits phénotypiques et explorant différents paramètres de l'environnement susceptibles d'induire des réponses plastiques.

IV. LES VILLES : DE POTENTIELS RÉPLICAS

L'essentiel du travail réalisé dans le cadre de cette thèse se fonde sur un seul couple de populations urbaine et forestière (excepté le **Manuscrit 4** fondé sur l'étude de trois réplicas), il est donc difficile de généraliser nos résultats. Dans le futur, il serait crucial de réitérer ces analyses sur plusieurs autres villes en Europe et sur plusieurs autres espèces pour voir si les patrons des sélections observés ici sont généralisables (**Figure bilan**).

De manière générale, il est indéniable que, tout autour du globe, les villes présentent plus de similitudes environnementales entre elles qu'avec les habitats « naturels » qu'elles ont remplacés. En effet, elles ont des caractéristiques intrinsèques communes telles que des fortes densités de population humaine, un recouvrement important des sols par des surfaces artificielles, des niveaux de perturbations sonores et lumineuses élevés, des concentrations de polluants importantes et une diminution drastique du couvert végétal. Ainsi, la vie en milieu urbain est susceptible d'entrainer les mêmes avantages, contraintes et pressions de sélection d'une ville à l'autre,

conduisant à des modifications similaires des processus écologiques et évolutifs s'y déroulant.

A l'heure actuelle, le nombre croissant d'études consacrées à la compréhension des processus éco-évolutifs en cours dans les milieux urbains permet de commencer à comprendre si ces processus sont similaires d'une ville à l'autre. En particulier, l'utilisation de réplicas urbains permet d'étudier la convergence (changements phénotypiques répétés et indépendants) et l'évolution parallèle (lorsque la convergence résulte de sélection agissant sur les mêmes bases moléculaires et développementales, Lambert et al. 2020). Au regard du phénotype des populations urbaines, bien qu'aucun patron de variation phénotypique associé au milieu urbain ne soit valable pour l'ensemble des espèces animales, certaines observations phénotypiques semblent tout de même être cohérentes au niveau de petits groupes taxonomiques ou au niveau d'espèces écologiquement proches vivant en milieu urbain. Pourtant ces tendances phénotypiques ne sont pas des universelles et bon nombre d'études trouvent des résultats contrastés ou même parfois opposés (voir par exemple Table 2 dans Sepp et al. 2017). On peut alors se demander si ces résultats non concordants entre villes viennent du fait que les populations répondent de manière différente à la vie en milieu urbain ou bien si les villes sont différentes entre elles et représentent des contraintes et pressions de sélection variables. De plus, les études varient souvent dans leur façon de quantifier l'urbanisation et dans les zones urbaines prises en compte. En effet, alors que certaines s'intéressent à des populations urbaines directement dans les rues et zones les plus urbanisées, d'autres se concentrent essentiellement sur des parcs urbains (Björklund et al. 2010; Markowski et al. 2013), et très peu couvrent la totalité du « gradient » d'urbanisation (mais voir par exemple Corsini et al. 2020).

Ainsi, si l'on veut comprendre les processus éco-évolutifs induits par l'urbanisation il est important de réaliser des études sur plusieurs couples de ville et forêt en parallèle, en prenant en compte l'hétérogénéité environnementale au sein des villes et en faisant en sorte d'étudier des zones aux niveaux d'urbanisation similaires. Des études de ce type sont en train d'être mises en places à plusieurs endroits du globe et font souvent intervenir des collaborations de dimensions sans précédents, entre plusieurs groupes de recherche (par exemple, le projet ANR URBANTIT initié en janvier 2020 à l'échelle de la France, ou le projet GLUE[8] à échelle mondiale dirigé par Marc Jonhson). Elles permettront peut-être de mieux comprendre un jour si et comment les populations sauvages s'adaptent au milieu urbain et dans quelle mesure leurs adaptations sont convergentes.

[8] GLobal Urban Evolution : http://www.globalurbanevolution.com/

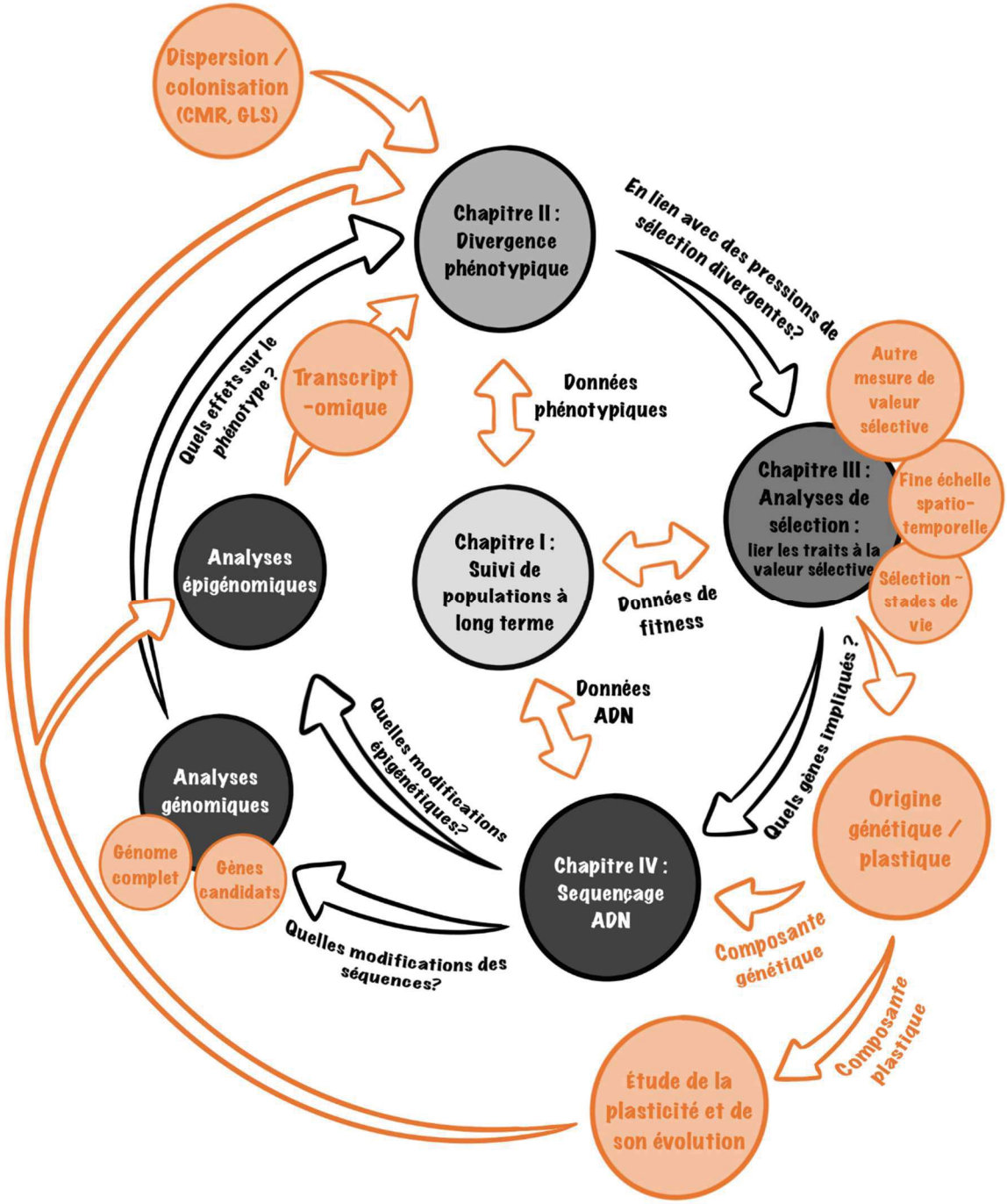

Figure Bilan | Résumé schématique des étapes pour l'étude de l'adaptation biologique au milieu urbain réalisées dans cette thèse (en nuances de gris) et les futures recherches importantes à développer en écologie évolutive pour affiner la compréhension des mécanismes éco-évolutifs associés au milieu urbain (en orange).

CHAPITRE BONUS

VULGARISATION



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– MA THESE EN 180 SECONDES –



Texte :

Imaginons la forêt… on sent l'air pur, la verdure et on voit des biches gambader. Imaginons maintenant un centre-ville, là c'est plutôt goudron, bouchons et klaxons. Les citadins parmi nous savent que vivre en ville ça a ses avantages mais c'est pas toujours facile. On est emportés par la foule, dérangés par les gaz d'échappements, assourdis par le bruit ambiant et éblouis par l'éclairage public. Et quand enfin on aperçoit de la verdure elle est ornementale. Rien de bien naturel tout ça ! Pourtant, si on tend bien l'oreille, on peut entendre une mésange chanter ! « Elle est bien loin de son habitat naturel » me direz-vous. Et vous avez raison.

Mais alors, qu'est-ce que ça fait d'être une espèce sauvage dans un monde artificialisé ? Comment mésange des champs est-elle devenue mésange des villes ?

C'est exactement les questions que je me pose pendant ma thèse. Et pour y répondre je m'intéresse aux mésanges charbonnières de Montpellier. Au printemps, mon équipe et moi-même partons étudier les mésanges qui nichent en ville. On les observe, on les attrape et on les mesure dans l'espoir de comprendre comment elles s'adaptent au milieu urbain.

Après plusieurs années de dur labeur, près de 1300 étudiées et 4800 poussins bagués des réponses commencent à émerger. En y regardant de plus près on voit que la mésange des villes a attrapé le syndrome urbain.

Et mon petit doigt me dit que ça va vous rappeler quelque chose.

Et bien, la mésange des villes est pressée. Elle pond ses œufs plus tôt que mésange des champs. C'est parce que le printemps arrive plus vite en ville car les températures y sont plus élevées en raison des activités humaines.

La mésange des villes a aussi l'air d'être surbookée, elle passe plus de temps à chercher de la nourriture pour ses petits et celle qu'elle trouve est de moins bonne qualité. De la malbouffe quoi. Elle a donc du mal à élever une famille nombreuse.

Bon, je vous l'avoue, sur ces aspects mésange des villes à l'air un peu détraqué.

Mais si on regarde ailleurs, la mésange des villes est en train de s'adapter. Il n'y a qu'à regarder son comportement ! La mésange des villes est audacieuse et ne craint plus l'homme, contrairement à la mésange des champs.

Ah et aussi, comme votre voisine de palier quand elle prend sa douche, la mésange des villes se prend pour la Castafiore. C'est qu'elle est obligée de chanter beaucoup plus haut pour se faire entendre malgré le vacarme ambiant.

Citadins, réjouissons-nous ! Ce travail est la preuve que la nature est à notre porte. Et encore, les mésanges ne constituent qu'une partie de la nature que l'on croise en ville. Mammifères, insectes et végétaux sont aussi en train de s'adapter à ce milieu parfois hostile. Regardez par exemple ces jolis pissenlits qui poussent au milieu des trottoirs. A l'heure où l'urbanisation empiète de plus en plus sur les forêts, c'est plutôt rassurant de voir que la nature résiste, vous ne trouvez pas ?

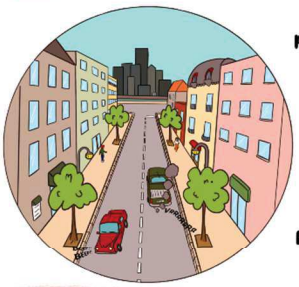
Car oui, la nature, jamais nous n'en serons maîtres, qu'on lui ferme la porte au nez, elle reviendra par les fenêtres.

– POSTER FÊTE DE LA SCIENCE –

Mésange des Villes & mésange des Champs
Quand la Recherche scientifique s'en mêle

L'urbanisation transforme les milieux naturels en zones bétonnées. Souvent considérées comme stériles, les villes abritent en réalité de nombreuses espèces sauvages. Parmi elles, certaines subissent la vie urbaine alors que d'autres réussissent à s'adapter. Mais comment ?

Les particularités de l'habitat urbain



Pollution lumineuse
Surfaces bétonnées
Pollution chimique
Pollution sonore
Ressources
Présence humaine
Température élevée
Végétation exotique
Fragmentation d'habitat

Vivre en ville : un défi pour les espèces sauvages

Nos objectifs

- Comprendre comment les espèces sont impactées par la vie urbaine

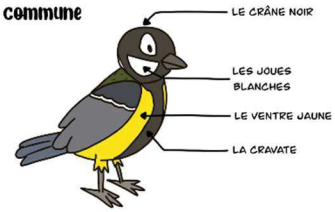
- Étudier leurs capacités d'adaptation face à l'urbanisation

Notre modèle d'études: La mésange charbonnière

Quelques infos :

- Répartition : Eurasie, espèce commune
- Poids : environ 16 grammes
- Taille : 12 à 14 centimètres
- Pond 1 à 2 nichées par an
- 6 à 12 oeufs par nichée
- Longévité : 3 à 5 ans
- Insectivore au printemps, granivore en hiver

Comment la reconnaître ?



Suivi de populations urbaine et rurale

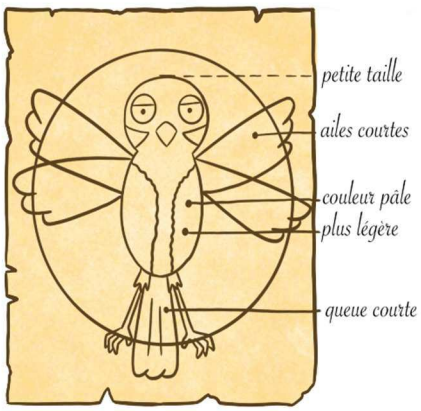
Nous étudions une population urbaine dans la ville de Montpellier et une population rurale dans une forêt proche. Ainsi, nous pouvons comparer les deux et comprendre quel est l'impact de l'urbanisation sur les mésange.



LES MÉSANGES SONT 'CAVICOLES' : ELLES NICHENT DANS DES CAVITÉS. ELLES AIMENT DONC S'INSTALLER DANS DES NICHOIRS QUE NOUS METTONS À LEUR DISPOSITION. PRATIQUE QUAND ON VEUT LES ÉTUDIER !

