THÈSE

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Connectivité fonctionnelle en paysage fragmenté : apport des données génétiques et démographiques pour étudier l'impact multi-spécifique des infrastructures linéaires de transport

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Résumé

La connectivité fonctionnelle est un processus essentiel permettant le maintien des populations dans les paysages. Cependant, dans les paysages fortement fragmentés par des Infrastructures Linéaires de Transport (ILT ; routes, voies ferrées, *etc.*), cette connectivité peut être rompue et la viabilité des populations compromise. De plus, la connectivité fonctionnelle varie selon l'espèce considérée et toutes les espèces n'ont pas la même susceptibilité d'être affectées par les ILT. Il est donc nécessaire d'appréhender cette connectivité via une approche multi-spécifique afin de garantir la mise en place efficace des réseaux de continuités écologiques. Ainsi, la connectivité de quatre espèces terrestres (l'Alyte accoucheur, la Couleuvre helvétique, la Féronie noire et le Myrtil) a été estimée dans un paysage fragmenté par six types d'infrastructures en Dordogne, France. Deux outils ont été utilisés : la génétique du paysage et des suivis par Capture-Marquage-Recapture (CMR). Les résultats ont montré que les ILT agissent principalement comme des barrières à la dispersion et qu'elles sont responsables de 38 % de la variabilité génétique expliquée à travers les espèces, les routes étant les éléments influençant le plus fortement les flux de gènes des organismes étudiés. Par ailleurs, cette thèse a été l'occasion de développer une nouvelle méthode d'estimation des effets barrières des ILT basée sur des suivis CMR. Cette méthode a été appliquée au Myrtil sur un site présentant à la fois une autoroute (A89) et une voie ferrée. Les résultats confirment que ces deux ILT limitent les événements de franchissement des papillons (six et deux fois moins de traversées de l'autoroute et de la voie ferrée qu'attendues en l'absence de ces infrastructures, respectivement). Enfin, le couplage des suivis génétiques et CMR appliqué à l'Alyte accoucheur a permis de montrer que les populations proches des infrastructures routières principales (route départementale et autoroute) ont une viabilité plus faible que les populations éloignées. Ces résultats indiquent que pour améliorer la connectivité de ces espèces dans ce paysage, davantage de structures favorisant le franchissement de la faune est nécessaire. Pour conclure, l'utilisation exclusive des suivis génétiques ou CMR n'apporte qu'une vision parcellaire de la connectivité fonctionnelle : le couplage de ces deux approches est en mesure d'améliorer fortement la compréhension des effets de la fragmentation sur la biodiversité.

Mots clés : connectivité ; dispersion ; génétique du paysage ; populations ; fragmentation ; fitness ; kernel ; capture-marquage-recapture

Abstract

Functional connectivity is a key concept which is linked to dispersal and sustains the viability of populations within landscapes. However, in highly fragmented landscapes, this connectivity can be impeded by Large-scale Transportation Infrastructures (LTIs) such as roads or railways, which represent a threat for the long term persistence of populations. In complex landscapes, LTIs might have various effects depending on the species under study. In addition, for a given species, some LTIs might impede connectivity while some others could enhanced dispersal. Therefore, multi-specific approaches are crucial in landscape ecology studies. Accordingly, the connectivity of four terrestrial species (the midwife toad, the grass snake, the ground beetle *Abax parallelepipedus* and the meadow brown) was studied in a landscape fragmented by six LTI types in south-western France. Two main tools were used: landscape genetics and Mark-Release-Recapture (MRR) surveys. The results showed that LTIs were acting mostly as dispersal barriers and that they were accounting for 38 % of the explained genetic variability across species with roads being the landscape feature that most affects organisms' gene flow. This thesis also aimed at developing a new method to estimate barrier effects of LTIs based on MRR data. This method was applied on meadow brown MRR data across two types of LTIs (a motorway and a railway) and revealed that crossing events throughout the motorway "A89" and the railway are sixfold and twofold reduced compared to a prediction where these LTIs are absent, respectively. Finally, the combination of genetic and MRR surveys applied on the midwife toad revealed that populations near main roads (country road and motorway) have a lower viability than populations further apart. These results indicate that, in order to enhance connectivity in this landscape, more crossing structures are required. To conclude, the use of genetic and MRR surveys independently only reflects a fragmented vision of functional connectivity. However, combining these two approaches in landscape studies will allow a deeper understanding of the effects of fragmentation on biodiversity.

Keywords: connectivity; dispersal; landscape genetics; populations; fragmentation; fitness; kernel; mark-release-recapture

Connectivité fonctionnelle en paysage fragmenté : apport des données génétiques et démographiques pour étudier l'impact multi-spécifique des infrastructures linéaires de transport

Avant-propos

Ces travaux ont été réalisés dans le cadre d'un contrat CIFRE (Convention Industrielle de Formation par la Recherche) au sein :

- Du bureau d'étude TerrOïko ; 14 Rue Ferlus, BP 26, 81540 Sorèze, France
- De la Station Écologique Théorique et Expérimentale de Moulis ; UMR5321 ; 2 route du CNRS, 09200 Moulis, France

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- De l'Agence de l'Environnement et de la Maitrise de l'Énergie (ADEME)

Bilan des actions de vulgarisation, valorisation et communications scientifiques

Articles scientifiques

- **Remon J.**, Moulherat S., Cornuau J. H., Gendron L., Richard M., Baguette M., Prunier J. G. Multi-specific gene flow in a fragmented environment. *Molecular Ecology, in prep.* (Chapitre 1)
- **Remon J.**, Chevallier E., Prunier J. G., Baguette M., Moulherat S. (2018). Estimating the permeability of linear infrastructures using recapture data. *Landscape Ecology* **33**(10): 1697-1710. doi: https://doi.org/10.1007/s10980-018-0694-0 (Chapitre 2)
- **Remon J.**, Moulherat S., Cornuau J. H., Besnard A., Prunier J. G. Low genetic diversity associated with fitness cost because of road proximity: an amphibian case study. *Conservation Genetics, in prep.* (Chapitre 3)

Articles de vulgarisation

- **Remon J.** Le projet CIRFE - Observatoire des infrastructures de transport et de la biodiversité en Dordogne - est lancé ! Bulletin municipal de la mairie de La Bachellerie. Avril 2015
- **Remon J.** PROJET CIRFE. Vivre a Brouchaud, numéro 57 - Décembre 2017 : 13-16 (Annexe H)

Communications orales

- **Remon J.**, Cornuau J. H., Baguette M., Moulherat S. Suivi génétique et par CMR de la Couleuvre à collier et de l'Alyte accoucheur à travers un cumul d'infrastructures linéaires de transport en Dordogne. Congrès annuel de la Société Herpétologique de France. Toulouse, 1er octobre 2015
- **Remon J.**, Chevallier E., Baguette M., Moulherat S. Are transportation infrastructures barriers to movements ? 13^{e} rencontre Ecologie et Comportement. Chizé, 23 juin 2017
- **Remon J.**, Moulherat S., Blanchet S., Baguette M., Prunier J. G. Genetic structure of *Natrix natrix* and *Alytes obstetricans* across five large-scale transportation infrastructures. Salzburg - Autriche. 19^{e} congrès de la société européenne d'herpé-

tologie. 19 septembre 2017

- **Remon J.**, Moulherat S., Cornuau J. H. Berggreen O. Communication vulgarisée de restitution des résultats du projet CIRFE en Dordogne. Limeyrat (Dordogne). 6 Novembre 2017

Posters

- Moulherat S., **Remon J.** CIRFE : Cumul d'Infrastructures Linéaires de transport terrestre et Relations Fonctionnelles Ecologiques. Colloque ITTECOP. Sophia-Antipolis, 1 et 2 octobre 2015
- **Remon J.**, Chevallier E., Moulherat S. Functional connectivity of biodiversity across an accumulation of large-scale transportation infrastructures in south-western France. IENE - 5th IENE International Conference on Ecology and Transportation. Lyon, 30 aout au 2 septembre 2016

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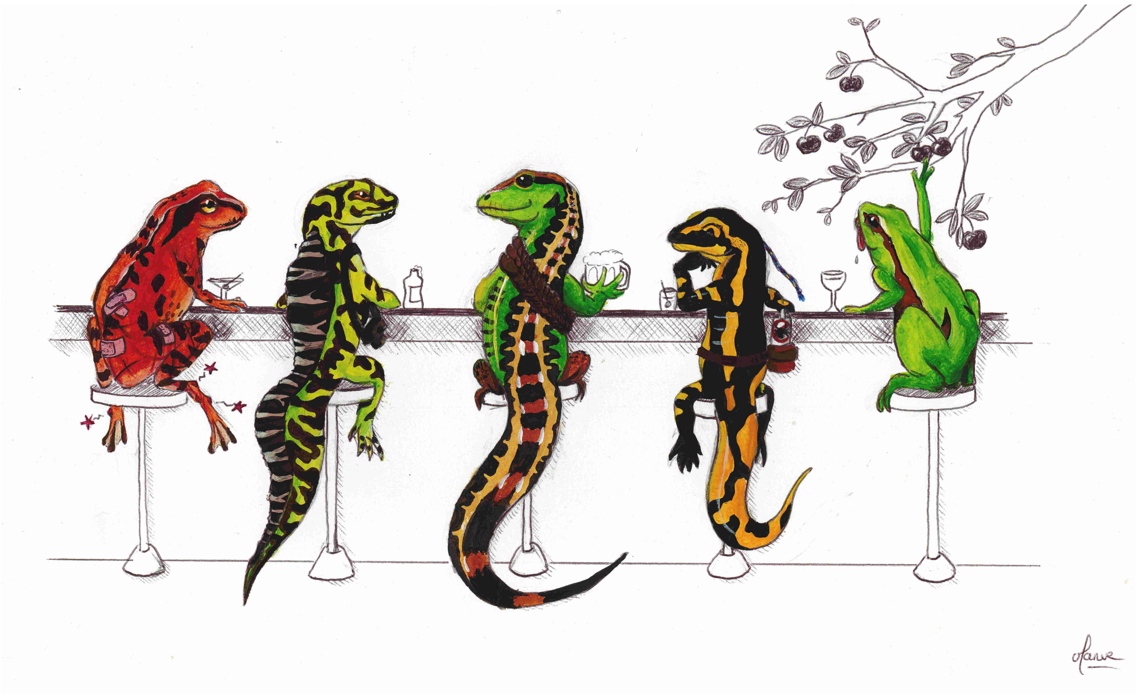