Quelques particularités des mésanges urbaines

Morphologie



Reproduction

- Ponte précoce
- Moins d'oeufs



Comportement

- Plus agressive
- Plus exploratrice
- Plus stressée



Centre d'Écologie Fonctionnelle & Évolutive
cnrs
OREME
ZOO MONTPELLIER
M

Contact:
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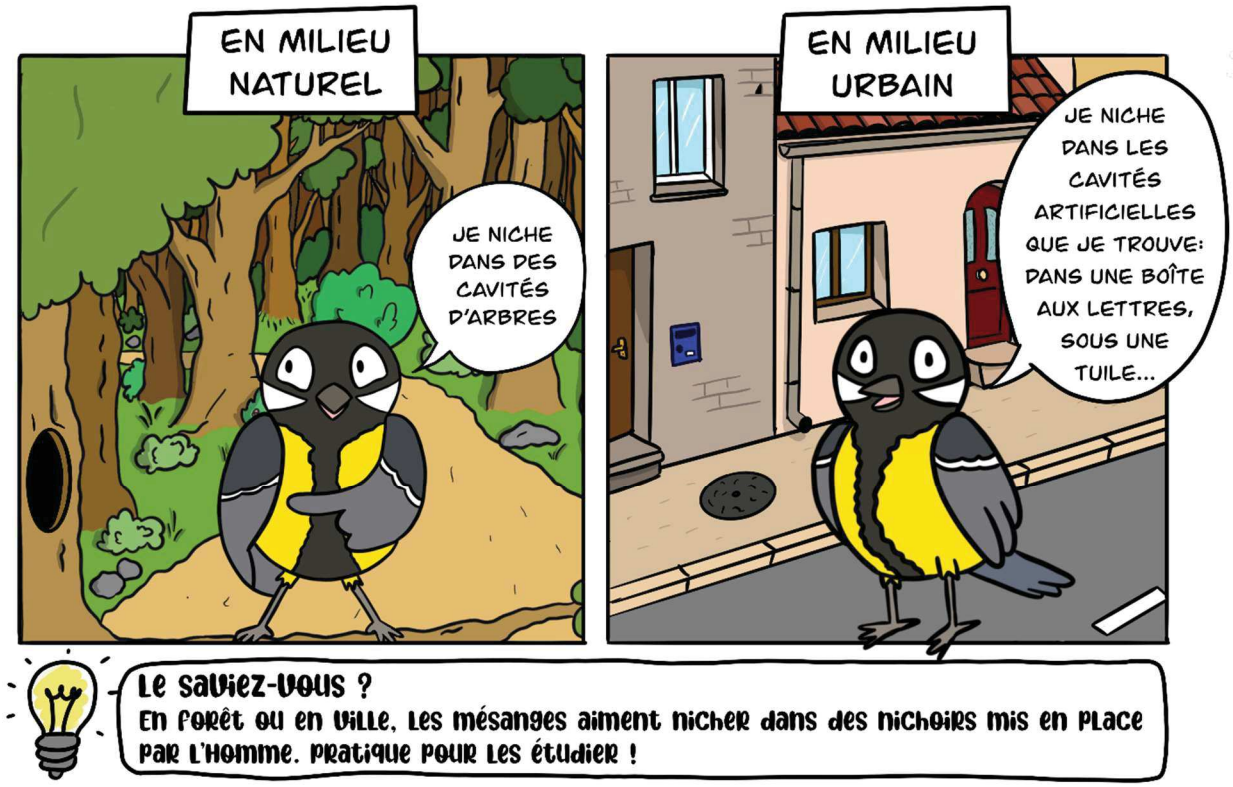
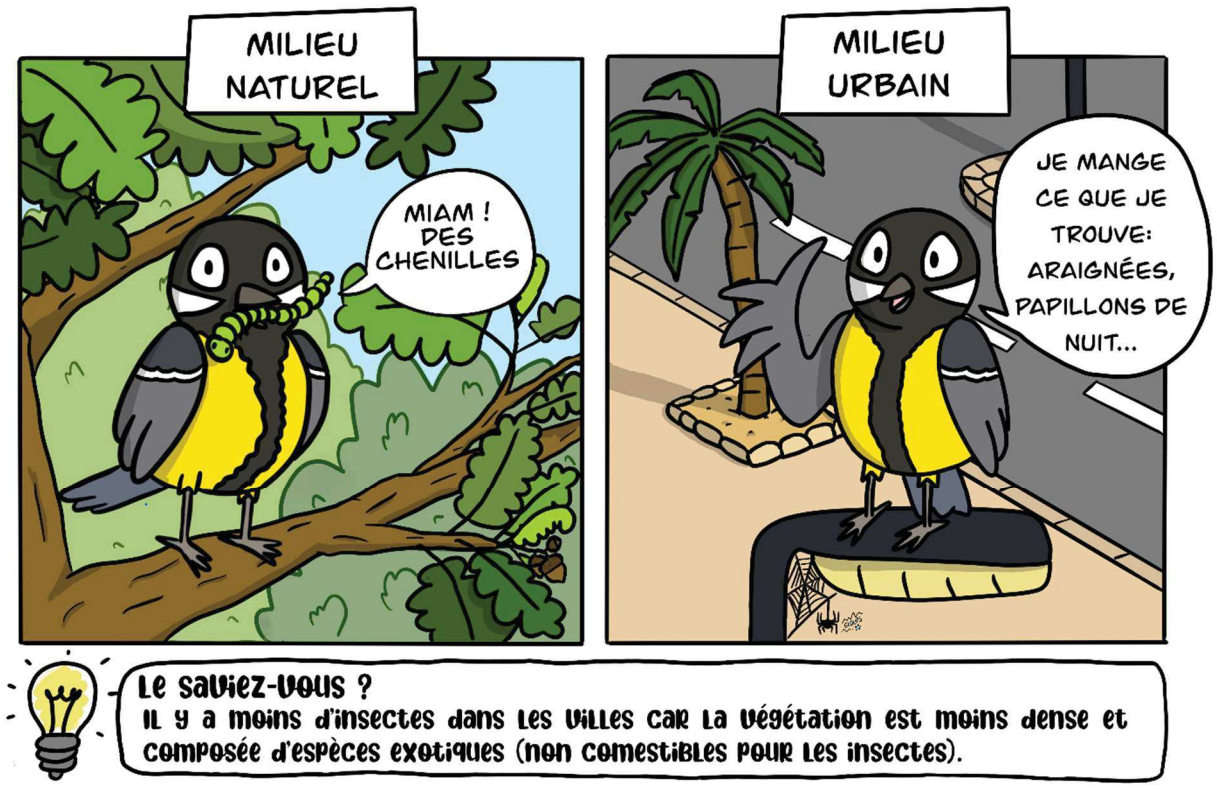
Où les mésanges nichent-elles ?



Que mangent-elles ?

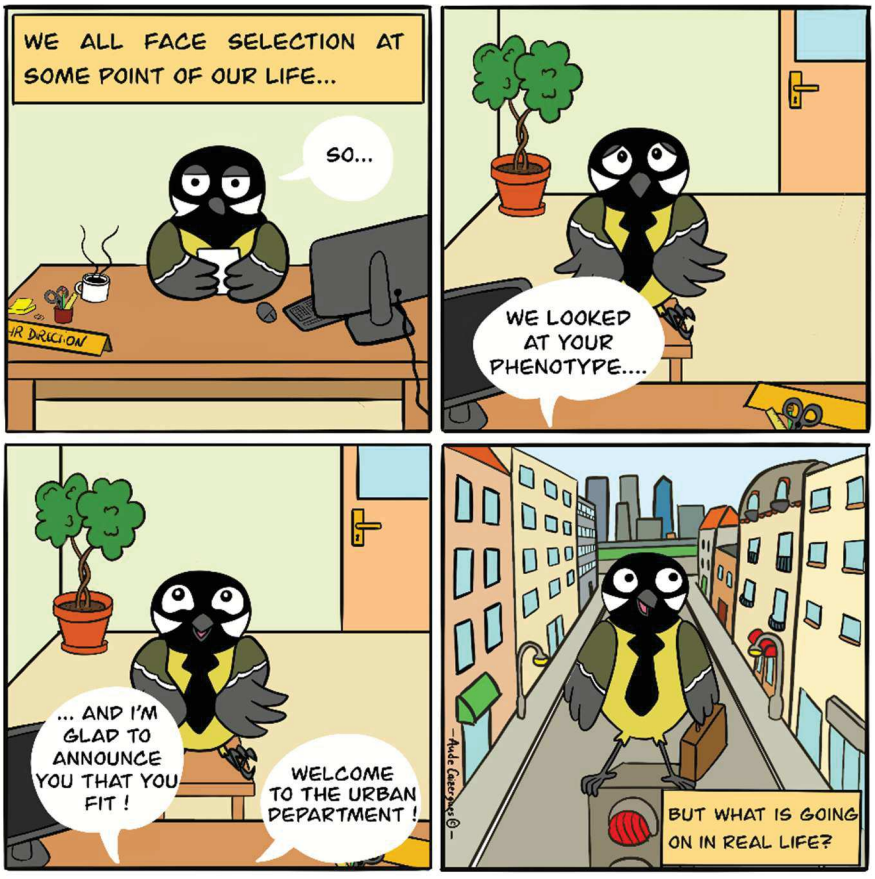


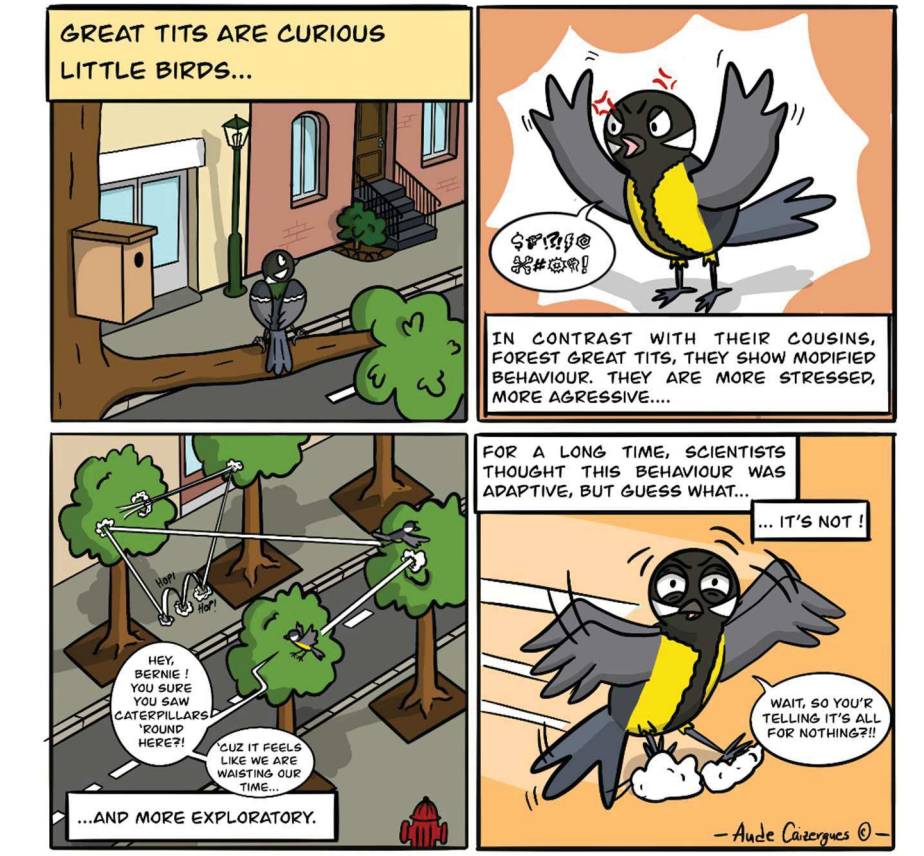
– MINI-BD ASSOCIÉES AUX ARTICLES –

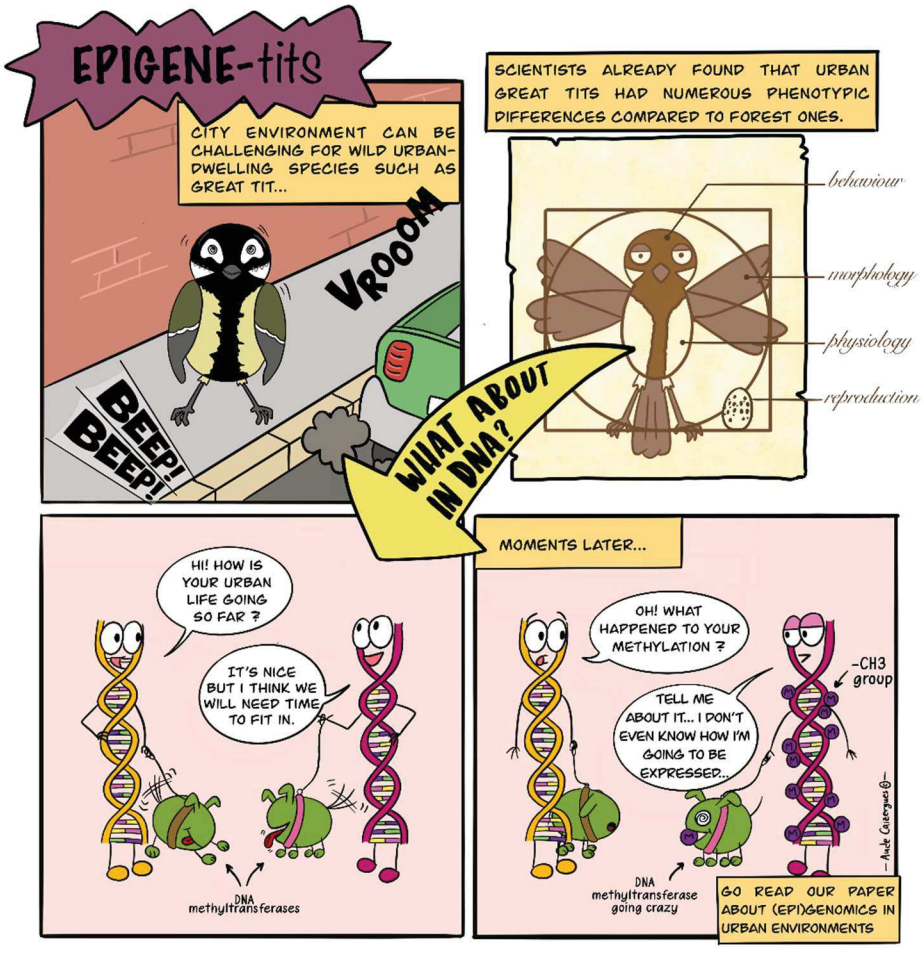
Je présente ici des mini-BD présentant les articles de ma thèse, l'idée étant de susciter la curiosité du lecteur en lui fournissant un « teaser » mignon et humoristique.




WE DIDN'T KNOW MUCH ABOUT SELECTION GOING ON IN URBAN ENVIRONMENTS. SO WE INVESTIGATED....
SELECTION
SO, WHAT IS YOUR PHENOTYPE ?
URBAN DEPARTMENT
I LAY MY EGGS EARLY AND I HAVE SMALL CLUTCH SIZE
WELL, I'M SORRY BUT YOU DON'T FIT...
JURY
NEXT !
THAT'S CRAZY ! THEY KEEP LAYING EARLIER AND FEWER EGGS EVEN IF IT'S NOT ADAPTIVE... I DON'T GET IT...
JURY
AND YOU? WHAT IS YOUR PHENOTYPE?
I AM SMALL, THIN. AND I HAVE SHORT WINGS AND TAIL
IT'S NOT A FASHION SHOW HERE, WE DON'T SELECT ON MORPHOLOGY.
JURY
WELL, IT SEEMS THAT URBAN PHENOTYPE IS NOT ADAPTIVE...
- Aude Caizergues ©


WE ALL FACE SELECTION AT SOME POINT OF OUR LIFE...
SO...
WE LOOKED AT YOUR PHENOTYPE....
... AND I'M GLAD TO ANNOUNCE YOU THAT YOU FIT !
WELCOME TO THE URBAN DEPARTMENT !
BUT WHAT IS GOING ON IN REAL LIFE?


GREAT TITS ARE CURIOUS LITTLE BIRDS...
IN CONTRAST WITH THEIR COUSINS, FOREST GREAT TITS, THEY SHOW MODIFIED BEHAVIOUR. THEY ARE MORE STRESSED, MORE AGRESSIVE....
Hop!
Hop!
HEY, BERNIE ! YOU SURE YOU SAW CATERPILLARS 'ROUND HERE?!
'CUZ IT FEELS LIKE WE ARE WAISTING OUR TIME...
...AND MORE EXPLORATORY.
FOR A LONG TIME, SCIENTISTS THOUGHT THIS BEHAVIOUR WAS ADAPTIVE, BUT GUESS WHAT...
... IT'S NOT !
WAIT, SO YOU'R TELLING IT'S ALL FOR NOTHING?!!
-Aude Caizergues ©-


EPIGENE-tits
CITY ENVIRONMENT CAN BE CHALLENGING FOR WILD URBAN-DWELLING SPECIES SUCH AS GREAT TIT...
VROOM
BEEP! BEEP!
SCIENTISTS ALREADY FOUND THAT URBAN GREAT TITS HAD NUMEROUS PHENOTYPIC DIFFERENCES COMPARED TO FOREST ONES.
behaviour
morphology
physiology
reproduction
WHAT ABOUT IN DNA?
HI! HOW IS YOUR URBAN LIFE GOING SO FAR ?
IT'S NICE BUT I THINK WE WILL NEED TIME TO FIT IN.
DNA methyltransferases
MOMENTS LATER...
OH! WHAT HAPPENED TO YOUR METHYLATION ?
TELL ME ABOUT IT... I DON'T EVEN KNOW HOW I'M GOING TO BE EXPRESSED...
-CH3 group
DNA methyltransferase going crazy
GO READ OUR PAPER ABOUT (EPI)GENOMICS IN URBAN ENVIRONMENTS
-Aude Caizergues ©-

– MA THESE EN BD–

QUAND LES MÉSANGES ARRIVENT EN VILLE



Aude Caizergues

Centre d'écologie fonctionnelle et évolutive (CEFE)

Passionnée depuis toujours par les petites bêtes que l'on retrouve dans les jardins et les mystères du fonctionnement de la vie, j'ai très tôt voulu devenir chercheuse pour pouvoir comprendre le monde qui m'entoure. Après une licence et un master en Écologie, je poursuis mes études par une thèse intitulée : « Potentiel adaptatif en milieu urbain : une étude chez la mésange charbonnière ». Au printemps, je parcours la ville de Montpellier, échelle sur l'épaule, pour étudier ce petit oiseau.

J'avais pourtant juré à mon père ornithologue que jamais au grand jamais je ne travaillerais sur les oiseaux, parce que « Les oiseaux c'est nul » (citation de moi-même à 8 ans), leur préférant les mammifères. Sauf que finalement, les oiseaux c'est génial : il y en a plein, ils sont diurnes et ils chantent super bien ! En plus il y en a partout, même dans les villes. Devant tant d'arguments, je ne pouvais que changer d'avis, et vous verrez que vous aussi !

Pour aller plus loin

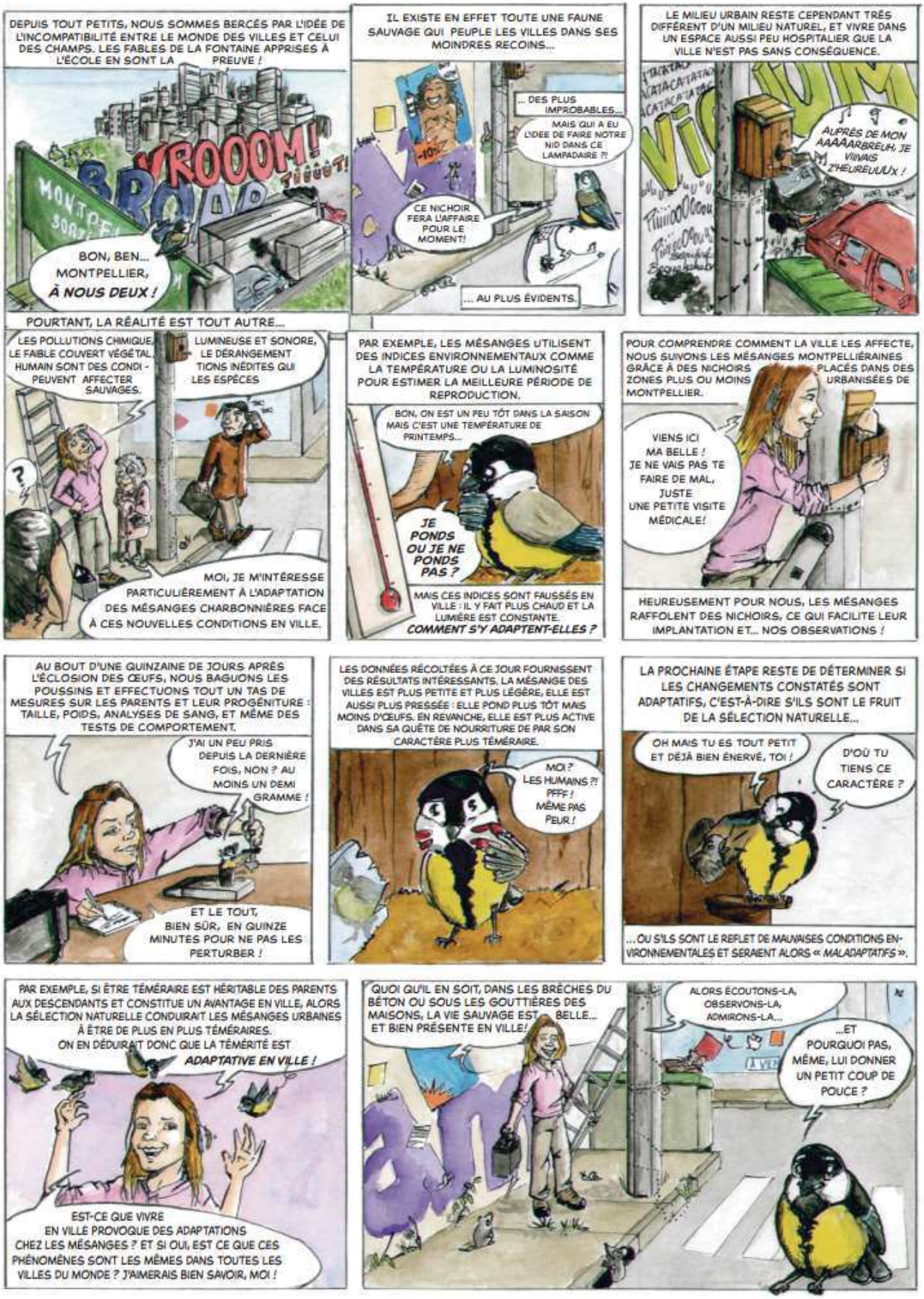


Vous connaissez le concours *Ma thèse en 180 secondes* ? J'y ai participé ! Comme son nom l'indique, des doctorants et doctorantes tentent en 3 minutes de présenter leur sujet de thèse de la manière la plus claire et accessible possible. Un véritable challenge surtout pour des passionnés comme moi !

C'est quoi ?



Oh, c'est trop mignon ! C'est un œuf de mésange charbonnière ! Elle peut en pondre de cinq à douze. Il s'agit donc de familles nombreuses. Les deux parents nourrissent les jeunes surtout avec des chenilles. Il faut donc que la ressource nutritive soit au bon moment d'où l'intérêt de ne pas naître trop tôt ou trop tard. Avec les changements climatiques, les cycles naturels se modifient progressivement, comment vont-elles s'adapter ?



En collabotation avec l'association Cosciences (Guillaume Bagnolini & Morgane Ganault), et le collège doctoral de l'UM. Illustratrice : Morgane Ganault (Mo')

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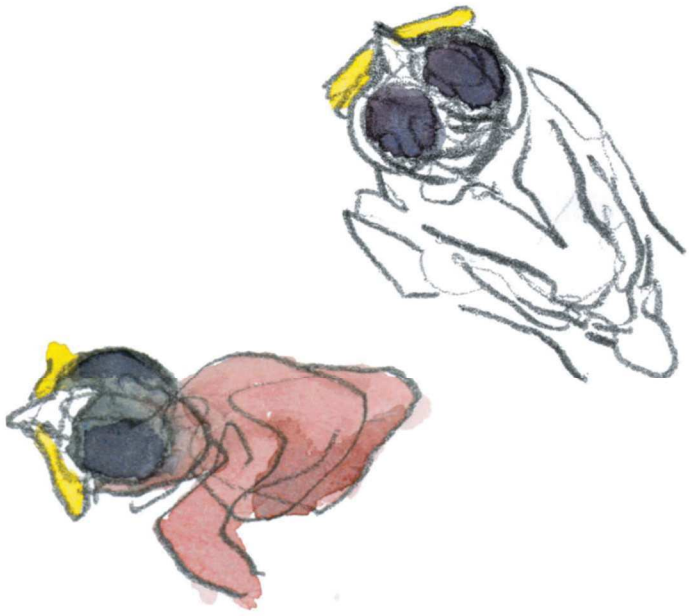

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ANNEXES



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– SUPPLEMENTARY MATERIALS – MANUSCRIT 3 –

Are behavioural and stress responses to urbanization adaptive? Great tits as a case study

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APPENDIX

a. Appendix 1: Impact of change of protocol on Breath Rate Index

For the period of 2013 to 2016 breath rate was estimated by the **number of chest moves during 30s (method 1)**. In 2017, the protocol was updated to measure the **time to complete 30 chest moves (method 2)**, more easy to perform. Measures from 2013 to 2016 were converted to approximate the time needed to complete 30 chests move to obtain similarly scaled measures as the 2017 measure. In order to test whether the shift in the method had a significant impact on the measure of breath rate, we explored potential differences in variances and means associated with the method. Measures of breath rate were significantly lower with method 2 ($mean_{method1}$ = 14.182, $mean_{method2}$ = 12.998, Wilcoxon test: W = 66902, P = $1.95x10^{-14}$).

The most likely explanation for a lower mean with method 2 is that method 1 of counting the number of chest moves during 30s took longer than method 2 (which took around 64 seconds), hence birds had time to calm down and reduce their breath rate during this relatively long handling time. Method 1 being quicker, we believe it better captured the immediate response to handling stress.

Based on these results, we performed a linear regression between breath rate measure and method and extracted the residuals. Residuals of this regression are used as Breat Rate Index (BRI) in the article.

b. Appendix 2: Estimating quadratic selection differentials/gradients on behavioural and stress response traits

In addition to estimating linear reproductive selection, we estimated non-linear (*i.e.* quadratic) selection using reproductive success as fitness proxy. While a linear selection differential/gradient provides an estimate of directional selection, a quadratic selection differential/gradient evaluates the existence and strength of stabilising or disruptive selection (Lande & Arnold, 1983). Quadratic selection differentials (γ) and selection gradients (γ_i for each trait x_i) as well as correlational selection gradients (γ_{ij} for traits x_i and x_j) were estimated for each trait x_i following these two types of models, ran on relative individual fitness ω :

$$\omega = \alpha + \beta x + (\gamma/2)x^2 + \varepsilon \qquad \text{(S1)}$$

$$\omega = \alpha + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + (\gamma_1/2)x_1^2 + (\gamma_2/2)x_2^2 + (\gamma_3/2)x_3^2 + \gamma_{12}x_1x_2 + \gamma_{23}x_2x_3 + \gamma_{13}x_1x_3 + \varepsilon \quad \text{(S2)}$$

In S1 and S2, α represents the intercept, β and β_i respectivelly represent the linear selection differential and the linear selection gradient for each trait x_i, and ε represents the error:

While a positive estimate of quadratic selection differential/gradient indicates disruptive selection, a negative estimate is indicative of stabilising selection.

The model selection procedure was performed following the method described in the main text.

Results for quadratic selection differentials

When estimating quadratic selection differentials on each of the three traits separately, model selection revealed that models with a trait×habitat interaction was equally supported (within 2 points of AICc) with the null model (see Appendix 2 - Table S1). These results suggest that quadratic selection differentials did vary between the two habitats for any of the three studied traits.

Results for quadratic selection gradients and correlational selection

For females, the model without any trait²*habitat interaction was among the best 3 equivalent models, along with a model including ES² *habitat and a model including BRI²*habitat (Appendix 2 – Table S2). This result suggests that quadratic selection acting on ES and BRI might vary between habitats although this habitat-specificity is not highly supported statistically. We provide quadratic selection gradients as well as correlational selection estimates in Appendix2 – Table S3, but we recommend interpreting them with care, considering that the relatively low statistical power conferred by 6 years of data on 938 individuals. For males, the best supported model was the model without any interaction involving habitat, suggesting that quadratic selection gradients did not differ between habitats. However, estimates of quadratic selection in urban and forest habitats combined, for BRI and ES were relatively large and significant. Hence, these analyses suggest diverging selection on male BRI ($\gamma/2 = 0.070 \pm 0.031$), meaning that fathers with low or high BRI produced more fledglings; as well as stabilising selection on male ES ($\gamma/2 = -0.088 \pm 0.060$), suggesting that intermediate values of exploration scores are favoured.

Appendix 2 - Table S1: Model selection for models estimating reproductive quadratic selection differentials for Handling Agression (HA), Breath Rate Index (BRI) and Exploration Score (ES) following equation (S1).

Models	# Par.	Deviance	AICc	Delta AICc
HANDLING AGGRESSION				
Females				
HA + habitat + HA* habitat + HA^2	8	1046.859	1063.236	0
HA + habitat + HA* habitat + HA^2 +HA^2*habitat	9	1046.3798	1064.852	1.616
Males				
HA + habitat + HA* habitat + HA^2	8	945.3111	961.7273	0
HA + habitat + HA* habitat + HA^2 +HA^2*habitat	9	944.4144	962.9361	1.2088
BREATH RATE INDEX				
Females				
BRI + habitat + BRI* habitat + BRI^2	8	1070.7236	1087.088	0
BRI + habitat + BRI* habitat + BRI^2 + BRI^2*habitat	9	1069.9585	1088.415	1.327
Males				
BRI + habitat + BRI* habitat + BRI^2	8	984.455	1000.857	0
BRI + habitat + BRI* habitat + BRI^2 + BRI^2*habitat	9	983.0907	1001.595	0.738
EXPLORATION SCORE				
Females				
ES + habitat + ES* habitat + ES^2	8	1709.8097	1726.044	0
ES + habitat + ES* habitat + ES^2 +ES^2*habitat	9	1709.4832	1727.777	1.733
Males				
ES + habitat + ES* habitat + ES^2	8	1564.9509	1581.208	0
ES + habitat + ES* habitat + ES^2 +ES^2*habitat	9	1564.514	1582.836	1.628

Appendix 2 - Table S2: Model selection for models estimating reproductive quadratic selection gradients and correlational selection for Handling Agression (HA), Breath Rate Index (BRI) and Exploration Score (ES) following equation (S2). Models in bold are equally supported.

Models	# Par.	Deviance	AICc	Delta AICc
Females				
HA + BRI + ES + habitat + HA^2 +BRI^2 +ES^2 + HA*BRI + HA*ES + BRI*ES	**14**	**999.082**	**1028.236**	**0**
HA + BRI + ES + habitat + HA^2 +BRI^2 +ES^2 + HA*BRI + HA*ES + BRI*ES + ES^2*habitat	**15**	**998.0076**	**1029.33**	**1.094**
HA + BRI + ES + habitat + HA^2 +BRI^2 +ES^2 + HA*BRI + HA*ES + BRI*ES + BRI^2*habitat	**15**	**998.8824**	**1030.205**	**1.969**
HA + BRI + ES + habitat + HA^2 +BRI^2 +ES^2 + HA*BRI + HA*ES + BRI*ES + HA^2*habitat	15	999.0468	1030.369	2.133
HA + BRI + ES + habitat + HA^2 +BRI^2 +ES^2 + HA*BRI + HA*ES + BRI*ES + BRI^2*habitat + ES^2*habitat	16	997.8281	1031.331	3.095
HA + BRI + ES + habitat + HA^2 +BRI^2 +ES^2 + HA*BRI + HA*ES + BRI*ES + HA^2*habitat + ES^2*habitat	16	997.9032	1031.406	3.17
HA + BRI + ES + habitat + HA^2 +BRI^2 +ES^2 + HA*BRI + HA*ES + BRI*ES + HA^2*habitat + BRI^2*habitat	16	998.8433	1032.346	4.11
HA + BRI + ES + habitat + HA^2 +BRI^2 +ES^2 + HA*BRI + HA*ES + BRI*ES + HA^2*habitat + BRI^2*habitat + ES^2*habitat	17	997.7187	1033.414	5.178
Males				
HA + BRI + ES + habitat + HA^2 +BRI^2 +ES^2 + HA*BRI + HA*ES + BRI*ES	**14**	**897.1598**	**926.4521**	**0**
HA + BRI + ES + habitat + HA^2 +BRI^2 +ES^2 + HA*BRI + HA*ES + BRI*ES + BRI^2*habitat	15	897.018	928.4994	2.0473
HA + BRI + ES + habitat + HA^2 +BRI^2 +ES^2 + HA*BRI + HA*ES + BRI*ES + ES^2*habitat	15	897.1023	928.5838	2.1317
HA + BRI + ES + habitat + HA^2 +BRI^2 +ES^2 + HA*BRI + HA*ES + BRI*ES + HA^2*habitat	15	897.1097	928.5912	2.1391
HA + BRI + ES + habitat + HA^2 +BRI^2 +ES^2 + HA*BRI + HA*ES + BRI*ES + BRI^2*habitat + ES^2*habitat	16	896.9814	930.6656	4.2135
HA + BRI + ES + habitat + HA^2 +BRI^2 +ES^2 + HA*BRI + HA*ES + BRI*ES + HA^2*habitat + BRI^2*habitat	16	896.9884	930.6726	4.2205
HA + BRI + ES + habitat + HA^2 +BRI^2 +ES^2 + HA*BRI + HA*ES + BRI*ES + HA^2*habitat + ES^2*habitat	16	897.0495	930.7337	4.2816
HA + BRI + ES + habitat + HA^2 +BRI^2 +ES^2 + HA*BRI + HA*ES + BRI*ES + HA^2*habitat + BRI^2*habitat + ES^2*habitat	17	896.9487	932.8493	6.3972

Appendix 2 - Table S3: Reproductive quadratic selection gradients and correlational selection acting on Handling Agression (HA), Breath Rate Index (BRI) and Exploration scores (ES), estimated using model averaging for the best equally supported models presented in Appendix 2 – Table S2.

			Quadratic selection gradients (γ/2)			Correlational selection		
sex	habitat	sample size	HA^2	BRI^2	ES^2	HA*BRI	HA*ES	BRI*ES
females	forest	103	0.030 ± 0.055	-0.051 ± 0.038	0.015 ± 0.067	-0.098 ± 0.046	0.081 ± 0.045	-0.044 ± 0.046
	city	276		-0.056 ± 0.031	-0.015 ± 0.069			
males	forest	93	0.018± 0.064	**0.070 ± 0.031**	**-0.088 ± 0.060**	-0.018 ± 0.048	0.033± 0.051	-0.006 ± 0.052
	city	247						

TABLES

Table S4: Bird handling aggression scale**.** See Figure S2 for an illustration of how the bird should be handled. Note that when a bird had one reaction specific to a score and another reaction specific to another score, the attributed score was an average of the two.

Score	Bird strickes handler	Tail spread	Wings spread
0	No	No	No
1	Yes, but only if provoked	No	No
2	Yes, spontaneously	Yes	No
3	Yes, spontaneously	Yes	Yes

Table S5: Model selection for the recapture probability (p) in relation to habitat, sex and Handling Aggression (HA) or Breath Rate Index (BRI) or Exploration Score (ES) of breeding great tits**.** Models are ranked according to QAICc values, and only the five best models (i.e. lowest QAICc) are presented for each trait-specific dataset. Supported models (ΔQAICc<2) are represented in bold. For all models, the survival probability is modelled as an additive function of habitat, sex and trait.

Models	# Par.	Deviance	QAICc	ΔQAICc
Handling aggression				
$p_{.}$	**5**	**1437.46**	**1173.9839**	**0**
p_{hab}	**6**	**1436.07**	**1174.8857**	**0.9018**
p_{sex}	**6**	**1437.4**	**1175.9562**	**1.9723**
$p_{hab+sex}$	7	1436.01	1176.8617	2.8778
p_{hab+HA}	7	1436.03	1176.878	2.8941
Breath Rate index				
$p_{.}$	**5**	**1272.61**	**897.5161**	**0**
$p_{hab+BRI}$	**7**	**1266.97**	**897.6333**	**0.1172**
p_{hab}	**6**	**1271.47**	**898.7423**	**1.2262**
$p_{sex+BRI}$	**7**	**1269.41**	**899.3393**	**1.8232**
p_{sex}	**6**	**1272.54**	**899.4879**	**1.9718**
Exploration Score				
$p_{.}$	**5**	**1264.91**	**875.776**	**0**
p_{hab}	**6**	**1263.81**	**877.045**	**1.269**
p_{sex}	**6**	**1264.87**	**877.77**	**1.99**
p_{hab+ES}	7	1263.75	879.032	3.256
$p_{hab+sex}$	7	1263.76	879.036	3.26

Table S6: Contrasting great tits Handling Aggression, Breath Rate Index and Exploration across habitat, year, sex, age, temperature and rank of capture. Estimates are calculated by model averaging of equally supported best models presented in Table 1. The upper section of the table indicates estimates for fixed effects. The lower section of the table presents variance components and sample sizes.