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Table des matières

Introduction

La principale cause du déclin de la biodiversité est la **perte d'habitats** (Hanski, 2011). Ces habitats peuvent être naturels (non modifiés par l'Homme) ou semi-naturels (telles que les zones pâturées). Ces derniers abritent une biodiversité qui peut, dans certains cas, surpasser celle des habitats naturels (Habel, Dengler, Janišová, Török, Wellstein and Wiezik, 2013). Ces habitats semi-naturels composent la vaste majorité des milieux que l'on peut trouver en paysages anthropisés. On estime ainsi que, depuis 1990, plus de la moitié des biomes terrestres a été convertie pour les pratiques humaines (Millenium Ecosystem Assessement, 2005). La détérioration et la perte de ces habitats, qui se sont accélérées depuis quelques décennies (Butchart et al., 2010 ; Ripple et al., 2017), s'avèrent catastrophiques pour la biodiversité (Foley et al., 2005 ; Hanski, 2011). La dégradation actuelle des habitats engendre un processus d'extinction future de nombreuses espèces appelé "dette d'extinction" (Tilman et al., 1994).

Ce phénomène amène certains scientifiques à considérer ce déclin de biodiversité comme la sixième extinction de masse sur Terre (Ceballos et al., 2017). Si le rythme actuel persiste, ce phénomène est susceptible d'être du même ordre (voire plus important) que les cinq extinctions majeures de ces derniers 550 millions d'années (Barnosky et al., 2011). Les profondes influences de l'Homme sur les écosystèmes amènent à désigner l'époque actuelle comme une nouvelle ère géologique appelée Anthropocène (Lewis and Maslin, 2015).

La perte de biodiversité a des effets délétères sans équivalent sur l'humanité (Cardinale et al., 2012). En effet, l'Homme dépend étroitement du bon fonctionnement des écosystèmes, ne serait-ce que pour assurer les services écosystémiques (pollinisation ou recyclage du CO_2 par exemple) (Millenium Ecosystem Assessement, 2005). D'autre part, le côté esthétique de la biodiversité a des effets bénéfiques pour l'Homme en procurant un certain bien-être (Tribot et al., 2016).

La destruction des habitats amène typiquement à la **fragmentation** (Forman and Godron, 1986 ; Turner, 1989 ; Fahrig, 2003). Cette dernière modifie de manière profonde les fonctions des écosystèmes et est responsable d'une réduction de 13 à 75 % de la biodiversité terrestre (Haddad et al., 2015). La fragmentation à l'échelle paysagère est un processus qui divise l'habitat en un ensemble de fragments (**tâches**) plus petits et isolés les uns des autres (Laurance, 2008) (Fig. A). Ces tâches d'habitat sont isolées par une matrice paysagère transformée par l'Homme, composée, par exemple de zones urbanisées ou de cultures intensives.

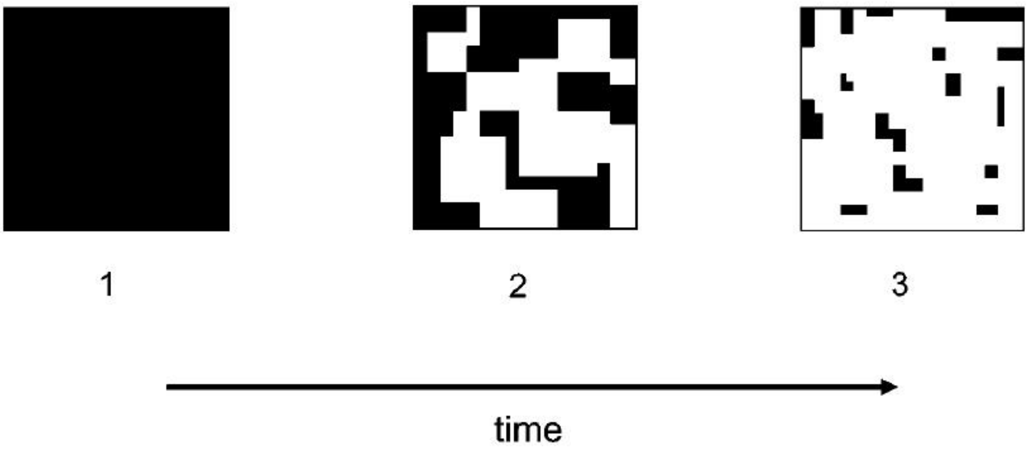

Figure A – Dynamique temporelle du processus de fragmentation. L'habitat (en noir) subit une division en de nombreux fragments (tâches) au cours du temps avec un isolement de plus en plus important entres ces tâches. Extrait de Fahrig (2003).

Les dynamiques des populations d'espèces vivant dans ces tâches petites et isolées peuvent être fortement affectées. La théorie des méta-populations (Levins, 1969) prédit qu'un ensemble de tâches est soumis à un processus dynamique d'extinction-colonisation. En effet, les fragments de petite taille possèdent de faibles capacités d'accueil et supportent donc des populations plus petites. Ces petites populations sont davantage sujettes au risque d'extinction, et ce pour deux raisons principales. Premièrement, les petites populations sont démographiquement plus sensibles aux aléas stochastiques (Legendre et al., 1999 ; Reed et al., 2002). Deuxièmement, les petites populations sont davantage exposées à la **dérive génétique** que les grandes populations (fluctuations aléatoires des fréquences alléliques), induisant une perte de **diversité génétique** et une augmentation de la **consanguinité** (Allendorf, 1986 ; Brook et al., 2002 ; Rowe and Beebee, 2003). Ces effets entraînent une accumulation d'allèles délétères (McCauley, 1991) et, par conséquent, réduisent la **fitness moyenne** de ces populations (Allentoft and O'Brien, 2010). Une faible fitness se traduira, par exemple, par une fécondité limitée et/ou une survie des individus appauvrie. Les populations ayant une fitness moyenne plus faible auront donc un taux d'accroissement plus lent. L'ensemble de ce processus qualifié de "vortex d'extinction" (McCauley, 1991 ; Fagan and Holmes, 2006) augmente le risque d'extinction locale des petites populations.

Ces extinctions peuvent toutefois être contrebalancées par des épisodes de **dispersion** à l'échelle paysagère, permettant le maintien des méta-populations. La dispersion est définie comme le mouvement d'individus qui engendre du **flux de gènes** dans un paysage (Ronce, 2007). Cette dernière permet la recolonisation de tâches laissées vacantes et le renforcement démographique et génétique des populations en déclin (phénomène qualifié de "rescue effect", Gotelli, 1991). Plus généralement, la dispersion assure un brassage génétique dans les paysages et permet le maintien du potentiel adaptatif des populations, les rendant ainsi moins sensibles à la stochasticité environnementale.

Face à cette problématique, les stratégies de conservation visent notamment à améliorer la **connectivité** entre les tâches d'habitats. La connectivité se définit comme le degré auquel le paysage facilite ou limite le mouvement des individus entre tâches (Tischendorf and Fahrig, 2000). Dans cette définition, il est nécessaire de dissocier **connectivité structurelle** et **connectivité fonctionnelle**.

La connectivité structurelle, tout d'abord, mesure uniquement la relation entre les éléments structurants du paysage, indépendamment des caractéristiques écologiques de l'espèce considérée (Tischendorf and Fahrig, 2000 ; Taylor et al., 2006). Cette connectivité est relativement aisée à appréhender via l'utilisation d'indices paysagers tels que la taille et la forme des fragments d'habitats ou la distance les séparant. Ainsi un paysage aura une faible connectivité structurelle si les tâches d'habitats sont petites et fortement isolées les unes des autres.

La connectivité fonctionnelle, quant à elle, est plus difficile à mesurer car elle s'intéresse aux mouvements de l'espèce étudiée et à son interaction avec les éléments paysagers (Kadoya, 2009). Pour pouvoir l'estimer, l'approche la plus directe consiste à suivre les gamètes ou les individus dans les paysages via l'emploi de suivis génétiques ou des techniques de localisation (CMR, télémétrie, *etc.*). Une deuxième approche permet d'appréhender la connectivité fonctionnelle en utilisant des modèles basés sur la "résistance" du paysage (LaPoint et al., 2015). Parmi eux, on peut citer les modèles utilisant les chemins de moindre coût (Adriaensen et al., 2003), la théorie des circuits (McRae, 2006) ou les modèles intégrant de l'information comportementale des individus (e.g. Palmer et al., 2011).

En France, les **Trames Vertes et Bleues** (TVB) ont été mises en place suite au

Grenelle de l'environnement de 2008 (Loi Grenelle 1, JORF n° 0179 du 5 août 2009 ; LOI n° 2009-967 du 3 août 2009 - Art 23). Les TVB visent à favoriser la connectivité via les Schémas Régionaux de Cohérence Ecologique (SRCE) qui fixent les grandes lignes directrices des TVB au niveau régional. Les objectifs de ces schémas sont ensuite appliqués dans les documents de planifications comme les Schémas Directeurs d'Aménagement et de Gestion des Eaux (SDAGE), les Schémas de Cohérence Territoriale (SCoT) et les Plans Locaux d'Urbanisme (PLU). Les Trames Vertes et Bleues ont pour objectif premier de contribuer à enrayer la perte de biodiversité, en participant à la préservation, à la gestion et à la remise en bon état des milieux nécessaires aux continuités écologiques (Allag-Dhuisme et al., 2010). En pratique, cette définition vise donc la connectivité structurelle via la mise en place de corridors écologiques. La validation fonctionnelle de ces corridors est importante pour la gestion et la conservation de la biodiversité. En effet, la connectivité structurelle n'engendre pas nécessairement de la connectivité fonctionnelle si les corridors ne sont pas utilisés par les espèces visées (Taylor et al., 2006). De la même manière, il peut très bien exister de la connectivité fonctionnelle dans un paysage sans qu'il n'y ait de connectivité structurelle si l'espèce en question est capable de traverser la matrice inhospitalière séparant les fragments d'habitats (Bélisle and Desrochers, 2002).