	Handling Aggression				Breath Rate Index				Exploration Score			
Fixed effects	**Est.**	**SE**	**z**	**P**	**Est.**	**SE**	**z**	**P**	**Est.**	**SE**	**z**	**P**
Intercept	1.775	0.172	10.325	**$< 2x10^{-16}$**	-0.689	0.310	2.220	**0.026**	3.775	0.857	4.400	**$1.1x10^{-5}$**
Habitat-Urban	0.419	0.108	3.937	**$8.3x10^{-5}$**	-0.450	0.143	3.144	**0.002**	2.494	0.472	5.282	**$1.0x10^{-7}$**
Year-2015	-0.143	0.105	1.356	0.175	0.257	0.201	1.280	0.201	n.s.	n.s.	n.s.	n.s.
Year-2016	-0.235	0.108	2.168	**0.030**	0.304	0.232	1.310	0.191	n.s.	n.s.	n.s.	n.s.
Year-2017	0.071	0.114	0.324	0.533	0.584	0.442	1.320	0.189	n.s.	n.s.	n.s.	n.s.
Year-2018	-0.070	0.114	0.615	0.539	0.736	0.230	3.156	**0.002**	n.s.	n.s.	n.s.	n.s.
Year-2019	-0.115	0.113	1.011	0.312	0.674	0.210	3.201	**0.001**	n.s.	n.s.	n.s.	n.s.
Sex-Female	-0.068	0.117	0.580	0.562	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Age-Yearling	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-0.023	0.123	0.185	0.854
Capture rank	n.s.	n.s.	n.s.	n.s.	-0.179	0.056	-3.225	**0.001**	-0.228	0.209	1.087	0.277
Temperature	0.001	0.006	0.143	0.886	0.047	0.010	4.541	**$5.6x10^{-6}$**	0.030	0.034	0.887	0.375
Hab-Urb × Sex-Fem	-0.464	0.141	3.292	**$9.9x10^{-4}$**	n.s.	n.s.	n.s.	n.s.	0.012	0.435	0.028	0.977
Hbt-Urb × Yr-2015	n.s.	n.s.	n.s.	n.s.	0.173	0.323	0.536	0.592	n.s.	n.s.	n.s.	n.s.
Hbt-Urb × Yr-2016	n.s.	n.s.	n.s.	n.s.	0.310	0.318	0.972	0.331	n.s.	n.s.	n.s.	n.s.
Hbt-Urb × Yr-2017	n.s.	n.s.	n.s.	n.s.	0.692	0.549	1.260	0.208	n.s.	n.s.	n.s.	n.s.
Hbt-Urb × Yr-2018	n.s.	n.s.	n.s.	n.s.	0.170	0.332	0.511	0.609	n.s.	n.s.	n.s.	n.s.
Hbt-Urb × Yr-2019	n.s.	n.s.	n.s.	n.s.	-0.382	0.292	1.305	0.192	n.s.	n.s.	n.s.	n.s.

Random effects	**Variance**	**Sample size**	**Variance**	**Sample size**	**Variance**	**Sample size**
Individual ID	0.44514	nb. Indiv=812	0.138	nb indiv=675	9.42	nd indiv=667
Manipulator ID	0.03648	nb. manip=22	0.138	nb manip=7	-	-
Residuals	0.56673	nb. Obs = 1302	1.414	nb obs=874	8.56	nb obs=850

Table S7: Estimation of variance for Handling Aggression, Breath Rate Index and Exploration Scores per habitat, and Fisher test exploring potential differences in variances, with associated P-values.

	Variance		F-test		
	Forest	Urban	F	d.f.	P
Handling Aggression	1.02	1.149	1.126	860	0.156
Breath Rate Index	1.839	1.54	0.839	666	0.107
Exploration Scores	16.549	18.378	1.111	641	0.369

Table S8: Estimates extracted from the best models of viability selection containing a "trait" effect (HA, BRI or ES). Values represent raw estimates output from CMR models, and are used are used to plot individual survival against value of a trait presented **Figure S4**. Individual values of survivals are calculated by the equation: $\phi_{ind}=(1/(1+\exp(-\beta_0+\beta_1*x_{ind}))$.

	$\beta_{0(rural)}$	$\beta_{0(urban)}$	β_1
Handling Aggression	0.063 (-0.194 - 0.320)	-0.183 (-0.365 - -0.001)	1.006 (0.713 - 1.420)
Breath Rate Index	0.292 (-0.002 - 0.585)	-0.070 (-0.258 - 0.117)	1.096 (0.746 - 1.449)
Exploration Score	0.221 (-0.080 - 0.521)	-0.047(-0.237-0.141)	1.110 (0.0756 - 1.462)

FIGURES

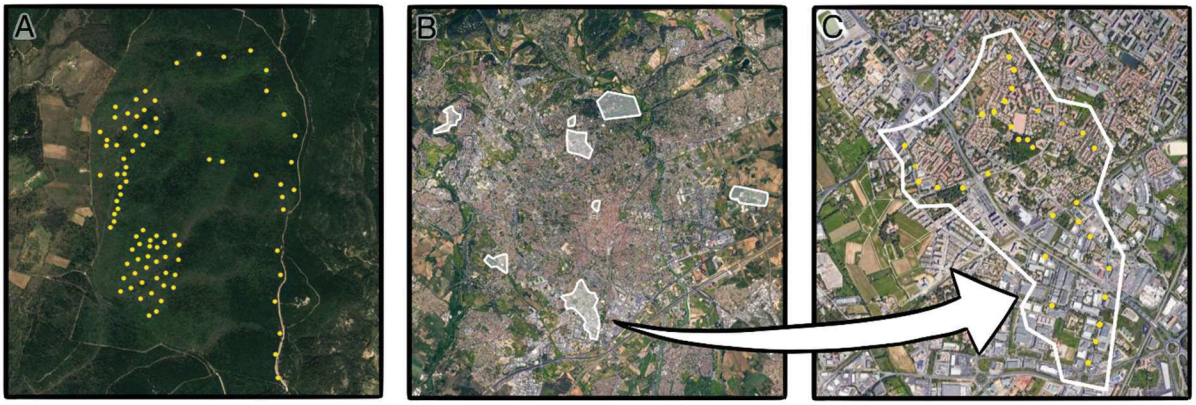

Figure S1 : Satellite images of the study sites**.** (A) The La Rouvière forest site near Montarnaud, France. (B) Map of Montpellier showing monitored zones of the city in purple. (C) Zoom of a section of the urban site, each yellow dot represents a nest-box. Yellow dots represent great tit nest-boxes. Note that the forest site is also equipped with blue tit nest-boxes (entrance whole of 28 mm). ©Google Earth.

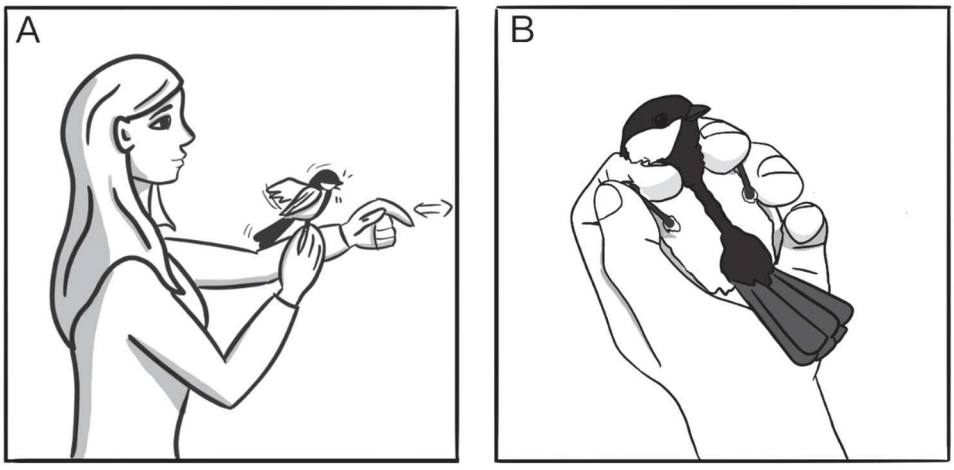

Figure S2: (A) Position of handler for the Handling Aggression test. During the test, the bird's legs are held still by one hand and the bird is nagged with a finger of the other hand. (B) Bird held on his back to perform the Breath Rate measure.

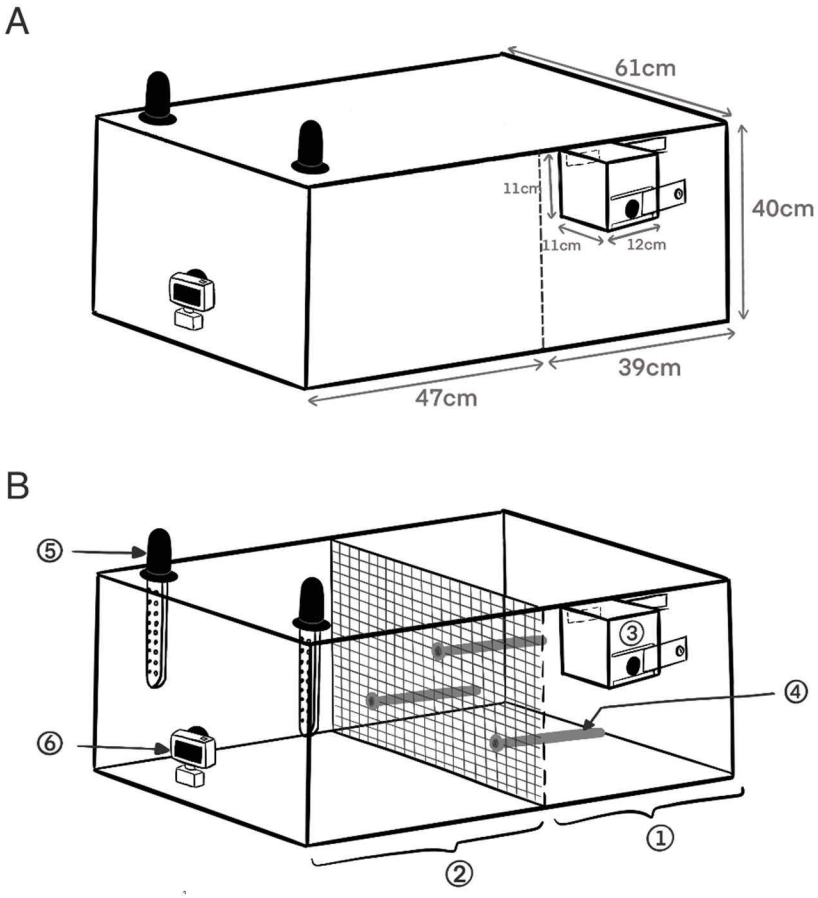

Figure S3: Drawing of the experimental open-field cage. (A) Outside view and detailed dimensions (B) Transparent view of the internal structure of the cage. 1: exploration room with three perches, 2: empty area inaccessible for the bird, 3: acclimatization compartment, 4: perch, 5: light and 6: camera. A sliding door connected the acclimatization compartment and the experimental area. External walls were made of expanded PVC sheets.

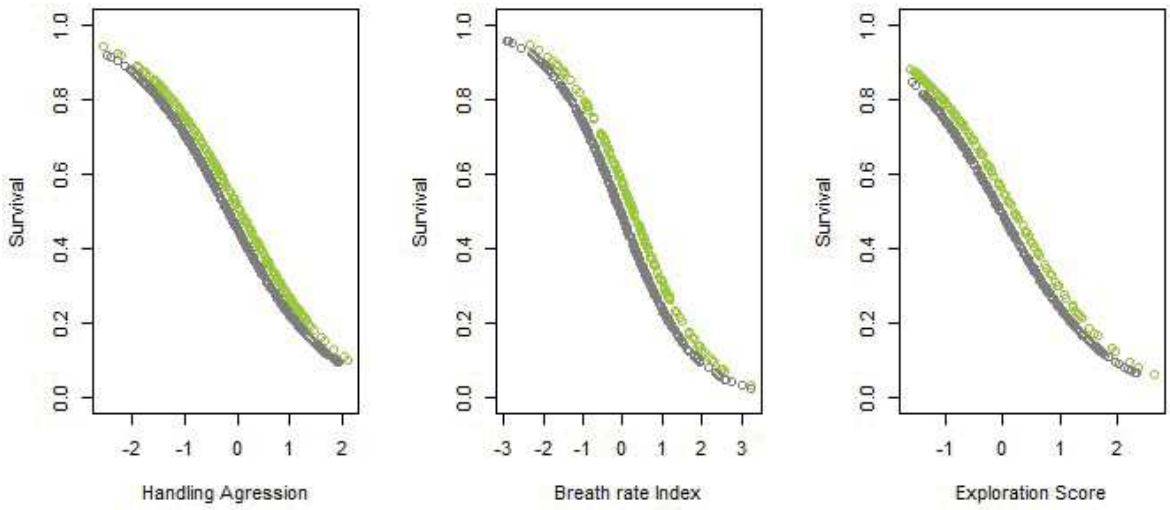

Figure S4: Variation in survival rate of urban (grey) and forest (green) great tits in relation to Handling Aggression, Breath Rate Index and Exploration Score. Individual values of survival are calculated based on β estimates (**Table S8**) from the CMR models containing the trait effect and the lower AICc (see **main text Table 4**).

– SUPPLEMENTARY MATERIALS – MANUSCRIT 4 –

Testing for parallel genomic and epigenomic footprints of adaptation to urban life in a passerine bird

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SUPPLEMENTARY TABLES

Table S1: Redundancy analysis (RDA) performed on genetic data (including Z chromosome).

	adjusted R-squared	P-value	RDA1	RDA2	RDA3	RDA4
Full RDA	0.018	0.001	% variance explained by axes			
			0.024	0.021	0.021	0.019
Variables			Biplot scores			
City – Montpellier		0.001	0.57	-0.819	0.059	-0.039
City – Warsaw			0.381	0.883	0.214	0.17
Habitat – Urban		0.001	-0.21	-0.1	0.968	-0.089
Sex – Male		0.004	0.184	0.143	-0.0358	-0.972
Partial RDA for city	0.012	0.001	% variance explained by axes			
			0.025		0.022	
Variable			Biplot score			
City – Montpellier		0.001	0.63		-0.776	
City – Warsaw			0.356		0.934	
Partial RDA for habitat	0.004	0.001	% variance explained by axes			
			0.022			
Variable			Biplot score			
Habitat – Urban		0.001	0.999			
Partial RDA for sex	0.002	0.004	% variance explained by axes			
			0.02			
Variable			Biplot score			
Sex – Male		0.005	-0.999			

Table S2: Redundancy analysis (RDA) performed on genetic data without Z chromosome.

	adjusted R-squared	P-value	RDA1	RDA2	RDA3	RDA4
Full RDA	0.016	0.001	% variance explained by axes			
			0.024	0.021	0.021	0.017
Variables			Biplot scores			
City – Montpellier		0.001	0.5725	-0.818	0.049	-0.0244
City – Warsaw			0.382	0.889	0.227	0.11
Habitat – Urban		0.001	-0.215	-0.119	0.967	-0.073
Sex – Male		0.26	0.14	0.096	-0.033	-0.985
Partial RDA for city	0.012	0.001	% variance explained by axes			
			0.025		0.022	
Variable			Biplot score			
City – Montpellier		0.001	0.624		-0.781	
City – Warsaw			0.362		0.932	
Partial RDA for habitat	0.004	0.001	% variance explained by axes			
			0.022			
Variable			Biplot score			
Habitat – Urban		0.001	0.999			
Partial RDA for sex	0.0004	0.373	% variance explained by axes			
			0.018			
Variable			Biplot score			
– Urban		0.369	-0.999			

Table S3: Fst estimation between pairs of subpopulations. 95% confidence computed intervals in parenthesis were computed using StAMPP package with 1000 bootstrap.

	Mont-Rur	Mont-Urb	Var-Rur	Var-Urb	Bar-Rur
Mont-Urb	0.012 (0.011 – 0.013)	-	-	-	-
Var-Rur	0.009 (0.009 – 0.010)	0.018 (0.017 – 0.019)	-	-	-
Var-Urb	0.018 (0.017 – 0.018)	0.026 (0.025 – 0.027)	0.018 (0.017 – 0.019)	-	-
Bar-Rur	0.009 (0.009 – 0.010)	0.019 (0.017 – 0.018)	0.009 (0.009 – 0.010)	0.018 (0.017 – 0.019)	-
Bar-Urb	0.025 (0.024 – 0.027)	0.034 (0.033 – 0.035)	0.025 (0.025 – 0.026)	0.034 (0.033 – 0.034)	0.018 (0.017 – 0.019)

Table S5: Fst estimation averaged on allosomes between pairs of subpopulations. 95% confidence computed intervals in parenthesis were computed using StAMPP package with 1000 bootstrap.

	Mont-Rur	Mont-Urb	Var-Rur	Var-Urb	Bar-Rur
Mont-Urb	0.017 (0.017 – 0.017)	-	-	-	-
Var-Rur	0.018 (0.018 – 0.018)	0.015 (0.014 – 0.015)	-	-	-
Var-Urb	0.014 (0.014 – 0.015)	0.011 (0.011 – 0.011)	0.015 (0.014 – 0.015)	-	-
Bar-Rur	0.018 (0.018 – 0.018)	0.015 (0.014 – 0.015)	0.018 (0.018 – 0.018)	0.014 (0.014 – 0.014)	-
Bar-Urb	0.011 (0.011 – 0.011)	0.008 (0.007 – 0.008)	0.011 (0.011 – 0.011)	0.008 (0.007– 0.008)	0.014 (0.014 – 0.014)

Table S6: Fst estimation averaged on Z chromosome between pairs of subpopulations. 95% confidence computed intervals in parenthesis were computed using StAMPP package with 1000 bootstrap.

	Mont-Rur	Mont-Urb	Var-Rur	Var-Urb	Bar-Rur
Mont-Urb	0.013 (0.006 – 0.018)	-	-	-	-
Var-Rur	0.016 (0.009 – 0.021)	0.020 (0.013 – 0.025)	-	-	-
Var-Urb	0.025 (0.019 – 0.031)	0.025 (0.020 – 0.032)	0.020 (0.015 – 0.025)	-	-
Bar-Rur	0.015 (0.010 – 0.019)	0.017 (0.011 – 0.022)	0.014 (0.009 – 0.019)	0.018 (0.015 – 0.024)	-
Bar-Urb	0.031 (0.026 – 0.036)	0.041 (0.032 – 0.047)	0.028 (0.022 – 0.034)	0.036 (0.030– 0.043)	0.014 (0.009 – 0.021)

Table S7: Redundancy analysis (RDA) performed on methylation data (including Z chromosome).

	adjusted R-squared	P-value	RDA1	RDA2	RDA3	RDA4
Full RDA	0.007	0.001	% variance explained by axes			
			0.021	0.019	0.018	0.016
Variables			Biplot scores			
City – Montpellier		0.001	0.21	0.259	-0.648	0.685
City – Warsaw			-0.603	-0.77	0.198	-0.067
Habitat – Urban		0.03	-0.256	0.286	0.669	0.637
Sex – Male		0.001	0.736	-0.562	0.263	0.269
Partial RDA for city	0.003	0.001	% variance explained by axe			
			0.021		0.018	
Variable			Biplot score			
City – Montpellier		0.001	-0.356		0.934	
City – Warsaw			0.986		-0.161	
Partial RDA for habitat	0.001	0.3	% variance explained by axe			
			0.019			
Variable			Biplot score			
Habitat – Urban		0.181	0.999			
Partial RDA for sex	0.003	0.001	% variance explained by axe			
			0.021			
Variable			Biplot score			
Sex – Male		0.001	0.999			

Table S8: Redundancy analysis (RDA) performed on methylation data without Z chromosome.

	adjusted R-squared	P-value	RDA1	RDA2	RDA3	RDA4
Full RDA	0.006	0.001	% variance explained by axes			
			0.021	0.019	0.018	0.016
Variables			Biplot scores			
City – Montpellier		0.001	0.231	-0.204	-0.666	0.679
City – Warsaw			-0.645	0.761	0.2467	-0.058
Habitat – Urban		0.019	-0.261	-0.365	0.632	0.632
Sex – Male		0.001	0.698	0.569	0.326	0.289
Partial RDA for city	0.003	0.001	% variance explained by axes			
			0.02		0.018	
Variable			Biplot score			
City – Montpellier		0.001	-0.357		0.933	
City – Warsaw			0.987		-0.159	
Partial RDA for habitat	0.001	0.176	% variance explained by axe			
			0.019			
Variable			Biplot score			
Habitat – Urban		0.168	0.999			
Partial RDA for sex	0.003	0.001	% variance explained by axe			
			0.021			
Variable			Biplot score			
Sex – Male		0.001	0.999			

Table S9: Table results of Type I ANOVA performed of mean methylation level of cytosines in a CpG context per individual, including : habitat (rural vs urban), city (Barcelona, Montpellier and Warsaw) and sex as explanatory variables. The first part of the table represents analysis on autosomes and the second part represents analysis on Z chromosome only.

Autosomes					
Effect	Df	Sum Sq	Mean Sq	F value	P
Habitat	1	0.416	0.416	0.511	0.478
Sex	1	1.041	1.041	1.278	0.263
City	2	5.408	2.704	3.319	0.044
Habitat* City	2	0.316	0.158	0.194	0.825
Residuals	53	42.174	0.8146		
Z chromosome					
Effect	Df	Sum Sq	Mean Sq	F value	P
Habitat	1	0.012	0.012	0.011	0.915
Sex	1	133.584	133.584	125.077	1.45x10-15
City	2	0.979	0.489	0.458	0.635
Habitat* City	2	1.1879	0.594	0.556	0.577
Residuals	53	56.605	1.068		

Table S10: Significantly enriched GO associated with genes overlapping 5kb windows around genomic outliers.

GO.ID	Term	Annotated	Significant	Expected	Weight	Associated Genes
GO:2000618	regulation of histone H4-K16 acetylation	5	3	0.34	0.003	ATG5, AUTS2, SIRT1
GO:1902287	semaphorin-plexin signaling pathway invo...	7	3	0.48	0.009	PLXNA1, PLXNA2, PLXNA4
GO:1901842	negative regulation of high voltage-gate...	3	3	0.21	0.000	DYSF, GEM, RRAD
GO:0090335	regulation of brown fat cell differentia...	10	4	0.69	0.005	FTO, PIM1, PRDM16, SIRT1
GO:0071391	cellular response to estrogen stimulus	6	3	0.41	0.006	AR, CRHBP, ESR1
GO:0071277	cellular response to calcium ion	28	7	1.93	0.002	CRHBP, ECT2, HPCA, RASAL1, SYT1, SYT11, SYT9
GO:0050953	sensory perception of light stimulus	57	6	3.92	0.026	CDH23, CRYBB2, NYX, PCDH15, RPE65, WHRN
GO:0050765	negative regulation of phagocytosis	7	3	0.48	0.009	ATG5, DYSF, SYT11
GO:0048846	axon extension involved in axon guidance	23	4	1.58	0.026	NRP2, PLXNA4, SEMA4G, SLIT3
GO:0048026	positive regulation of mRNA splicing vi…	6	3	0.41	0.006	HMX2, NCBP1, NUP98
GO:0045777	positive regulation of blood pressure	9	3	0.62	0.026	ADRA1D, GRIP2, WNK1
GO:0044351	macropinocytosis	6	3	0.41	0.006	DOCK2, MAPKAPK2, SNX33
GO:0043410	positive regulation of MAPK cascade	197	15	13.56	0.013	ADRA1D, ADRB2, APP, AR,AXIN1, EPHA8, FGFR3, MTURN, NRG1, PDGFRB, PLCB1, RET, ROR1, ROR2, SH3RF2
GO:0043087	regulation of GTPase activity	194	17	13.36	0.025	AGAP3, ARHGAP17, ARHGAP45, ARHGEF12, DOCK2, ECT2, IQGAP2, MLST8, PLCB1, PLXNA1, PLXNA2, PLXNA4, RASAL1, RDX, RGS8, SIPA1L1, WNK1
GO:0042535	positive regulation of tumor necrosis fa...	6	3	0.41	0.006	APP, HSPB1, MAPKAPK2
GO:0042311	vasodilation	13	4	0.89	0.010	ADRB2, ATG5, CPS1, NOS1
GO:0035418	protein localization to synapse	26	5	1.79	0.009	BSN, GPC4, GRIP2, HSPB1, NRXN1
GO:0033555	multicellular organismal response to str...	37	6	2.55	0.006	BRINP1, DBH, NOS1, NR4A2, PPP3CA, RET
GO:0021987	cerebral cortex development	56	8	3.86	0.013	CCDC85C, CNTNAP2, DCX, HIF1A, NRP2, PAX5, PLCB1, TRAPPC9
GO:0016339	calcium-dependent cell-cell adhesion via...	15	4	1.03	0.016	CDH13, CDH17, CDH19, CDH20
GO:0009755	hormone-mediated signaling pathway	87	12	5.99	0.014	AR, ASIP, CRHBP, ESR1, ESRRB, KMT2D, NR5A1, PPARG, PRLR, REN, RNF14, SIRT1
GO:0008344	adult locomotory behavior	46	7	3.17	0.006	APP, CHL1, FGF12, NR4A2, NTAN1, PCDH15, SEZ6L
GO:0007274	neuromuscular synaptic transmission	9	4	0.62	0.002	ADARB1, CHRNA1, FCHSD2, SLC5A7
GO:0007171	activation of transmembrane receptor pro...	8	3	0.55	0.014	DGKQ, NRG1, PRLR
GO:0007156	homophilic cell adhesion via plasma memb...	57	11	3.92	0.001	CDH13, CDH17, CDH19, CDH20, CDH23, CNTN4, DSCAML1, IGSF11, MYOT, PCDH15, RET
GO:0007155	cell adhesion	508	53	34.97	0.015	ADAMTS12, ADGRL3, AMIGO3, ASS1, ASTN2, CDH13, CDH17, CDH19, CDH20, CDH23, CHL1, CLDN23, CNTN4, COL8A1, CTNNA1, CTNNA3, DSCAML1, DTX1, DYSF, EGFLAM, EPHA8, GPC4, IGSF11, ITGA11, ITGB2, LAMA3, LSAMP, MACF1, MAD1L1, MEGF9, MYH9, MYOT, NCAM1, NEGR1, NFKBIZ, NRXN1, PARD3B, PARVA, PCDH15, PLXNA1, PLXNA2, PLXNA4, PPP3CA, RDX, RET, STAB2, STK10, TLN1, TMEFF2, TNC, VTN, VWF, ZDHHC2
GO:0003073	regulation of systemic arterial blood pr...	39	6	2.68	0.003	ADRA1D, ADRB2, AR, NCALD, REN, WNK1
GO:0001967	suckling behavior	7	3	0.48	0.009	APP, CNTFR, UBE2Q1
GO:0001778	plasma membrane repair	7	3	0.48	0.009	DYSF, MYH9, SYT11

Table S11: Significantly enriched GO associated with genes overlapping 5kb windows around habitat linked DMRs.

GO.ID	Term	Annotated	Significant	Expected	Weight	Barcelona	Montpellier	Warsaw
GO:0050804	modulation of chemical synaptic transmis...	190	10	2.92	0.016	CACNG3, CPLX2, OPHN1, PPP1R9B, PRKCB	CDH11	DRD3, EPHB1, NAPB, SNAPIN
GO:0042981	regulation of apoptotic process	619	8	9.52	0.004	DIP2A, HERPUD1, NR4A1, SOD1, ZBTB7A		ANP32E, RSL1D1, UACA
GO:0007626	locomotory behavior	122	6	1.88	0.005	ADCY6, ELAVL4, SOD1		CDH23, DRD3, SEZ6L
GO:0006816	calcium ion transport	173	6	2.66	0.024	CACNB3, CACNG3, PRKCB		CDH23, DRD3,STIM1
GO:0050728	negative regulation of inflammatory resp...	61	5	0.94	0.011	PSMB4, SOD1,	CD276, WFDC1	PBK
GO:0046677	response to antibiotic	91	5	1.4	0.012	ADCY6, SOD1	RSRC1	AOC1, DRD3
GO:0043087	regulation of GTPase activity	237	5	3.65	0.025	GAPVD1, OPHN1, RANBP3L, SOD1	PROM2	PROM2
GO:2000300	regulation of synaptic vesicle exocytosi...	40	4	0.62	0.017	CPLX2	PRKCB	NAPB, SNAPIN
GO:0008217	regulation of blood pressure	74	4	1.14	0.040	SOD1	POMC	ADRA1B, DRD3
GO:0044060	regulation of endocrine process	22	3	0.34	0.012	NR3C1,	POMC	RAB11FIP3
GO:0055072	iron ion homeostasis	42	3	0.65	0.014	NEO1, SOD1	HMOX2	
GO:0071559	response to transforming growth factor b...	113	3	1.74	0.031	NR3C1, ZBTB7A, ZFHX3		
GO:0050852	T cell receptor signaling pathway	52	3	0.8	0.046	CACNB3, SKAP1	CD276	
GO:0007200	phospholipase C-activating G protein-	40	3	0.62	0.048	ADRA1B	P2RY1	DRD3

Table S12: Significantly enriched GO associated with genes overlapping 5kb windows around sex linked DMRs.

GO.ID	Term	Annotated	Significant	Expected	Weight	Barcelona	Monpellier	Warsaw
GO:1903706	regulation of hemopoiesis	172	8	2.28	0.050	KAT7, RARA, RUNX1, TJP2	CARD11, KAT7, PTN, RASSF2,	
GO:1902930	regulation of alcohol biosynthetic proce...	20	3	0.27	0.039	SCAP	SCAP, SOD1, VDR	
GO:1902652	secondary alcohol metabolic process	53	4	0.7	0.013	LSS, SCAP	LSS, SCAP, SOD1	FGFR1
GO:0060173	limb development	123	4	1.63	0.028	RARA	PTN, TBX3	FGFR1, TBX3
GO:0060117	auditory receptor cell development	13	3	0.17	0.026	WHRN	SOD1	FGFR1
GO:0046887	positive regulation of hormone secretion	46	3	0.61	0.016			BLK, FGFR1, NMB
GO:0046849	bone remodeling	46	3	0.61	0.023	TPH1	PTN, RASSF2	
GO:0043087	regulation of GTPase activity	237	6	3.15	0.006	MLST8	PROM2, SOD1	LRRK2, PROM2, RGS14, STARD8
GO:0042981	regulation of apoptotic process	619	19	8.22	0.002	DIP2A, RARA, UBE2Z	BAG5, CARD11, CIDEC,DIP2A, GRK5, RASSF2, SOD1, TBX3	ANP32E, BLK, FGFR1, HERPUD1, KDM2B, LRRK2, PDIA3, PHLDA1, TBX3
GO:0035264	multicellular organism growth	93	4	1.23	0.033	RARA, SELENOM	ANKRD11, SOD1	ANKRD11, SELENOM
GO:0032436	positive regulation of proteasomal ubiqu...	46	4	0.61	0.018		CSNK1A1	CSNK1A1, HERPUD1, LRRK2, STUB1
GO:0032091	negative regulation of protein binding	50	3	0.66	0.028		ZFPM1	LRRK2, STUB1
GO:0031647	regulation of protein stability	152	5	2.02	0.048	LSS	BAG5, LSS, RASSF2	LRRK2,STUB1
GO:0009790	embryo development	625	15	8.3	0.049	MDFI, PCDH8, RARA, WHRN	ANKRD11, HOXB9, PTN, SOD1,TBX1,TBX 3, ZFPM1	ANKRD11, ASCL2, DNMT3A, FGFR1, KDM2B, PCDH8, TBX3
GO:0009267	cellular response to starvation	85	4	1.13	0.044	PIK3C2B	XPR1	LRRK2,WDR24
GO:0007218	neuropeptide signaling pathway	46	3	0.61	0.023			GALR2, NMB, POMC
GO:0003151	outflow tract morphogenesis	46	3	0.61	0.046	RARA	TBX3, ZFPM1	TBX3
GO:0001666	response to hypoxia	91	4	1.21	0.023	NPEPPS, SCAP	NPEPPS, SCAP	ASCL2, LIMD1
GO:0000122	negative regulation of transcription by ...	454	12	6.03	0.017	RARA	CBFA2T2, SCAF4, TBX1, TBX3, VDR, ZFPM1	ASCL2, DNMT3A, FGFR1, KDM2B, MAF, TBX3

Table S13: Number habitat linked DMRs found in females and males separately. See Supplementary Analysis 2. "Hypermethylated" or "Hypomethylated" refer to hyper- or hypomethylated DMR in urban habitat compared to forest habitat.

	Females		
	Barcelona	Montpellier	Warsaw
Hypermethylated	240	275	205
Hypomethylated	224	133	316
Total	464	408	521
	Males		
	Barcelona	Montpellier	Warsaw
Hypermethylated	107	347	315
Hypomethylated	317	229	218
Total	424	576	533

Table S14: Number of shared habitat linked DMRs found in females and males separately. See Supplementary Analysis 2.