⇒ *Dans cette thèse, nous nous focaliserons uniquement sur la connectivité fonctionnelle en paysage fragmenté.*

Les espèces généralistes et spécialistes face à la fragmentation des habitats

Comme énoncé précédemment, les conditions changeantes de l'environnement et notamment la fragmentation des habitats sont susceptibles d'impacter fortement la viabilité des populations. Certains éléments indiquent que les **espèces généralistes** peuvent subir plus fortement ces effets que les **espèces spécialistes** (Habel and Schmitt, 2018). Ce constat s'appuie sur des différences génétiques entre ces deux types d'espèces.

Les espèces spécialistes ont généralement une diversité génétique plus faible que les espèces généralistes (e.g. Habel, Rödder, Lens and Schmitt, 2013). Ce phénomène s'explique par le fait que les espèces spécialistes se répartissent en petites populations souvent isolées au sein des paysages (Thomas, 2016). De fortes pressions de sélection contre les individus homozygotes possédant des allèles délétères entretiennent ainsi cette faible diversité génétique (Reed and Frankham, 2003 ; Habel and Schmitt, 2018). Au contraire, les populations d'espèces généralistes sont plus répandues dans les paysages et sont moins sensibles aux éventuelles pressions de sélection locales. Par conséquent, tant que la connectivité est maintenue, les populations d'espèces généralistes jouissent d'une plus forte diversité génétique que les populations d'espèces spécialistes.

On admet ainsi couramment que les espèces spécialistes présentant des comportements sédentaires sont plus sensibles à la perte d'habitats que les espèces généralistes car elles manquent de ressources pour s'adapter (faible diversité génétique) ou pour disperser (faible capacité de se déplacer).

Cependant, il est à noter que les espèces spécialistes se maintiennent depuis longtemps sous forme de populations démographiquement isolées et génétiquement appauvries. Elles sont donc, sans doute, moins sensibles à l'accélération actuelle de la fragmentation que des espèces généralistes qui formaient au préalable de larges réseaux de populations bien connectés. Les espèces généralistes subissent soudain, de plein fouet, la perte de diversité génétique associée à la perte d'habitat et de connectivité fonctionnelle (Habel and Schmitt, 2018).

De ce fait, la préservation de la connectivité entre fragments d'habitat semble plus importante pour les espèces à tendances généralistes que pour les espèces spécialistes (Habel and Schmitt, 2018). Cette tendance a d'autant plus d'importance en matière de conservation puisque les espèces généralistes possèdent généralement des espaces vitaux vastes et leurs niches écologiques incluent celles de nombreuses autres espèces. Les espèces généralistes coïncident donc souvent avec les **espèces "parapluies"**, dont la protection bénéficiera à de nombreuses autres espèces partageant le même type d'habitat (Noss, 1990 ; Barua, 2011).

⇒ *Cette thèse se focalise donc sur des espèces "parapluies", à tendances généralistes.*

Les infrastructures linéaires de transport

De manière générale, les Infrastructures Linéaires de Transport terrestre (ILT) participent fortement à la fragmentation des habitats (Forman and Alexander, 1998 ; Trombulak and Frissell, 2000 ; Balkenhol and Waits, 2009). Dans les régions les plus développées, les réseaux denses d'ILT ont de profonds impacts sur les écosystèmes (Dulac, 2013 ; Laurance et al., 2014) (Fig. B).

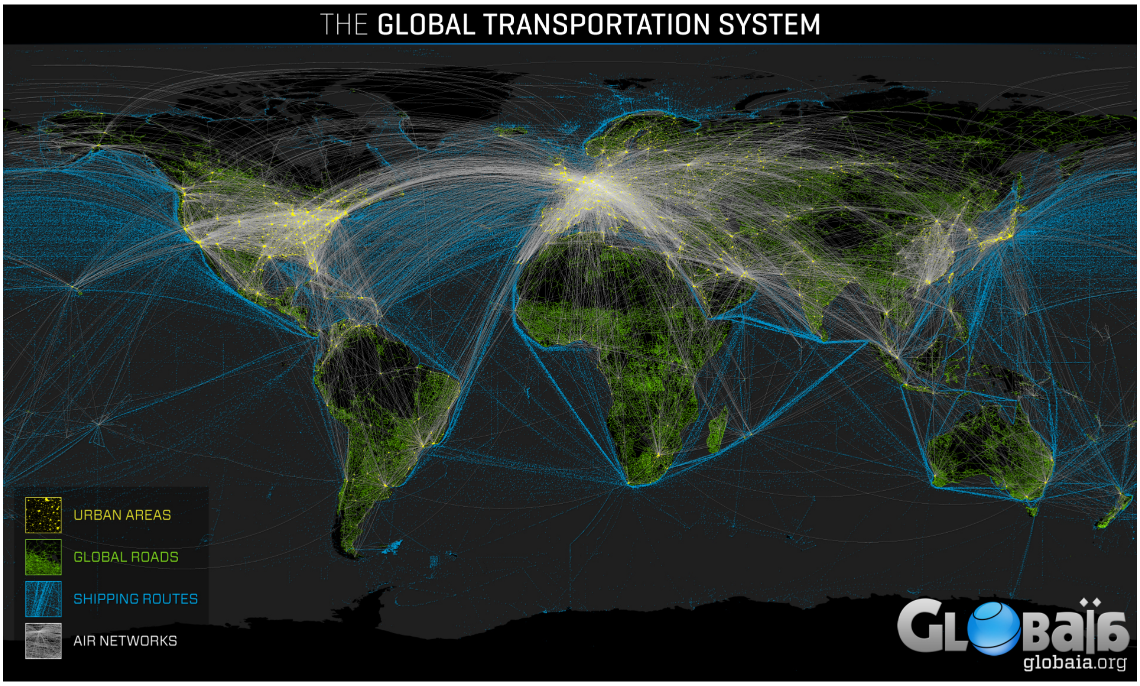

FIGURE B – Distribution globale du réseau de transports sur Terre.

Les ILT ont pour rôle le transport de personnes, de marchandises ou d'énergies. Elles permettent une mobilité accrue et un développement économique notoire des zones qu'elles relient. Les ILT les plus communes sont les routes, autoroutes et voies ferrées ; les canaux, gazoducs, lignes électriques et pipelines constituent des ILT moins répandues. Le réseau routier est le plus développé. On compte environ 37 millions de km de routes sur Terre (CIA The World Factbook, extrait le 16 Janvier 2018), ce qui est suffisant pour faire 50 fois l'aller-retour jusqu'à la Lune ! Il existe peu d'endroits sur Terre qui ne subissent pas les effets de la fragmentation due aux routes (Ibisch et al., 2016) (Fig. C).

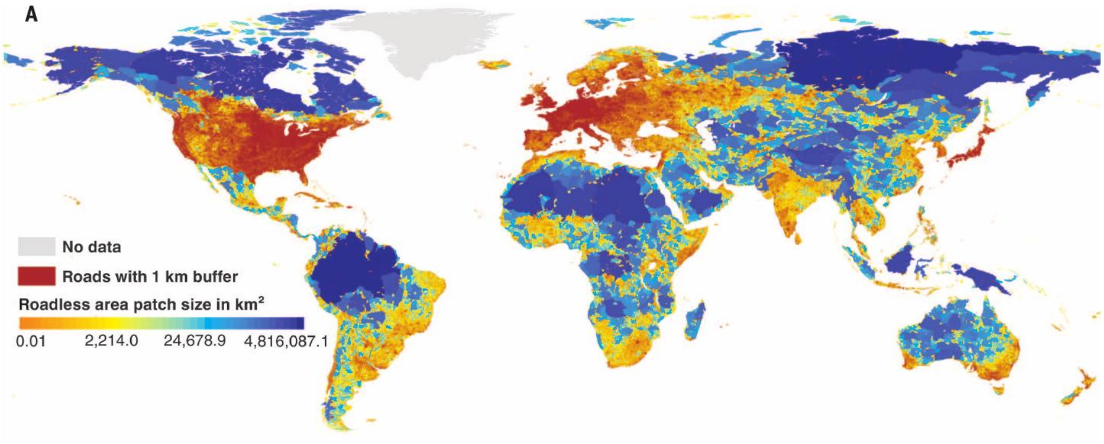

Figure C – Distribution mondiale de la taille des fragments sans routes. En rouge les zones les plus fortement touchées. Extrait de Ibisch et al. (2016).

En Europe, le réseau formé par les ILT s'est fortement développé depuis les années 70, et a des conséquences importantes sur les milieux où elles sont implantées (Girardet, 2013). En France, on compte environ 11 599 km d'autoroutes, 28 987 km de voies ferrées et 1 074 619 km de routes (Eurostat, 2015). Les directives européennes exigent l'estimation des effets que les ILT engendrent sur la biodiversité. Cependant, les études d'impacts se limitent essentiellement aux effets directs des ILT, notamment en se basant sur des estimations de mortalité d'individus par collision. Pourtant, les effets peuvent être plus subtils et dépendent étroitement du contexte paysager et de l'écologie des espèces.

L'effet direct le plus évident et visible des ILT est la mortalité par collision (Trombulak and Frissell, 2000) (Fig. D). La plupart des organismes terrestres est affectée quel que soit le taxon considéré (Forman and Alexander, 1998 ; Trombulak and Frissell, 2000 ; Balkenhol and Waits, 2009 ; Fahrig and Rytwinski, 2009 ; Borda-de Agua et al., 2017).

FIGURE D – Quelques exemples de mortalités par collision sur des infrastructures de transport observées en Dordogne durant le terrain lié à la thèse. De haut en bas et de gauche à droite : écureuil roux, vipère aspic, fouine, couleuvre verte et jaune, chevreuil, chouette hulotte.