	Number of DMRs found in females and males	
	same or opposite direction	same direction only
Barcelona	37	20
Montpellier	79	15
Warsaw	56	28

SUPPLEMENTARY FIGURES

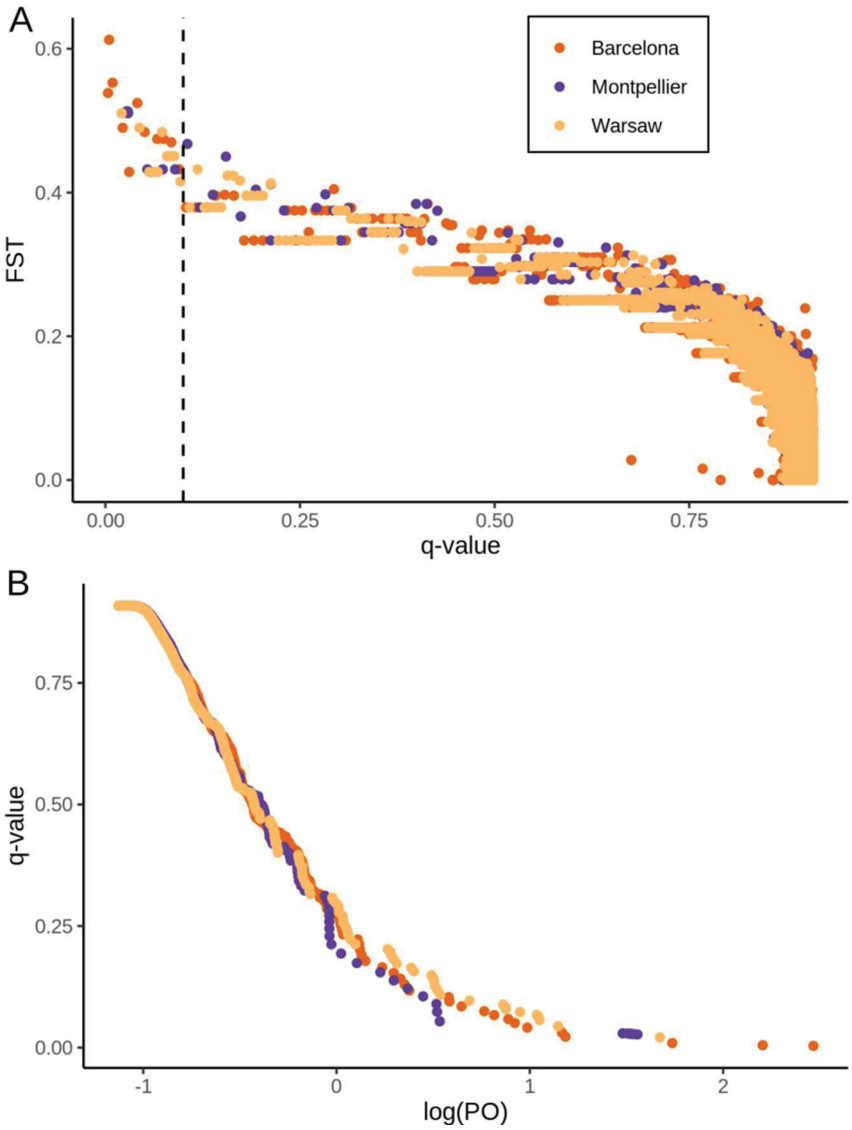

Figure S1: Bayescan plots of biallelic markers for the comparison between forest and urban great tits. (A) Distribution of F_{ST} values per position in function of q-value. (B) Distribution of q-value in function of log(PO).

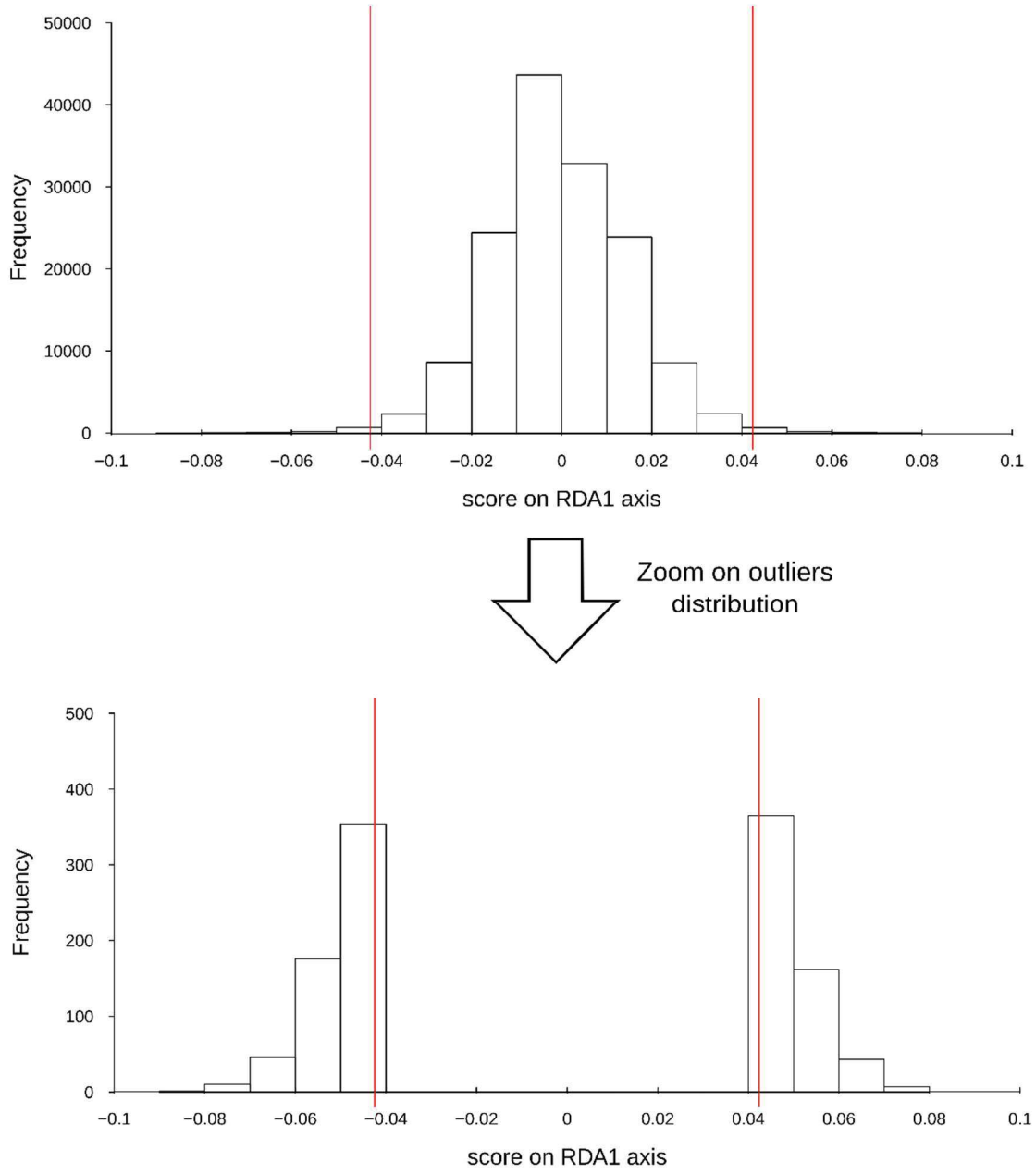

Figure S2: Distribution of genomic RDA loadings and outlier. Vertical red lines represent the thresholds used to select outliers.

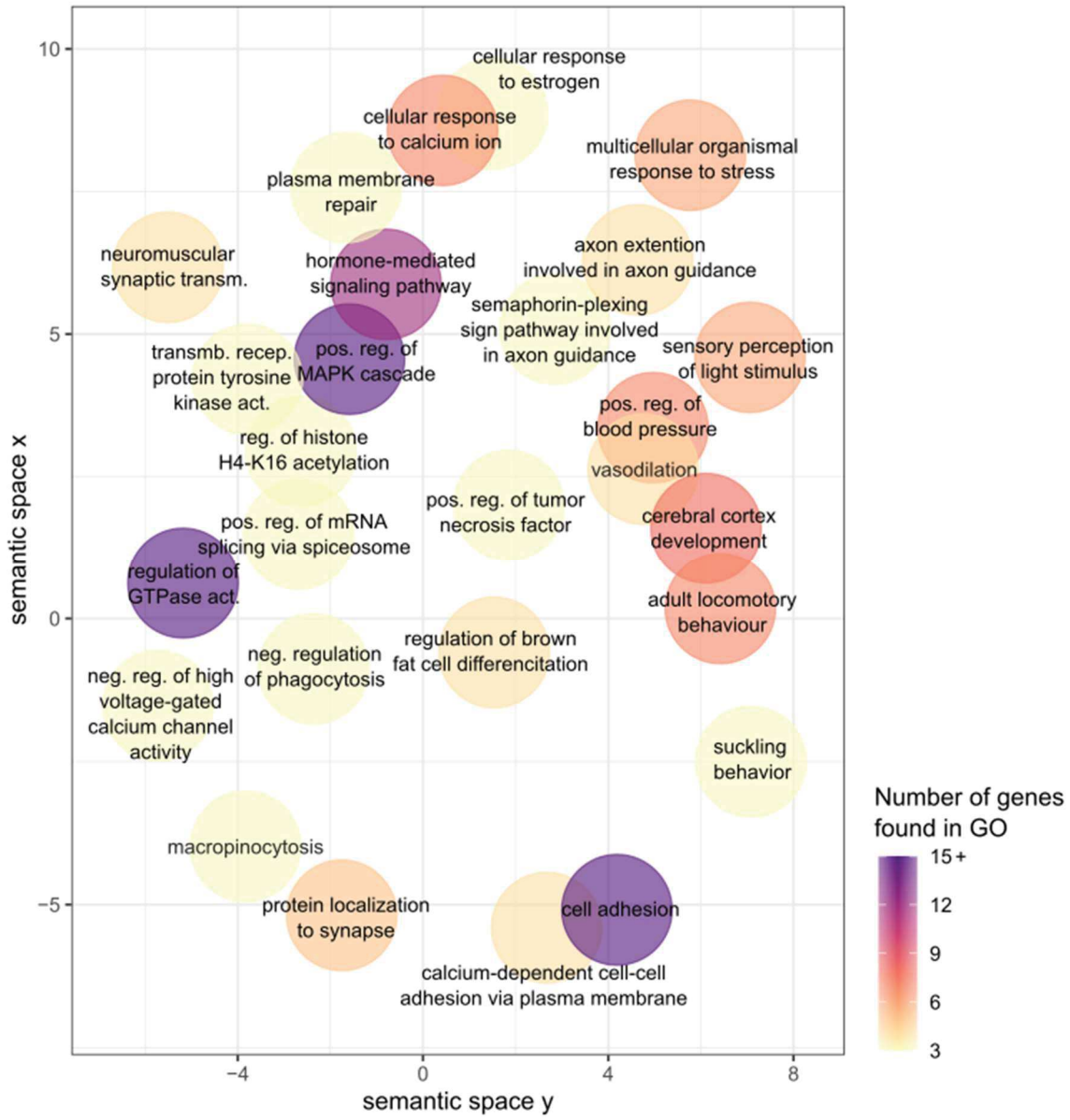

Figure S3: Spatial representation of enriched GO terms from genes associated with genetic outlier. Semantic space coordinates are calculated based on similarity of GO term word composition by REVIGO online software. Color gradient represent the number of genes involved in each GO term.

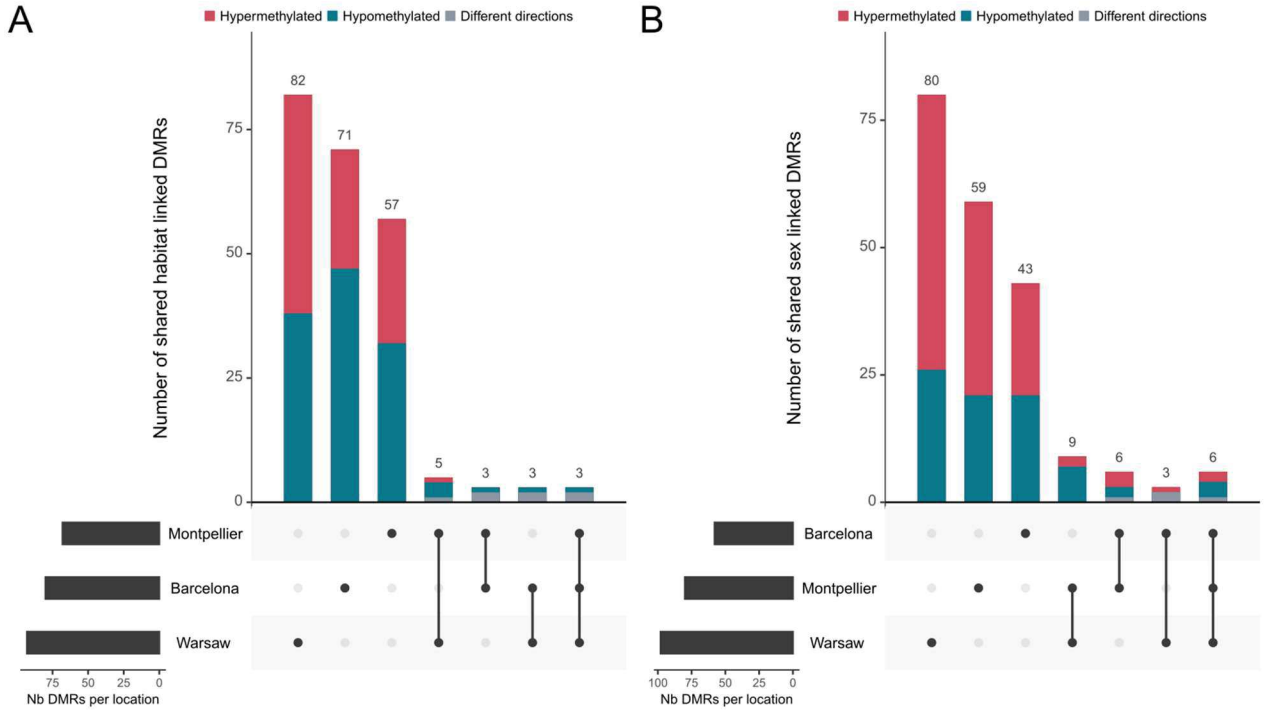

Figure S4: Number of significant DMRs between A) forest and urban, B) females and males great tits found in each location and shared between 2 or 3 locations. Hypermethylated DMRs (A) in urban, B) in males) are shown in red, hypomethylated in blue and for shared DMRs, grey represent cases where a DMRs was found in multiple locations but for which the direction of methylation was opposite.

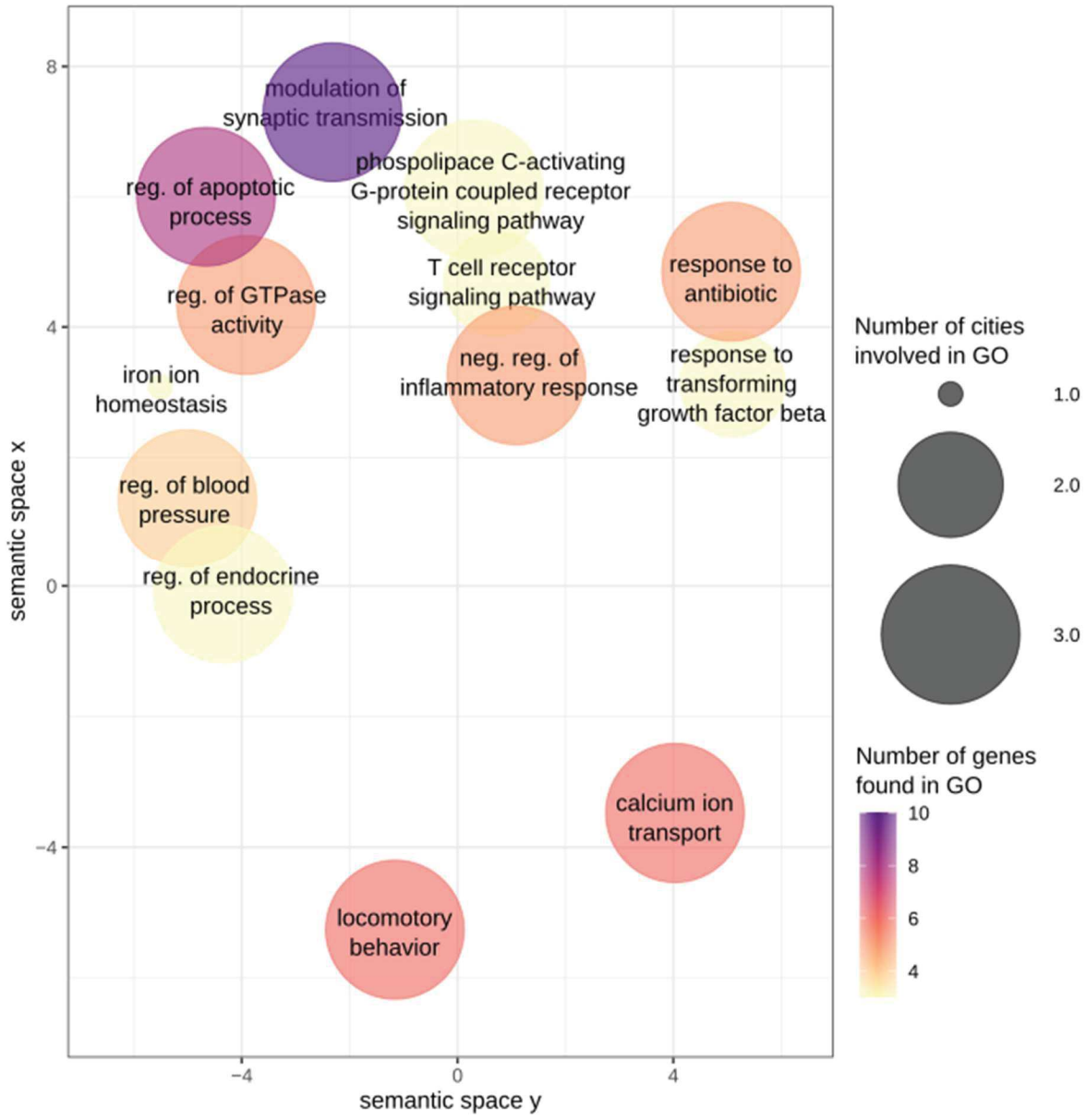

Figure S5: Spatial representation of enriched GO terms from genes associated with urbanization-linked DMRs. Semantic space coordinates are calculated based on similarity of GO term word composition by REVIGO online software. Color gradient represent the number of genes involved in each GO term and circle size, the number of city contributing to each category.