Des effets indirects peuvent également empêcher les organismes de franchir les ILT, notamment via l'utilisation de structures d'exclusions comme des grillages le long de certaines infrastructures. Par ailleurs, l'évitement comportemental est courant (Ascensao et al., 2016). Il peut être le résultat d'une perception du danger, de nuisances sonores et visuelles, d'une modification de l'habitat naturel, de la perturbation du succès reproducteur ou de l'altération de processus physiologiques (Trombulak and Frissell, 2000). Les amphibiens par exemple peuvent être fortement affectés par la mortalité routière (Beebee, 2013) (effet direct) tout en évitant les zones à proximité des ILT à cause du bruit du trafic qui interfère avec le chant des mâles (Bee and Swanson, 2007) (effet indirect).

Ces effets directs et indirects induisent une réduction des événements de franchissement des ILT et limitent donc la dispersion à l'échelle d'un paysage. Cependant, toutes les infrastructures ne sont pas nécessairement des barrières à la dispersion. Certaines n'affectent pas la mobilité des organismes ou peuvent même favoriser la connectivité.

Les **routes** (et **autoroutes**) sont de loin les infrastructures les plus étudiées. Dans une revue de la littérature publiée par Fahrig and Rytwinski (2009), la grande majorité des études montre que les routes sont des éléments limitant les flux de gènes. Il existe toutefois quelques exceptions. A titre d'exemple, Prunier et al. (2014) identifient une autoroute comme un potentiel corridor longitudinal pour la dispersion du Triton alpestre (*Ichthyosaura alpestris*). De la même manière, la connectivité de l'escargot Petit-gris (*Cornu aspersum*) semble favorisée par les routes à l'échelle du paysage (Balbi et al., 2018). Bien que ce ne soit pas l'asphalte en elle-même qui entraine ces effets positifs sur la dispersion, les emprises longeant ces infrastructures comme les bandes enherbées sont souvent impliquées.

Les **voies ferrées** sont responsables d'effets très variés selon les espèces considérées. En effet, elles peuvent constituer des barrières fortes (Whittington et al., 2004 ; Bartoszek and Greenwald, 2009 ; Breyne et al., 2014 ; Yu et al., 2017) ou bien créer des corridors de dispersion utilisables par la faune ; c'est le cas de serpents (Graitson, 2006) ou de loups (Paquet and Callagan, 1996). Ce type d'ILT peut également améliorer la richesse spécifique et les abondances des populations proches des rails (Li et al., 2010). Enfin, certaines espèces ne semblent pas impactées par des lignes à grande vitesse (e.g. papillon *Pyronia tithonus*, Vandevelde et al., 2012).

Par leur effet d'ouverture du milieu, les **lignes électriques** sont souvent décrites comme des infrastructures permettant la création de corridors, notamment pour certaines plantes (Lampinen et al., 2015), des abeilles (Russell et al., 2005) ou des loups (Paquet and Callagan, 1996). Elles peuvent également favoriser certains rapaces en fournissant de bons perchoirs pour les activités de chasse (Morelli et al., 2014). Les oiseaux peuvent cependant être négativement affectés en cas d'électrocution avec les lignes (Loss et al., 2014, 2015). La plupart des études cherchant à identifier les effets barrières des lignes électriques trouve une absence d'effet (Latch et al., 2011 ; Bartzke et al., 2015 ; Jahner et al., 2016) à l'exception de Pruett et al. (2009) qui mettent en évidence un impact négatif sur deux espèces de Tétras. Ces oiseaux franchissent moins souvent les lignes électriques comparativement à un mouvement aléatoire.

Les **barrages hydroélectriques** représentent des barrières importantes pour la faune aquatique (e.g. Meldgaard et al., 2003 ; Hansen et al., 2014) mais également pour certains grands carnivores terrestres (Kaya Özdemirel et al., 2016). Enfin, les **canaux** ont été identifiés comme pouvant limiter la connectivité des cerfs (Coulon et al., 2006 ; Breyne et al., 2014).

Les infrastructures ne sont pas localisées aléatoirement. Elles sont souvent construites aux endroits les plus logiques en termes de contraintes économiques et typologiques (fonds de vallées, bords de mer). De ce fait, lors de la construction d'une nouvelle ILT, elle est souvent placée à proximité immédiate d'une infrastructure déjà existante. Si ces ILT ont des effets barrières, ceux-ci peuvent se cumuler et rendre impossible tout franchissement (Bélisle and St. Clair, 2001 ; Connelly, 2011). Alternativement, les infrastructures en situation de cumul peuvent avoir des effets antagonistes ; certaines favorisant la dispersion tandis que d'autres limitent la connectivité (Bartzke et al., 2015). Dans un paysage fragmenté par de multiples ILT, il est donc nécessaire d'estimer les effets de chaque infrastructure indépendamment (Balkenhol, Gugerli, Cushman, Waits, Coulon, Arntzen, Holderegger, Wagner, Arens, Campagne, Dale, Nicieza, Smulders, Tedesco, Wang and Wasserman, 2009). La question clef réside dans la manière d'estimer la capacité de franchissement de ces infrastructures pour différentes espèces.

⇒ *Cette thèse vise donc à étudier les effets de plusieurs types d'ILT dans une situation de cumul via une approche multi-spécifique.*

Estimation de la connectivité fonctionnelle

La connectivité fonctionnelle vise à estimer l'importance des flux dans un paysage (Kadoya, 2009). Cette estimation est centrée sur l'espèce et cherche à comprendre les effets des éléments paysagers sur la réponse du mouvement (Taylor et al., 2006). Comme vu précédemment, deux approches peuvent être utilisées : l'approche des suivis directs sur le terrain et l'approche utilisant des techniques de modélisation de la résistance du paysage (LaPoint et al., 2015). La première approche permet de mettre en évidence la **connectivité réelle** qui fournit une estimation directe du lien qui existe entre le mouvement des individus et les éléments paysagers (Fagan and Calabrese, 2006). La seconde, quant à elle, estime la **connectivité potentielle** en intégrant des informations indirectes du paysages sur les capacités de mouvement des espèces étudiées. La première approche garantie donc un meilleur niveau de précision de la connectivité fonctionnelle mais nécessite une plus grande quantité de données. Nous nous focaliserons ici sur la première approche permettant d'estimer la connectivité fonctionnelle réelle, via l'emploi de la génétique du paysage et des suivis directs par Capture-Marquage-Recapture.

Génétique du paysage

La génétique du paysage est un outil récent, en pleine expansion, permettant de répondre à des problématiques relatives à la connectivité fonctionnelle (Manel and Holderegger, 2013). Ce domaine de recherche combine la génétique des populations, l'écologie du paysage ainsi que des approches statistiques spatialisées afin d'explorer de quelle manière les éléments paysagers influencent la variabilité génétique (Manel et al., 2003 ; Holderegger and Wagner, 2008 ; Manel and Holderegger, 2013). Cette variabilité génétique est estimée à l'aide de marqueurs génétiques pouvant cibler plusieurs types de gènes (ou locus). Tout d'abord, elle peut être mesurée en ciblant des gènes n'ayant aucun effet sur la fitness, correspondant à une variabilité génétique neutre. Ceux-ci ne sont pas affectés par la sélection naturelle et sont donc sélectivement neutre (Holderegger et al., 2006). Les marqueurs neutres, couramment utilisés en génétique du paysage, appartiennent à deux catégories. Premièrement, les marqueurs à évolution lente (ADN mitochondrial, allozymes), qui sont de bons indicateurs de processus à larges échelles spatiale et temporelle (phylogéographie) comme les phénomènes de spéciation. Deuxièmement, les marqueurs ciblant des locus à fréquence de mutations rapide (microsatellites, SNPs). Ils sont utilisés à l'échelle du paysage pour informer sur des processus locaux, comme les effets barrières des infrastructures. D'autre part, il existe aussi des marqueurs non neutres qui sont qualifiés d'adaptatifs ou de sélectifs. Ils sont liés à la fitness et sont donc sujets aux pressions de sélection environnementale (Holderegger et al., 2006).

En réalité, il existe une bonne corrélation entre les variabilités génétiques neutre et adaptative (Reed and Frankham, 2001). De ce fait, la plupart des études explorant la variabilité génétique à l'aide de marqueurs neutres montre également une forte corrélation avec la fitness individuelle (Reed and Frankham, 2003).

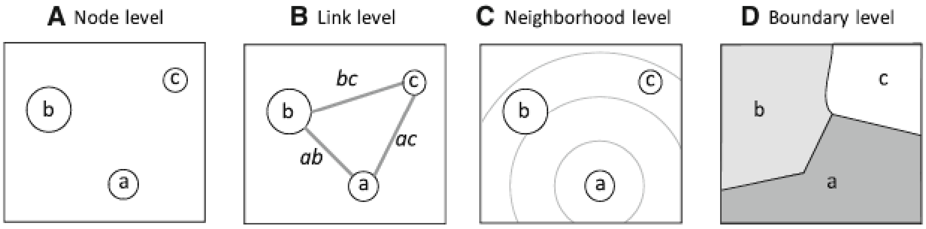

Figure E – Les quatre types d'approches en génétique du paysage pour appréhender les effets des éléments paysagers sur la variabilité génétique. Extrait de Wagner and Fortin (2013).

En génétique du paysage, on peut distinguer quatre approches principales pour mesurer les effets du paysage sur la variabilité génétique (Wagner and Fortin, 2013)(Fig. E). La première ("node level"), cherche à comparer la variabilité génétique de chaque noeud (population) avec les conditions environnementales présentes à chaque noeud. La seconde ("link level") est celle qui sera la plus employée durant cette thèse. Elle vise à comparer des distances génétiques entres paires (de populations ou d'individus) avec des distances environnementales liées au paysage séparant chaque paire. La troisième ("neighborhood level") étudie la relation existant entre la variabilité génétique et le contexte paysager environnant. Enfin la quatrième ("boundary level"), cherche à détecter des délimitations spatiales entre des populations discrètes via l'utilisation d'algorithmes de regroupement bayésiens. La superposition de ces regroupements spatiaux avec les éléments paysagers permet de déterminer les potentielles barrières aux flux de gènes.

L'hypothèse commune aux analyses génétiques cherchant à estimer les flux de gènes dans les paysages est l'isolement par la distance (IBD) (Wright, 1943). Cette hypothèse soutient que lorsque les flux de gènes et la dérive génétique sont à l'équilibre, il existe une relation monotone positive entre distance euclidienne géographique et distance génétique (Hutchison and Templeton, 1999). Cette relation d'IBD s'observe généralement jusqu'à une certaine limite spatiale. En effet, les flux de gènes sont plutôt dominants à faible échelle tandis que la dérive génétique est plus importante à large échelle spatiale. Dans les paysages complexes, l'isolement par résistance (IBR) (McRae, 2006) est susceptible d'expliquer la variabilité génétique de manière complémentaire à l'IBD. L'hypothèse sous-jacente est que chaque élément du paysage (forêts, prairies, villes, *etc.*) possède une valeur de résistance propre qui va favoriser ou limiter les flux de gènes. Par exemple, l'élément "forêt" facilitera les flux de gènes d'une espèce forestière comme l'écureuil roux (faible valeur de résistance) alors que l'élément "ville" les limitera (forte valeur de résistance). Enfin, l'isolement par barrière (IBB) s'intéresse aux éléments linéaires (routes, rivières...)

susceptibles de réduire les flux de gènes. En ce sens, les approches basées sur les flux de gènes sont pertinentes pour estimer les effets barrières des infrastructures linéaires de transport (Holderegger and Di Giulio, 2010). Ce type d'approche, et de manière plus générale le domaine de la génétique du paysage, est donc d'une importance capitale pour la gestion des espèces et leur conservation car il permet d'estimer le lien entre connectivité (flux de gènes) et éléments du paysage (Segelbacher et al., 2010). Il offre de nombreux avantages, comme la possibilité de suivre des espèces de petite taille et/ou difficilement détectables. Il permet également de réaliser des suivis non invasifs (analyses génétiques sur les fèces, poils, mues, *etc.*).

En tant que domaine de recherche récent, la génétique du paysage propose des méthodologies florissantes en constante évolution grâce aux avancées moléculaires et statistiques (Richardson et al., 2016). Parmi ces développements notoires, on peut citer l'outil prometteur de l'échantillonnage individu-centré appliqué aux populations à répartition non-continue. En effet, beaucoup d'espèces terrestres ont une distribution (au moins partiellement) discontinue (Prunier et al., 2013). C'est le cas, par exemple, de nombreux amphibiens, qui se regroupent en "agrégats" au sein de sites aquatiques en période de reproduction. Cependant, le reste de l'année, ils possèdent une distribution terrestre bien moins délimitée ("patchy distribution"). Dans de tels cas de figures, un échantillonnage classique par population ne permettra pas une couverture optimale du paysage, en raison à la fois du nombre restreint d'agrégats échantillonnés et de la présence d'agrégats non-échantillonnés (Broquet and Petit, 2009 ; Lowe and Allendorf, 2010). En effet, l'échantillonnage population-centré requiert généralement 30 échantillons par agrégat. Si les moyens alloués à l'étude ne permettent d'échantillonner que 300 individus, seuls 10 agrégats pourront être échantillonnés dans le paysage étudié. L'approche individu-centrée permet au contraire de contourner ces limites en augmentant le nombre d'agrégats échantillonnés dans l'espace puisque moins d'individus sont nécessaires par site d'échantillonnage (3 à 4 individus sont généralement conseillés). Cela permet à la fois de capturer une plus forte part de la variabilité génétique et d'optimiser la représentation de l'hétérogénéité du paysage (Prunier et al., 2013). Le design individu-centré a été appliqué dans plusieurs cas d'études avec succès (e.g. Prunier et al., 2014 ; Villemey et al., 2016). Cependant, bien que Prunier et al. (2013) défendent l'idée que le design individu-centré est plus efficace que le design population-centré, une comparaison entre ces deux approches sur le même modèle d'étude reste encore à réaliser (Luximon et al., 2014).

Les méthodes statistiques ont, elles aussi, évolué. Une des principales limites des analyses génétiques basées sur les distances génétiques (link-level) est la non-indépendance des données (Legendre and Fortin, 2010), qui aura pour effet d'augmenter le risque d'erreurs de type I (rejet de l'hypothèse nulle alors que celle-ci est vraie). En effet, dans ce type d'analyses, les distances génétiques sont calculées entre chaque paire d'individus ou de populations échantillonnés. Si l'on considère un exemple de trois populations : a, b et c (Fig. E), il en découle trois paires : ab, ac et bc. Les paires ab et ac partagent la population a et ne sont donc pas indépendantes statistiquement. Il en va de même pour les autres paires. Comment surmonter ce problème ?

Il existe un grand nombre de méthodes statistiques permettant d'estimer les liens existant entre distances génétiques et distances paysagères (Balkenhol, Waits and Dezzani, 2009 ; Guillot et al., 2009 ; Richardson et al., 2016). On peut les classer en trois catégories principales. La première est basée sur les tests de Mantel (Mantel, 1967) ou les tests partiels de Mantel (Smouse et al., 1986 ; Cushman et al., 2013). La deuxième catégorie se base sur des modèles mixtes (Linear mixed effects model, LME) (Clarke et al., 2002). Enfin la troisième utilise des régressions multiples sur des matrices de distances (RDM) (Legendre et al., 1994). La première a été vivement critiquée car l'utilisation des tests de Mantel (partiels ou non) ne satisfait pas les hypothèses de non-indépendance évoquées plus haut. Par conséquent, les estimations de significativité sont biaisées (Guillot and Rousset, 2013 ; Legendre et al., 2015). De plus, ces modèles sont généralement moins performants pour expliquer la variabilité génétique que les autres types de modèles (Shirk et al., 2017). Les modèles mixtes (deuxième catégorie) cherchent justement à tenir compte de la non-indépendance des données ; ils ont été appliqués depuis peu avec succès en génétique du paysage (e.g. Villemey et al., 2016 ; Balbi et al., 2018). Le principe de ces modèles mixtes est d'estimer la part de variance expliquée par des effets fixes (variables d'intérêt), tout en contrôlant la variation associée à d'autres niveaux d'organisation avec des effets aléatoires (Richardson et al., 2016). La troisième catégorie consiste à réaliser une régression multiple d'une matrice de distance génétique dépendante avec plusieurs matrices indépendantes (Balkenhol, Waits and Dezzani, 2009). La matrice dépendante correspond à des paires de différentiation génétique (Fst par exemple) et les matrices indépendantes correspondent à des données de différentiation spatiale entre paires (distance euclidienne, chemin de moindre coût, *etc.*). Dans un récent article, Shirk et al. (2017) démontrent que les modèles mixtes sont les plus performants en terme d'ajustement de modèles (R^2). Ils nuancent cependant leurs propos et précisent que les modèles de RDM basés sur les R^2

(troisième catégorie) sont quasiment aussi performants dans la plupart des cas.

Il semblerait donc que l'utilisation des modèles mixtes soit l'outil le plus adapté en génétique du paysage. Pourtant, cette méthode ne permet pas une estimation de la multicollinéarité (non-indépendance entre les prédicteurs), autre point très sensible en statistiques. En effet, si ces prédicteurs sont fortement corrélés entre eux, les résultats issus des régressions seront biaisés (Prunier et al., 2015). Une bonne compréhension de la multicollinéarité est nécessaire et peut être atteinte grâce aux analyses de commonalités (qui seront détaillées dans le chapitre 1). Ces dernières permettent une compréhension fine de la fiabilité des prédicteurs utilisés dans les modèles pour expliquer la variable dépendante au regard de la collinéarité (Prunier et al., 2015).

Au stade actuel, seules les régressions multiples sur des matrices de distances (RDM) permettent une estimation des commonalités (Prunier et al., 2015) et représentent donc la méthode la plus robuste pour les analyses en génétique du paysage.

⇒ *Dans cette thèse, nous emploierons principalement des analyses de commonalités sur des régressions multiples de distances (RDM) appliquées à des données collectées par des approches population et individu-centrées.*

Capture-Marquage-Recapture

La génétique du paysage engendre un coût financier non négligeable que certaines structures locales ne sont pas prêtes à dépenser ainsi qu'un coût éthique lié au prélèvement d'ADN sur les individus. De plus, le développement continuel des méthodologies d'analyses nécessite des compétences spécifiques et peut représenter un frein dans la mise en place de suivis génétiques opérationnels. Le décalage temporel entre la mise en place d'une barrière à la dispersion (construction d'une route par exemple) et la détection de son impact sur les populations en terme de structuration génétique constitue également une limite conséquente (Epps and Keyghobadi, 2015). En effet, un certain temps est nécessaire pour que la variation génétique atteigne un équilibre après une perturbation dans le paysage (Landguth et al., 2010). De ce fait, des changements récents de la connectivité ne seront pas immédiatement reflétés dans les paramètres génétiques. Par exemple, une nouvelle infrastructure peut constituer une barrière importante dans un paysage, mais le signal génétique, et donc les effets de l'infrastructure, ne seront pas détectables dans l'immédiat. Les causes de ce décalage sont nombreuses et complexes, rendant l'anticipation de la durée du décalage difficile (Epps and Keyghobadi, 2015). Par exemple, Prunier et al.

(2014) ont mis en évidence qu'une voie ferrée de 29 ans était trop récente pour détecter un effet dans la signature génétique du Triton alpestre. Plusieurs autres études démontrent néanmoins que ce décalage génétique peut être court (1 à 3 générations) (Landguth et al., 2010). C'est le cas de la voie ferrée Qinghai-Tibet qui représente une barrière importante pour les flux de gènes de la Gazelle de Przewalski (*Procapra przewalskii*) (Yu et al., 2017). Cette infrastructure a été grillagée en 2006, soit environ 10 ans (5 générations) avant la réalisation de l'étude (Yu et al., 2017).