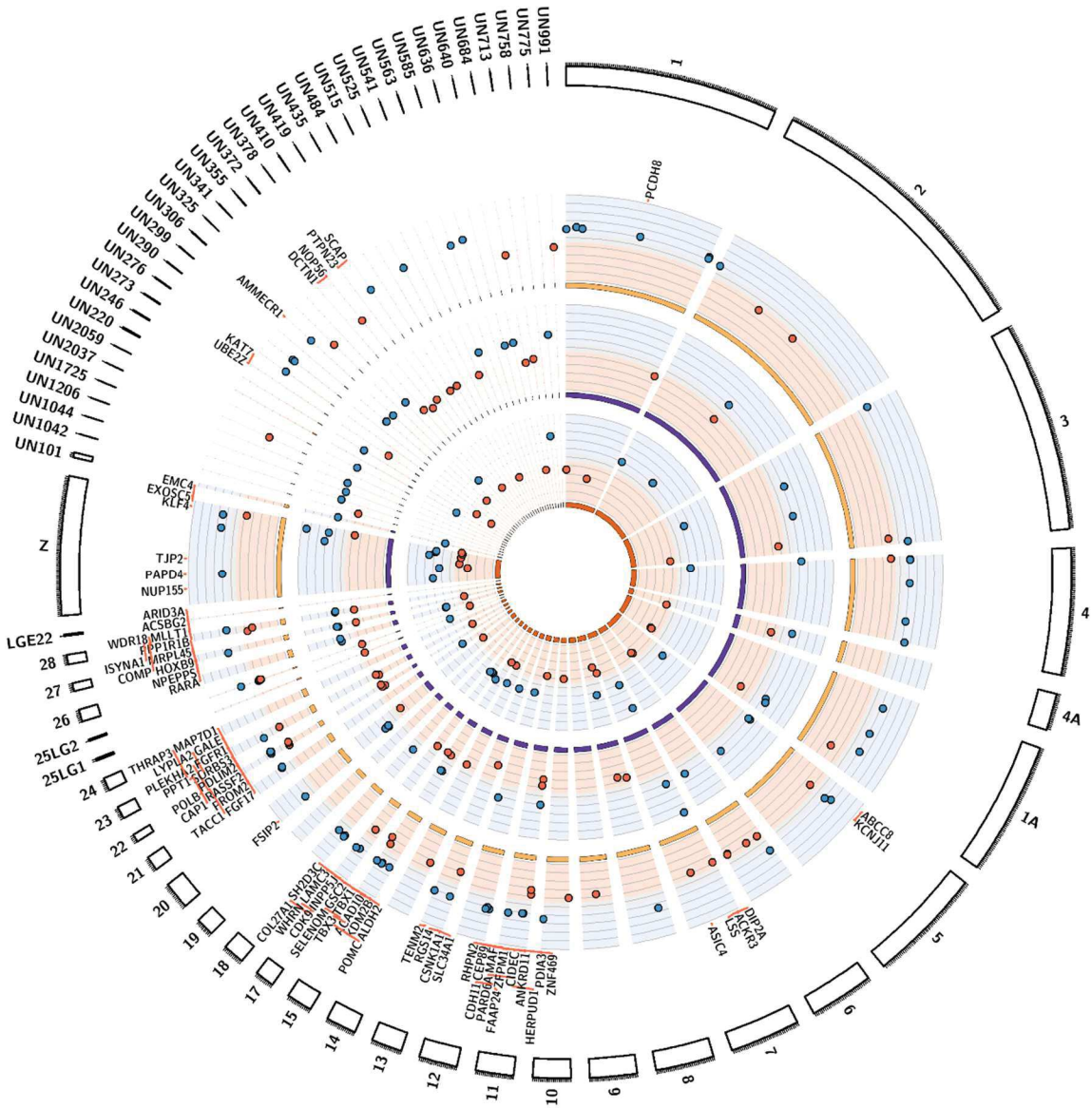

Figure S6: Circos plot of differentially methylated regions (DMRs) identified between females and males great tits in and near Barcelona, Montpellier and Warsaw (from inner to outer circles). Red points show hypermethylated regions in female great tits relatively to males, and blue points show hypomethylated regions. For graphical clarity, only a subset of genes are represented: genes associated with the 10% most extreme DMR. Names of the genes found within 5 kb of the represented DMRs are given.

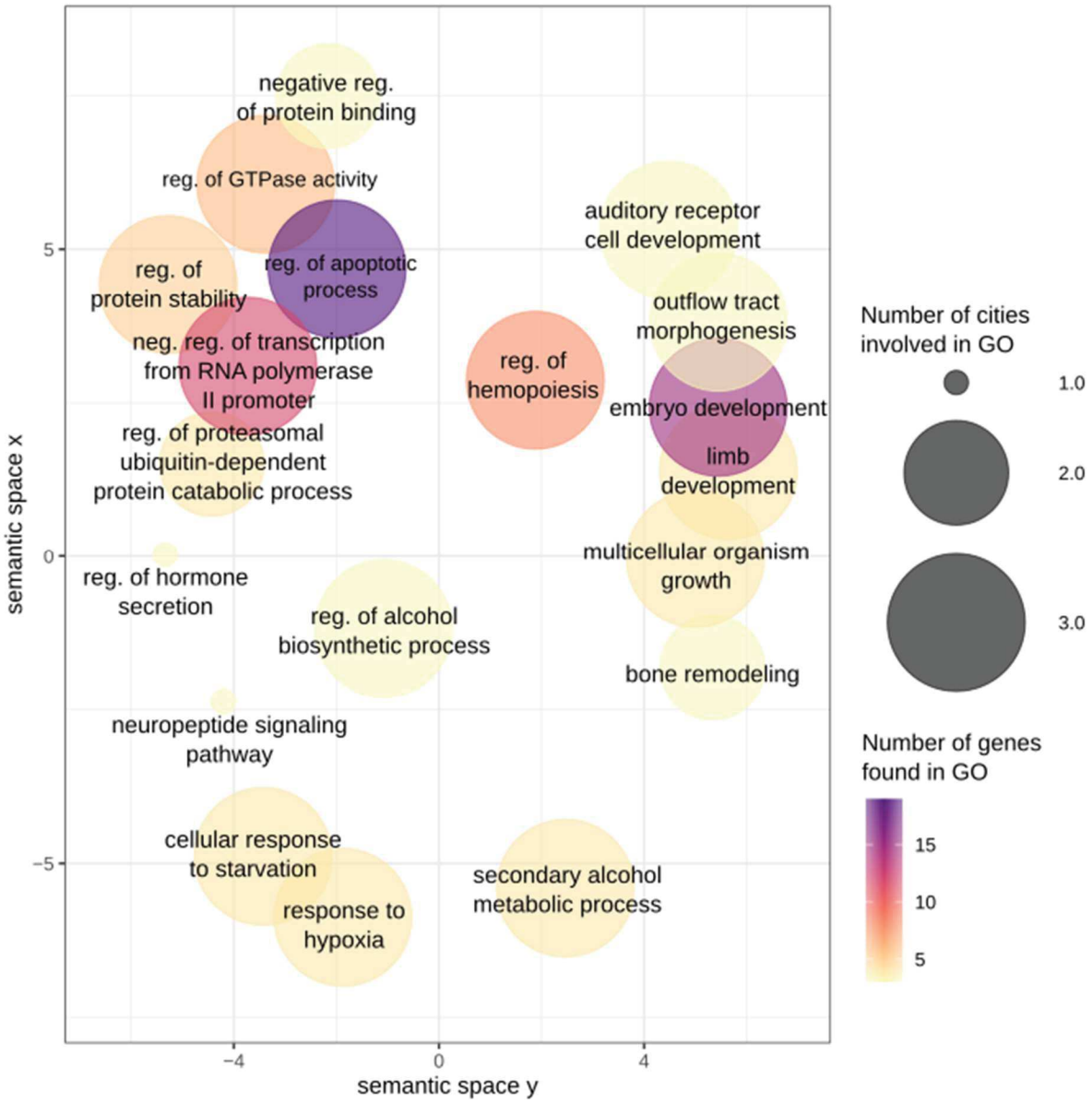

Figure S7: Spatial representation of enriched GO terms from genes associated with sex-linked DMRs. Semantic space coordinates are calculated based on similarity of GO term word composition by REVIGO online software. Colour gradient represent the number of genes involved in each GO term and circle size, the number of city contributing to each category.

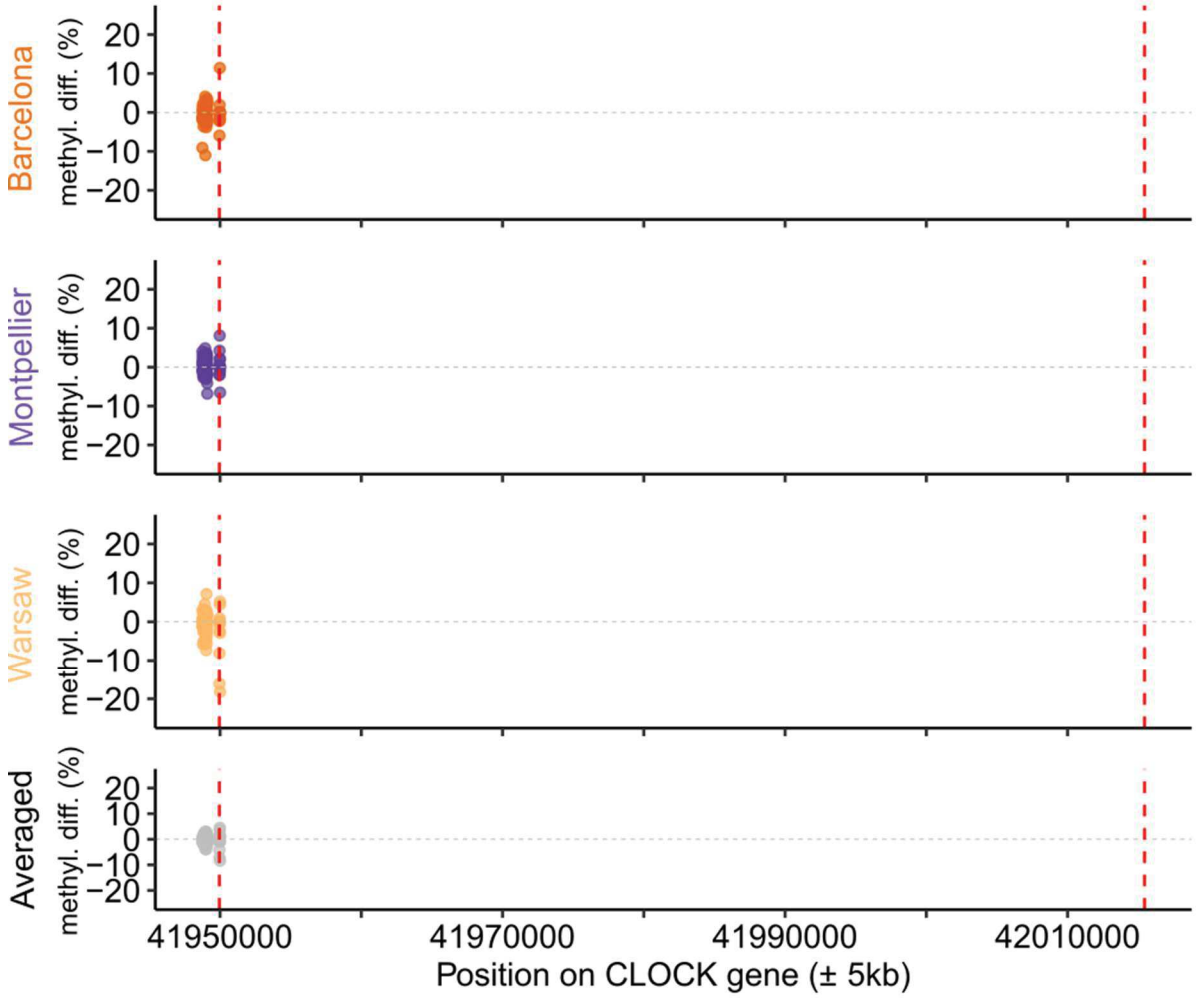

Figure S8: Mean methylation difference per base along the CLOCK gene. Vertical dashed lines materialised start and end of the gene.

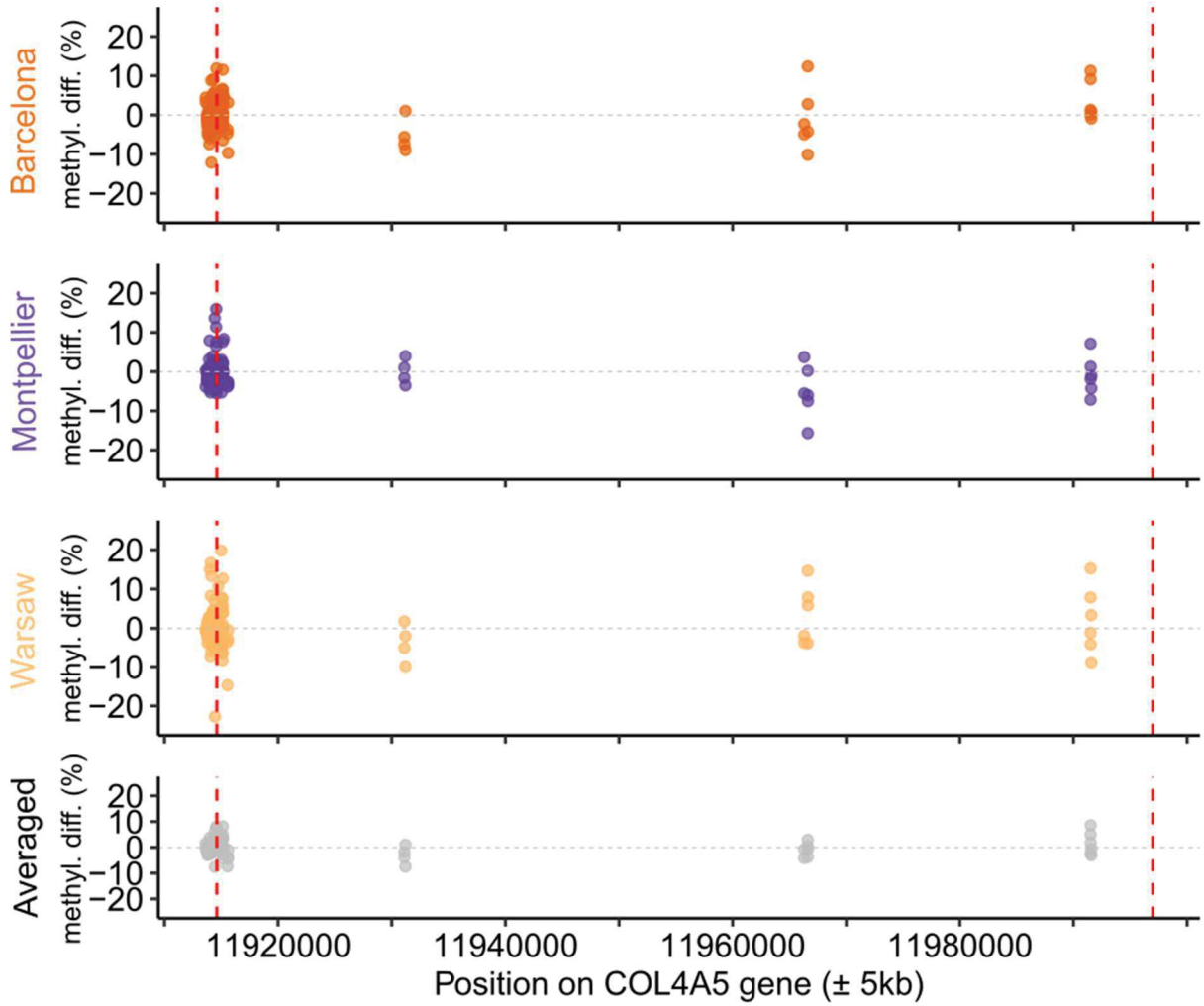

Figure S9: Mean methylation difference per base along the COL4A5 gene. Vertical dashed lines materialised start and end of the gene.

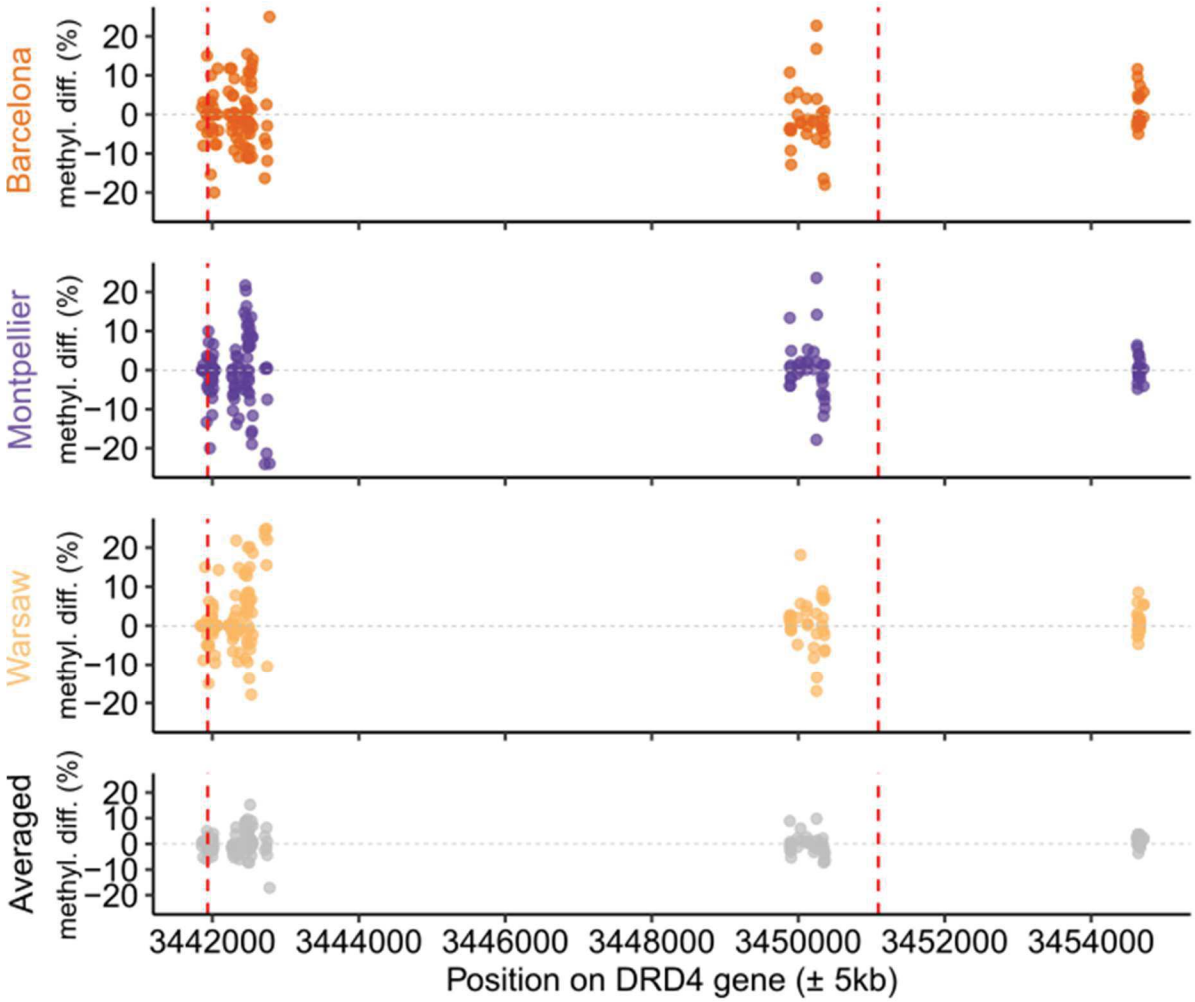

Figure S10: Mean methylation difference per base along the DRD4 gene. Vertical dashed lines materialised start and end of the gene.

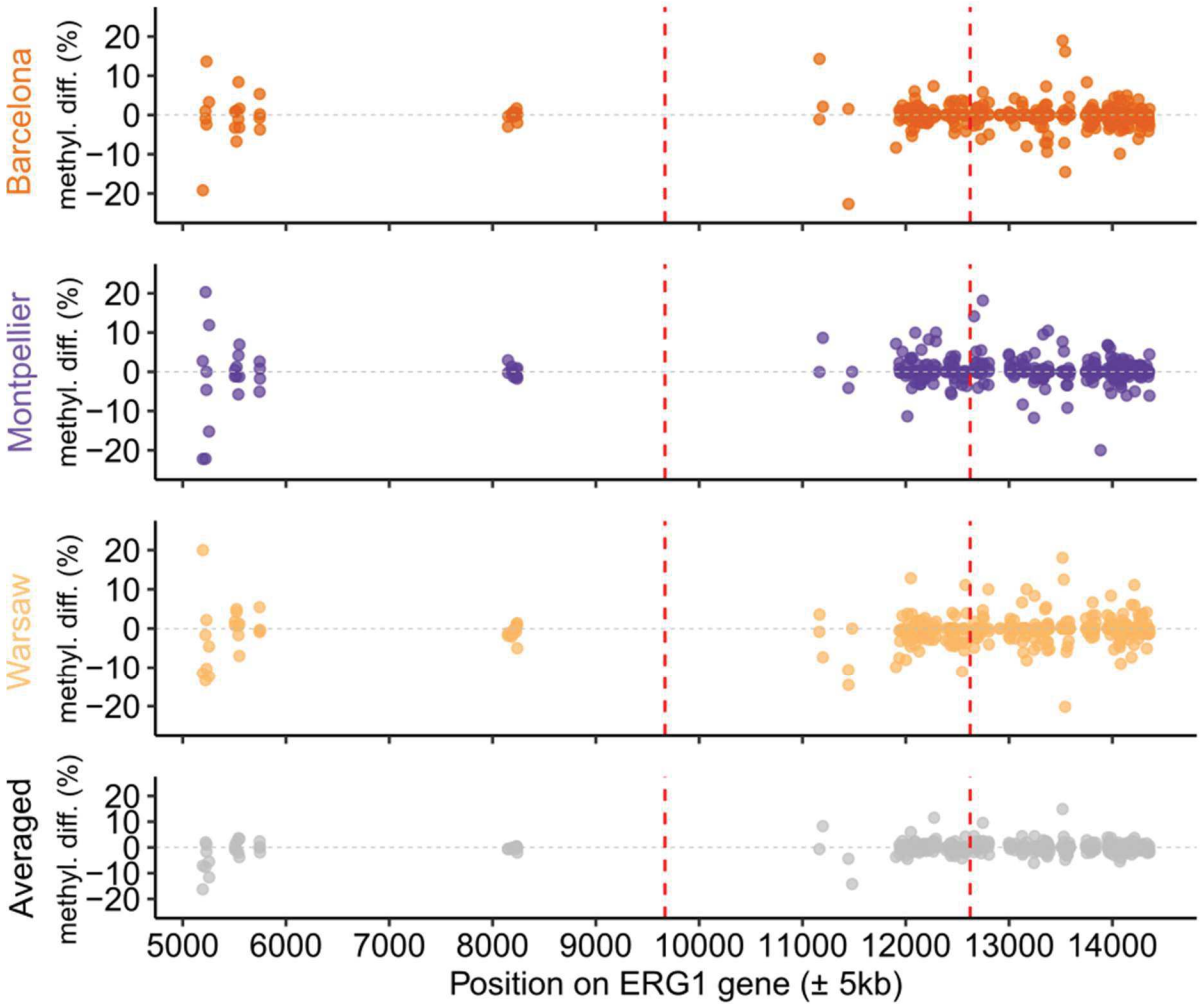

Figure S11: Mean methylation difference per base along the ERG1 gene. Vertical dashed lines materialised start and end of the gene.

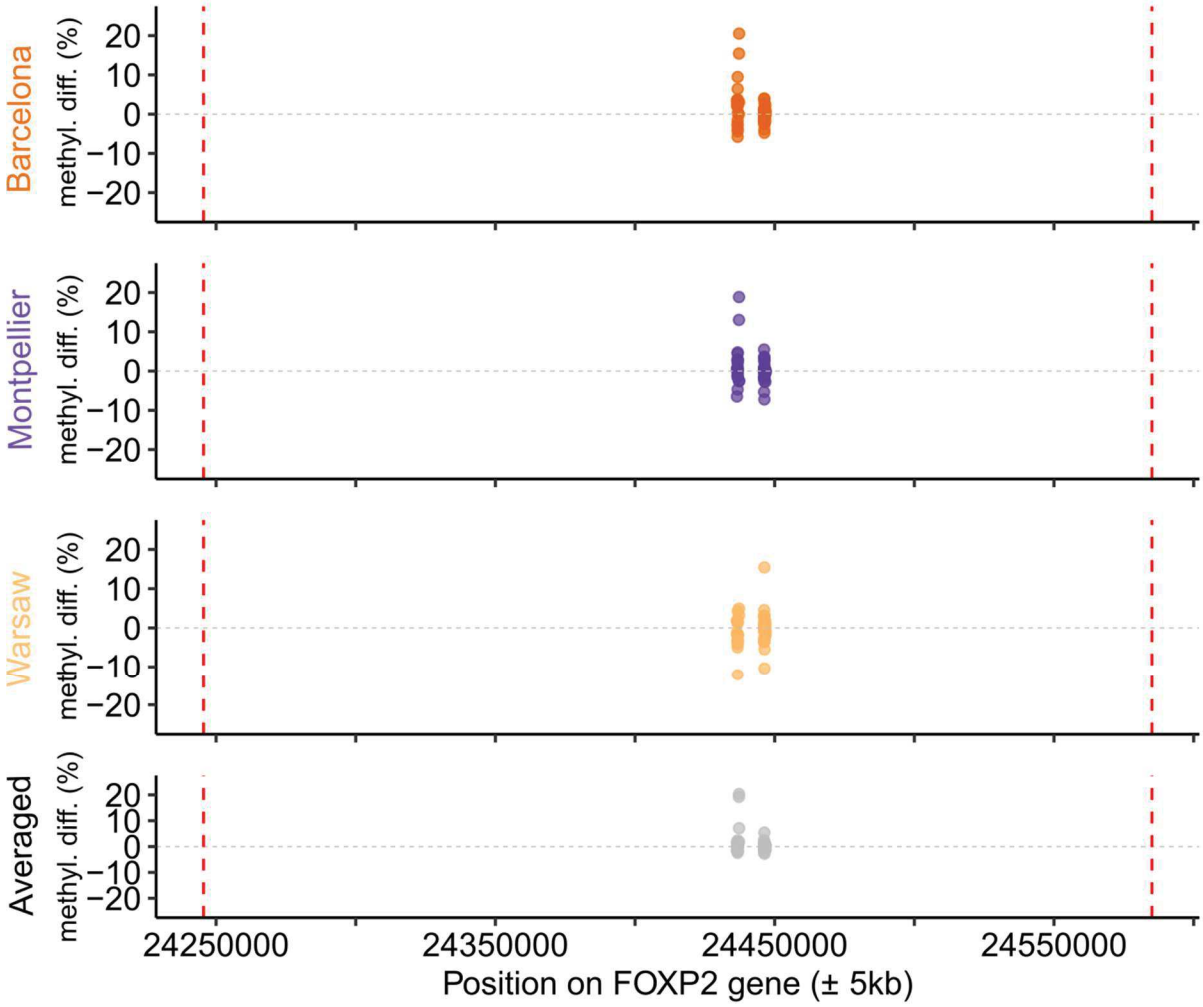

Figure S12: Mean methylation difference per base along the FOXP2 gene. Vertical dashed lines materialised start and end of the gene.

SUPPLEMENTARY ANALYSES

Differentially Methylated Regions (DMRs) between Females and Males

A total of 238 DMR were found between sexes : 58 in Barcelona, 81 in Montpellier and 99 in Warsaw (Figure S6). Warsaw presented significantly more hyper than hypomethylated DMR (χ^2=5.878, P= 0.015), but it was not the case for Barcelona (χ^2=0, P=1) nore Montpellier (χ^2=1.25, P=0.264). DMR were distributed on 29 chromosomes and 35 unplaced scaffolds.

In total, 181 DMR were located on genes or in 5kb upstream/downstream regions around genes. A gene ontology enrichment analysis performed on the pooled list of genes associated with DMR revealed significantly enriched modules associated with metabolism, development and hormonal function (see details Figure S4B and S7, and Table S12).

Differentially Methylated Regions between associated with urbanization in females and males

To explore the potential sex dependent effects of urbanization on methylation patterns, we searched for DMRs between forest and urban habitats in females and males separately. Number of significant DMRs per sex per location are reported in Table S13.

In females, 101 DMRs (7.9%) were shared between 2 or more location and 45 (3.5%) showed the same direction of variation in all cities.

Similarly, in males 134 DMRs (9.7%) were shared by multiple location, and 58 (4.18%) showed the same direction of variation.

Within location, sexes showed shared urban-linked DMRs, see Table S13.

− RÉSUMÉ −

L'urbanisation est un phénomène mondial convertissant des milieux naturels en milieux artificialisés. Les zones urbaines constituent un nouvel environnement majoritairement façonné par l'homme avec des conditions environnementales inédites pouvant directement impacter les espèces vivant en milieu urbain ainsi que leurs trajectoires évolutives. A l'heure actuelle, les processus évolutifs associés au milieu urbain sont peu connus bien qu'ils déterminent l'histoire passée, présente et future des espèces concernées. Ainsi, l'objectif de cette thèse est d'étudier l'impact de la vie urbaine sur les espèces sauvages et de comprendre si et comment les espèces s'adaptent à ce nouvel environnement. A travers l'étude de populations de mésanges charbonnières (*Parus major*) urbaine et forestière du sud de la France (Montpellier) ce travail explore dans un premier temps les différences phénotypiques associées au milieu urbain et met en évidence l'existence d'un phénotype urbain caractérisé par une taille réduite, des pontes précoces et réduites, un comportement plus exploratoire et plus agressif ainsi qu'une réponse accrue au stress. Dans un second temps, des analyses de sélection indiquent que ces différences phénotypiques ne résultent pas de nouvelles pressions de sélection associées au milieu urbain et ne sont pas adaptatives. Dans un troisième temps, une approche (épi)génomique dans trois villes (Montpellier, Barcelone et Varsovie) révèle l'existence de traces de sélection dans les génomes ainsi que de régions différentiellement méthylées en milieu urbain, mais peu de parallélisme entre villes. Parmi ces marqueurs (épi)génomiques, nombreux se révèlent associés à des gènes ayant des fonctions impliquées notamment dans le système nerveux, l'immunité, les processus hormonaux ainsi que le comportement, suggérant le potentiel rôle majeur de ces fonctions dans l'adaptation face aux conditions urbaines. Enfin, après avoir proposé une feuille de route pour tout écologue voulant étudier l'adaptation au milieu urbain, ce travail souligne certains points importants à aborder et approfondir dans le domaine, pour construire une compréhension plus vaste et détaillée de l'évolution biologique en milieu urbain.

− ABSTRACT −

Urbanization is a worldwide process irreversibly converting natural habitats into artificialized areas. Cities represent a new, human-made environment with novel environmental conditions that might directly impact urban-dwelling species and their evolutionary trajectories. Although they are of major importance for species past, present and future history, evolutionary processes ongoing in urban habitats remain poorly known. Hence, the main objective of the present work is to study city life impacts on wild species and to investigate if and how species adapt to the urban environment. Using a pair of urban and forest populations of great tits (*Parus major*) from south of France, I first explore phenotypic differences associated with urban life. Results show the existence of an urban phenotype characterized by a reduction of the birds' size, earlier laying dates, reduced clutch sizes, higher exploratory behaviour, higher aggressive behaviour and higher acute response to stress. Second, selection analyses on these morphological and behavioural traits reveal that phenotypic shifts associated with urbanization are not aligned with reproductive and survival selection, suggesting they are not adaptive. Third, an (epi)genomic approach across three cities unveils genomic footprints of adaptation as well as differentially methylated regions associated with city life, but little parallelism between cities. A majority of these (epi)genomic markers are associated with genes with functions involved in the nervous system, immunity, hormonal process and behaviour, suggesting the potential major role of these functions in avian adaptation to urban habitats. Finally, after proposing a roadmap for studying adaptation to urban habitats, this work highlights key future directions to build stronger and wider knowledge in urban evolutionary biology.