Au vu des limites de la génétique du paysage, il est important de combiner les suivis génétiques avec des suivis directs des organismes dans leur milieu afin d'évaluer leur devenir face aux changements environnementaux (Balkenhol and Waits, 2009 ; Safner et al., 2011). Les suivis par Capture-Marquage-Recapture (CMR) offrent de nombreux avantages et sont un complément idéal à la génétique du paysage afin d'appréhender la connectivité fonctionnelle des organismes au sein des paysages (Lowe and Allendorf, 2010).

Les méthodes dites de CMR sont utilisées pour réaliser de l'inférence statistique afin d'estimer les paramètres démographiques des populations (nombre d'individus dans les populations, recrutement, survie, probabilité de capture, *etc.*). Le principe des suivis par CMR consiste à capturer des individus et de les marquer afin de les reconnaître ultérieurement. Les marques peuvent être diverses, il peut s'agir de bagues pour les oiseaux, de puces sous-cutanées, d'un marquage colorisé visible à l'oeil nu ou de caractéristiques propres à chaque individu comme des tâches ou colorations particulières. La recapture d'individus précédemment marqués apporte une grande quantité d'informations sur l'écologie de l'espèce suivie. On peut ainsi obtenir des mesures de connectivité en analysant les déplacements individuels. Par exemple, à l'aide de ces méthodes, il est possible d'étudier comment le mouvement des papillons est affecté à proximité des routes (Brakefield, 1982 ; Valtonen and Saarinen, 2005), à travers une ligne à grande vitesse (Vandevelde et al., 2012) ou bien en paysage agricole fragmenté (Billeter et al., 2003).

Les sessions de captures ou de recaptures des individus sont plus ou moins espacées dans le temps selon le type de problématique et le modèle CMR utilisé. A chaque session, les individus sont recherchés et ceux nouvellement capturés sont marqués. Ces histoires de captures permettent, en plus de fournir de l'information sur le mouvement des individus (connectivité fonctionnelle), d'estimer des paramètres démographiques des populations étudiées (Lebreton et al., 1992).

Les premiers modèles CMR développés visaient essentiellement à estimer le nombre d'individus dans les populations (taille des populations). Ces modèles considéraient la population comme fermée (pas d'émigration, immigration, naissance ou mortalité) (Otis et al., 1978). Afin que cette hypothèse soit valide, les sessions devaient être resserrées dans le temps.

Par la suite, des modèles dits "ouverts" se sont développés comme celui, très répandu, de Cormack-Jolly-Seber (Cormack, 1964 ; Jolly, 1965 ; Seber, 1965). Ces modèles ne considèrent plus les populations comme "fermées" et permettent d'estimer la probabilité de survie des individus d'une session à l'autre. Le modèle de Cormack-Jolly-Seber a été énormément appliqué mais pas toujours adapté aux jeux de données étudiés. De plus, il aurait tendance à sous estimer les densités, le rendant non adapté pour les études à long terme (Amori et al., 2017).

Une alternative prometteuse à ce modèle comprend les modèles robustes (Robust design). Ceux-ci ont été développés plus récemment (Kendall, 2001) et visent à coupler les modèles "fermés" et "ouverts". Il s'agit de réaliser des sessions dites primaires, espacées dans le temps, où les populations sont considérées comme ouvertes. Chacune de ces sessions primaires est composée de plusieurs sessions secondaires rapprochées dans le temps ; la population est alors considérée comme fermée. Ce procédé a l'avantage de permettre l'estimation de plusieurs paramètres démographiques, notamment la survie et le recrutement grâce aux sessions primaires et la taille de la population avec les sessions secondaires. Ainsi, la combinaison des modèles "ouverts" et "fermés" offre un avantage considérable. Ces méthodes robustes apportent le meilleur ajustement aux modèles dans de nombreux cas et sont les plus adaptées aux suivis à long terme (Amori et al., 2017).

L'application des modèles CMR aux suivis de terrain doit répondre à un certain nombre d'hypothèses sans quoi les paramètres mesurés seront biaisés. Premièrement, le marquage des individus doit être visible par l'expérimentateur et durable (pas de perte des marques). Deuxièmement, les individus marqués doivent se mélanger aléatoirement dans la population. Enfin, tous les individus doivent avoir la même probabilité d'être capturés. Cette dernière hypothèse est cependant violée dans de nombreux cas ; il est alors possible d'inclure des covariables (sexe, classes d'âge) afin de contrôler cette hétérogénéité (Pollock, 1982). Par ailleurs, la probabilité de recapture d'un individu peut être affectée si celui-ci a été précédemment marqué. En effet, il peut adopter un comportement "trap shy" engendrant une diminution de sa probabilité de recapture. Il en résultera une sur-estimation de la taille de la population. A l'inverse, un comportement "trap happy" (probabilité de recapture plus forte que la probabilité de capture) aura pour effet de sous-estimer la taille de population. Pour estimer cet effet, il est possible d'inclure dans les modèles une probabilité de capture (c) différente de la probabilité de recapture (p).

⇒ *Dans cette thèse, nous emploierons, en plus des suivis génétiques, des méthodes de CMR (dont des robust designs) afin d'appréhender la connectivité fonctionnelle réelle à travers plusieurs types d'ILT.*

Objectifs et organisation du manuscrit

Cadre de l'étude

La connectivité résulte de plusieurs processus complexes mais indispensables à appréhender afin de réaliser des réseaux de continuités écologiques. Le cadre législatif français oblige les aménageurs à appliquer des mesures pour éviter, réduire et compenser les impacts des projets de construction ou de requalification sur la biodiversité. L'évaluation de l'impact réel reste complexe, souvent estimé à dire d'expert. Les mesures mises en place pour limiter l'impact de ces constructions visent principalement à rétablir la connectivité structurelle. Or, c'est la connectivité fonctionnelle qui permet le maintien des populations en assurant un brassage génétique à large échelle. Il est donc d'une importance capitale d'estimer comment cette connectivité fonctionnelle est perturbée dans les paysages fragmentés par les infrastructures de transport.

Comme vu précédemment, les suivis génétiques sont souvent employés pour répondre à ce type de problématique. Pourtant, ces suivis empiriques sont généralement monospécifiques (Balkenhol and Waits, 2009 ; Segelbacher et al., 2010). Cette approche monospécifique est critiquée, car elle apporte peu d'informations transposables en terme de mesures de conservation (Keller et al., 2015 ; Richardson et al., 2016). Une approche multi-spécifique est primordiale pour inférer les effets des ILT sur la biodiversité.

Dans certains cas de figures, les suivis CMR peuvent s'avérer plus pertinents pour mesurer la connectivité fonctionnelle à travers les ILT. Cependant, des méthodes permettant de mettre en évidence les effets barrières des ILT basées sur les kernels de dispersion des organismes suivis par CMR sont manquantes (Pépino et al., 2012, 2016). Par ailleurs, ces deux approches s'intéressent à des dynamiques temporelles différentes. Tandis que les suivis CMR permettent d'identifier des processus de dispersion instantanés, les suivis génétiques informent d'événements de dispersion antérieurs qui ont générés du flux de gènes (Landguth et al., 2010 ; Epps and Keyghobadi, 2015).

Enfin, les suivis génétiques et CMR pris indépendamment ne s'intéressent qu'à certains aspects de la connectivité : connectivité génétique d'une part et connectivité démographique d'autre part (Lowe and Allendorf, 2010). La confrontation entre ces deux approches est rarement explorée mais susceptible d'apporter une meilleure compréhension des statuts de conservation des populations confrontées aux changements environnementaux (Safner et al., 2011).

Dans ce contexte, ma thèse s'articule autour de l'écologie du mouvement et de la connectivité fonctionnelle. L'objectif consiste à mieux comprendre comment les éléments paysagers en général et les infrastructures de transport plus particulièrement influencent la connectivité fonctionnelle dans un milieu fragmenté. J'ai donc utilisé des outils de génétique du paysage et de suivis directs par CMR pour appréhender les processus influençant le mouvement. J'ai utilisé une approche multi-spécifique en m'intéressant à quatre espèces présentant des écologies très variées (deux insectes et deux vertébrés). Ces espèces ont été suivies dans un paysage fragmenté par un cumul de six types d'infrastructures.

Les données employées ont été entièrement récoltées dans le cadre de ma thèse. Au total, douze mois ont été nécessaires pour la collecte de données de terrain et environ dix mois pour les extractions, amplifications et génotypages en laboratoire.

Organisation du manuscrit

Le **premier chapitre** synthétise le suivi génétique des quatre espèces en Dordogne à travers le cumul d'infrastructures de transport. L'originalité de ces travaux est d'utiliser une approche multi-spécifique. Dans ce chapitre, j'utilise des développements récents en génétique du paysage (analyses individus-centrés, distances génétiques hiérarchiques, échelles spatiales adaptées, analyse des commonalités) afin de répondre à trois questions principales :

- La connectivité des espèces étudiées est-elle plutôt influencée par des éléments "naturels" du paysage ou par les éléments anthropisés incluant les ILT ?
- Les espèces sont-elles toutes impactées de la même manière par les ILT ?
- Quels types d'infrastructures sont les plus limitantes pour la connectivité de ces espèces ?

Le **deuxième chapitre** est plus méthodologique et vise à estimer les effets barrières des infrastructures en utilisant des données issues de suivis CMR. En effet, dans certains cas, l'utilisation de suivis génétiques n'est pas envisageable. Les méthodes par CMR constituent alors une alternative intéressante. Nous avons donc développé une méthode permettant d'estimer la probabilité attendue de franchissement d'une infrastructure linéaire. Cette probabilité attendue peut être comparée aux données empiriques provenant de suivis CMR. Nous avons utilisé des simulations pour estimer la robustesse de notre méthode et l'avons appliquée sur un cas d'étude : le Myrtil à travers deux types d'infrastructures. Les questions posées dans ce chapitres sont :

- Cette méthode est-elle robuste ? Quelles sont ses limites ?
- Les infrastructures étudiées sont-elles des barrières pour les mouvements du Myrtil ?

Le **troisième chapitre** vise à coupler l'approche génétique avec des suivis CMR sur plusieurs populations d'Alyte accoucheur. Neuf populations ont été échantillonnées génétiquement pour obtenir des informations sur leur diversité génétique ainsi que sur leurs conditions corporelles et leur fécondité. En parallèle, trois populations ont été suivies par CMR, permettant d'estimer le taux de croissance des populations et leur survie. Le lien entre paramètres démographiques (fitness) et diversité génétique est exploré. L'environnement étant susceptible d'influer sur ces deux composantes (fitness et diversité génétique), j'ai étudié la relation qui les lie à la fragmentation due aux routes ; les questions soulevées étant :

- Existe-t-il un lien entre diversité génétique et fitness chez les populations d'Alyte accoucheur ?
- Quel est le rôle des routes avoisinant les populations sur ces deux composantes (fitness et diversité génétique) ?

Aspects Méthodologiques

Zone d'étude

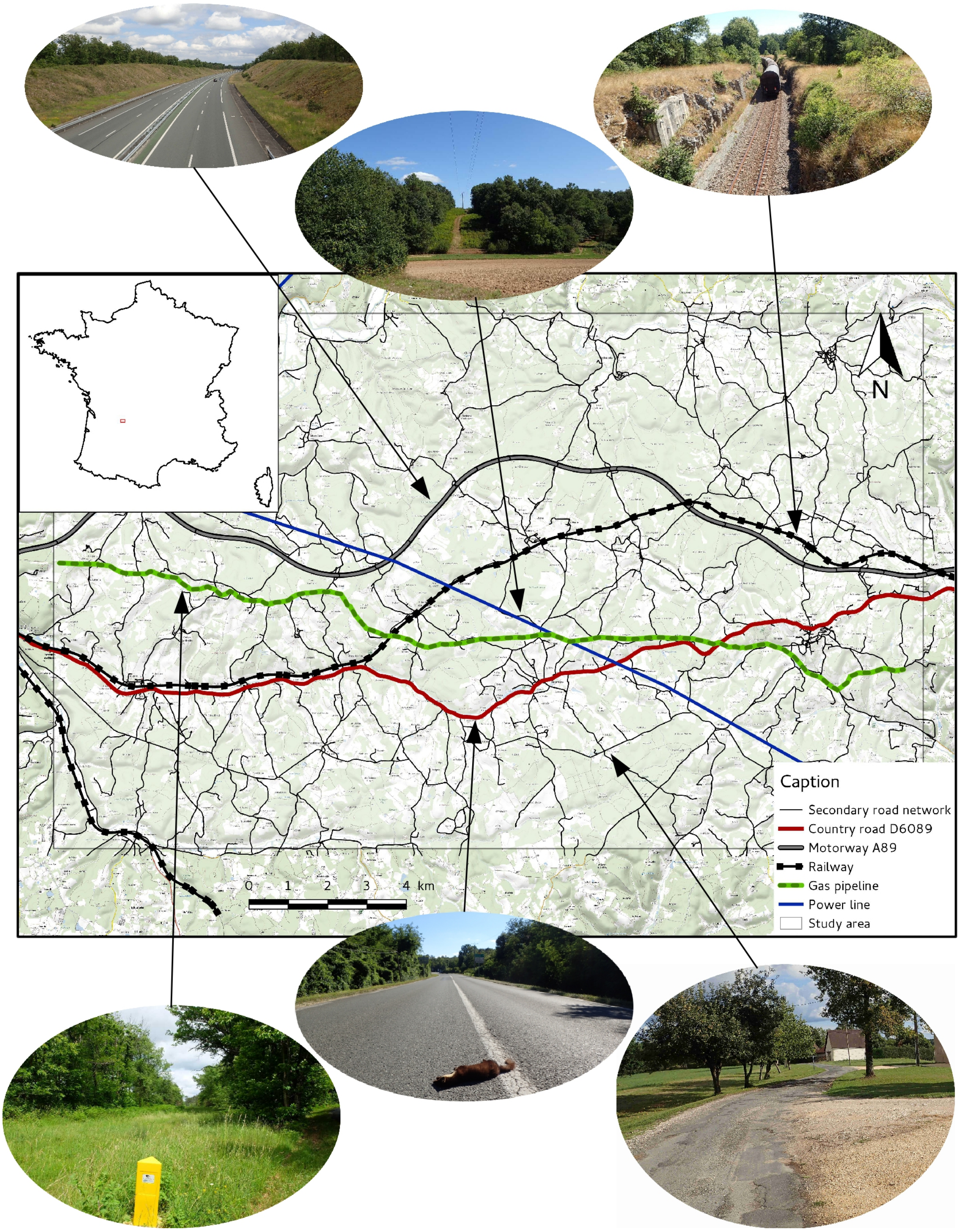

Figure F – Zone d'étude en Dordogne avec les six types d'ILT étudiés.

Nous avons choisi de cibler notre étude sur une zone géographique présentant un cumul d'ILT, à savoir une région de causses calcaires au sud-est de Périgueux entre Saint-Laurent-sur-Manoire et Thénon (Fig. F). Ce secteur a l'avantage d'être traversé par de nombreuses infrastructures linéaires, à savoir :

- La ligne TER entre Périgueux et Brive-la-Gaillarde (voie ferrée non clôturée), construite au XIX^e siècle
- La route D6089 entre Périgueux et Brive-la-Gaillarde, présente depuis le $XVIII^e$ siècle
- La canalisation GRT Gaz entre Périgueux et Brive-la-Gaillarde, mise en place en 1955
- Une ligne à moyenne tension du réseau RTE, construite en 1962
- L'autoroute A89 (clôturée), en service depuis 2004
- Un réseau de 1370 km de routes secondaires

Cette zone d'étude d'environ 300 km^2 (45°07'31.8"N ; 0°58'56.9"E) comprend des terrains cultivés (blé, maïs, tournesol, fraises, noix..), des prairies de fauche, des pâturages, des forêts de feuillus et une quinzaine de communes de moins de 2000 habitants chacune. L'hydrologie se limite à quelques rivières (la Manoire au Sud, Le Blâme au Nord) ainsi que des mares artificielles ou naturelles. L'altitude varie entre 91 et 294 m.

Espèces étudiées

Figure G – Les quatre espèces étudiées durant cette thèse : Alyte accoucheur (mâle avec ses oeufs), Couleuvre helvétique, papillon Myrtil (femelle) et Féronie noire.

Afin de caractériser l'impact des infrastructures sur la biodiversité, les modèles retenus sont des espèces possédant de grandes aires de répartition (espèces "parapluies"). Elles ont l'avantage de posséder des abondances suffisantes pour réaliser un suivi fiable. Par ailleurs, nous avons choisi des espèces pour lesquelles des marqueurs génétiques neutres ont été précédemment développés. Nous avons retenu des marqueurs microsatellites car ce sont ceux qui sont le plus couramment utilisés dans les analyses de génétique du paysage (Storfer et al., 2010). Nous souhaitions également étudier des espèces présentant des traits d'histoire de vie variés. Par conséquent, deux insectes et deux invertébrés ont été sélectionnés (Fig. G) :

— L'Alyte accoucheur, *Alytes obstetricans*
— La Couleuvre helvétique, *Natrix helvetica*
— Le Myrtil, *Maniola jurtina*
— La Féronie noire, *Abax parallelepipedus*

L'**Alyte accoucheur** est un petit anoure de la famille des Alytidae. Sa répartition s'étend sur l'ouest européen (péninsule ibérique, France, Suisse, Allemagne, Luxembourg, Belgique et Pays-Bas) (Arnold and Ovenden, 2010). Cette espèce possède un mode de reproduction unique en Europe. L'accouplement s'effectue sur terre ; les mâles récupèrent le chapelet d'oeufs et le transportent sur leur dos. Les oeufs se développent ainsi pendant plusieurs semaines avant que les mâles ne les libèrent dans un point d'eau où les têtards sortiront rapidement. Il s'agit d'un rare cas de soin parental chez un amphibien. L'habitat de l'Alyte accoucheur est souvent lié aux milieux anthropisés : zones ouvertes, proches de points d'eau, terres cultivées, pentes rocheuses, carrières, éboulis, *etc.* (Arnold and Ovenden, 2010). En terme de conservation, cette espèce souffre d'une sensibilité importante au champignon Chytride *Batrachochytrium dendrobatidis* (Bosch et al., 2001). La fragmentation des habitats est aussi une menace car les populations d'Alyte fonctionnent comme des entités relativement indépendantes avec une forte structuration génétique entre populations (Tobler et al., 2013 ; Maia-Carvalho et al., 2014 ; Albert et al., 2015). Cette espèce s'est également révélée sensible à la fragmentation due aux routes. Dans une étude espagnole, le réseau routier secondaire agit comme une forte barrière aux flux de gènes chez cette espèce (Garcia-Gonzalez et al., 2012). Enfin, les capacités de dispersion de cet amphibien sont peu connues. Une distance maximale de dispersion de 500 m a été reportée (Trochet et al., 2014).

Le reptile étudié est la **Couleuvre helvétique**. Cette espèce était appelée Couleuvre à collier jusqu'à récemment. Les récents travaux génétiques effectués sur le genre *Natrix* (Kindler et al., 2013 ; Pokrant et al., 2016 ; Kindler et al., 2017, 2018) démontrent que la précédente sous espèce *Natrix natrix helvetica* doit être maintenant élevée au rang d'espèce. Elle se rencontre en France, en Grande-Bretagne, en Belgique, aux Pays-Bas, en Suisse et au Nord de l'Italie (Arnold and Ovenden, 2010). Cette couleuvre affectionne les milieux humides où elle trouve sa principale source de nourriture, à savoir tous types d'amphibiens (Gregory and Isaac, 2004). Elle évolue donc à proximité des milieux aquatiques où elle thermorégule et attrape ses proies principalement dans l'eau (Arnold and Ovenden, 2010). Le domaine vital d'un adulte s'étend sur environ 40 ha (Wisler et al., 2008). Cette espèce possède de bonnes capacités de dispersion avec des distances mesurées dépassant le kilomètre en moins d'un mois (Pettersson, 2014). Une étude précédente en génétique du paysage a montré que dans un territoire d'agriculture intensive d'environ 100 km^2, ce serpent ne possédait aucune structuration génétique, indiquant probablement une bonne capacité de dispersion dans les paysages fragmentés (Meister et al., 2010).

Le **Myrtil** est un papillon univoltin très commun en Europe qui possède localement de fortes abondances. Cette espèce est inféodée aux milieux herbacés tels que les pelouses, les prairies, les pâturages, les lisières de forêts. Sa durée de vie médiane est d'environ 6,5 jours et la période de vol s'étale sur environ 67 jours entre juin et septembre (Bubová et al., 2016). Les chenilles se nourrissent d'un large spectre de plantes avec des préférences pour *Poa spp.*, *Agrostis spp.* et *Lolium spp.* (Brakefield, 1982 ; Thomas and Lewington, 1991). *Maniola jurtina* possède une capacité de dispersion intermédiaire avec des distances moyennes de dispersion variant entre 50 et 300 m (Schneider et al., 2003 ; Ouin et al., 2008 ; Stevens et al., 2013). Cependant, certaines distances de plus de 2,5 km ont déjà été reportées (Öckinger and Smith, 2007).

Le carabe forestier ***Abax parallelepipedus*** est une espèce carnivore opportuniste (Loreau, 1983). Ce coléoptère d'environ 2 cm de long est inféodé aux milieux boisés et vit dans la couche supérieure de la litière forestière (Loreau, 1987). Les haies en milieux agricoles sont également d'importants refuges (Fournier and Loreau, 1999) et corridors (Petit and Burel, 1998). Cette espèce présente un taux de croissance lent et une forte longévité ($>$ 2 ans) pour un carabe (Chaabane et al., 1997). Une précédente étude dans une forêt de hêtre en Belgique a estimé la densité d'*A. parallelepipedus* à environ 2000 individus par hectare (Loreau and Nolf, 1993). Une étude similaire dans une forêt mixte fragmentée en Suisse a identifié des densités variant entre 632 et 1707 individus par hectare. La distance moyenne parcourue quotidiennement par ce carabe est comprise entre 0,6 et 2,3 m (Brouwers and Newton, 2009) avec un domaine vital d'environ 660 m^2 (Loreau and Nolf, 1993). De ce fait, les capacités de dispersion d'*A. parallelepipedus* sont faibles avec un comportement d'évitement des milieux ouverts (Charrier et al., 1997) et une forte sensibilité à la fragmentation due aux routes (Keller et al., 2004).

Chapitre 1

Multi-specific gene flow in a fragmented environment

Avant-propos

Les Infrastructures Linéaires de Transport (ILT) peuvent représenter des barrières importantes à la dispersion des organismes, particulièrement lorsqu'elles sont en situation de cumul dans les paysages. La génétique du paysage est très utile pour mesurer la connectivité fonctionnelle à travers les ILT, via l'étude des flux de gènes entre populations ou individus. Cependant, cette approche reste majoritairement mono-spécifique ce qui limite la portée des résultats transposables en terme de mesures de conservation.

Par conséquent, nous nous sommes intéressés aux effets de six types d'ILT sur les flux de gènes de quatre espèces dans un paysage fragmenté en Dordogne. Nous avons utilisé des méthodologies récentes en génétique du paysage (échantillonnage individu-centré, distances génétiques hiérarchiques, analyses de commonalités sur des matrices de distances génétiques) afin d'identifier comment la dispersion de ces espèces est impactée par les ILT. Les résultats mettent en évidence la variabilité existante entre les espèces vis à vis des effets des ILT. Ainsi, cette étude permet une meilleure compréhension de la connectivité de la faune terrestre dans un paysage fortement fragmenté par les ILT.

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Multi-specific gene flow in a fragmented environment

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Abstract

Barrier effects of Large-scale Transportation Infrastructures (LTIs; roads, railways, *etc.*) are among the main factors contributing to habitat fragmentation. Dispersal reduction across LTIs can drive small, local populations to extinction. Barrier effects detection is now facilitated by the field of landscape genetics. However, a main limitation in genetic studies is the focus on a single species. Multi-specific approaches are required when trying to understand how biodiversity is affected by landscape features in general and by LTIs in particular.

Accordingly, we surveyed two vertebrates species (the grass snake *Natrix helvetica* and the midwife toad *Alytes obstetricans*) and two insect species (the butterfly *Maniola jurtina* and the ground-beetle *Abax parallelepipedus*) in a landscape fragmented by six types of infrastructures: a secondary road network, a country road, a motorway, a railway, a gas pipeline and a power line.

Using multiple linear regressions and commonality analyses on two types of genetic distances (classical and hierarchical genetic distances), we showed that LTIs accounted for 47 % of the explained variance in *A. obstetricans* genetic distances, 100 % in *N. helvetica*, 0 % in *M. jurtina* and 49 % in *A. parallelepipedus.* More precisely, we found that roads (country road and secondary road network) were acting as major barriers to gene flow in *A. obstetricans* and *A. parallelepipedus* but the secondary road network was enhancing gene flow in the snake *N. helvetica.* The motorway limited *N. helvetica* dispersal but promoted gene flow in *A. obstetricans.* The railway impeded gene flow in *A.*

obstetricans but enhanced *N. helvetica* dispersal. The gas pipeline reduced gene flow in *A. parallelepipedus* and the power line had no effect on gene flow in any species. We also assessed how other landscape elements (various landscape features, isolation-by-distance and altitude) affect gene flow in these four species.

Our results revealed that infrastructures were mostly acting as barriers to gene flow in terrestrial species (85 % of the averaged unique contributions across data sets) but that they could also somehow promote it, because of alternative favourable landscape features provided by right-of-ways. We also confirm that roads are acting as a major threat to biodiversity. Specific efforts are required for current and planned roads in order to offset their negative effects on gene flow. Considering the high variability of infrastructure effects depending on the species under study, we encourage future researches to target species with various life-history traits in order to cover a wide amount of variability in ecosystems. Building large data bases which link infrastructure effects and life history traits of species (such as dispersal ability) would help to implement appropriate conservation planning in fragmented landscapes.

Key-words: connectivity; fragmentation; dispersal; individual-based; hierarchical genetic distance; commonality analysis; spatial scale

1.1 Introduction

The fragmentation of natural habitats is one of the main cause driving the global biodiversity collapse (Fahrig, 2003; Haddad et al., 2015). The most ubiquitous form of habitat fragmentation is large-scale transportation infrastructures (LTIs) (Forman and Alexander, 1998; Trombulak and Frissell, 2000; Balkenhol and Waits, 2009). LTIs are linear infrastructures allowing the transportation of goods, vehicles or energy. In urbanised areas, they are expending considerably, creating dense transportation networks with deep impacts on natural ecosystems (Dulac, 2013; Laurance et al., 2014).

The most visible detrimental effect of LTIs is direct vehicular collisions with wildlife (Trombulak and Frissell, 2000). Most animals are affected by collisions, from small insects to large mammals (Forman and Alexander, 1998; Trombulak and Frissell, 2000; Balkenhol and Waits, 2009; Fahrig and Rytwinski, 2009; Borda-de Agua et al., 2017). Besides collision, LTIs also induce behavioral modifications; leading to infrastructure avoidance (Ascensao et al., 2016). They avoid LTIs because of several reasons such as traffic noise, modification of their natural habitat, perturbation of their reproductive success or perturbation of their physiological state (Trombulak and Frissell, 2000). For example, reproductive success of amphibians can be perturbed by main roads due to sound interferences covering up calling calls of males (Bee and Swanson, 2007). These disturbances lead to a limitation of crossing events through LTIs and limit effective dispersal (the movement of individuals that sustains gene flow within landscapes (Ronce, 2007)). Barrier effects due to LTIs may create geographical isolation of populations which are not any more connected by dispersal (Fahrig and Rytwinski, 2009; Beyer et al., 2016). When populations are isolated and small, they exhibit higher rates of inbreeding and genetic drift, resulting in a decrease in heterozygosity and in an increased risk of population extinction (McCauley, 1991; Fagan and Holmes, 2006).

In practice, LTIs do not always impede organism's dispersal but their effects are context dependent. Classical LTIs are roads, motorways, railways, power lines, pipelines and canals. Roads and motorways are the most studied infrastructures. They have strong barrier effects on a large range of animal species (Fahrig and Rytwinski, 2009). Railways have various effects. They can represent barriers for certain species (Whittington et al., 2004; Bartoszek and Greenwald, 2009; Breyne et al., 2014), create corridors (Penone et al., 2012) or be neutral to movement (Vandevelde et al., 2012). They also can increase species richness and abundance near infrastructures (Li et al., 2010). Power lines create openings in woodlands environments. Sometimes, wildlife avoid power lines (e.g. prairie grouse (Pruett et al., 2009)); but few studies were able to detect a consistent effect of this infrastructure type on animal movements (Latch et al., 2011; Bartzke et al., 2015; Jahner et al., 2016). Power lines can even attract wildlife by providing perches for hunting activities of birds (Morelli et al., 2014). The other types of LTIs (gas pipelines, canals, *etc.*) have been seldom studied and require more investigations (but see Dyer et al., 2002; Coulon et al., 2006; Breyne et al., 2014; Kaya Özdemirel et al., 2016).

Large-scale infrastructures building may be restricted by landscape features such as valleys and coastlines. At such places, LTIs are built parallel and close to each other because of technical and economic reasons. Effects of each LTI may add up and result in a "cumulative" barrier effect. Alternatively, the various LTIs present might have antagonist effects with some promoting dispersal and others impeding gene flow (Bartzke et al., 2015). For example, Paquet and Callagan (1996) followed wolves in a Canadian landscape fragmented by a railway, a major motorway and power lines. They found that the motorway was as strong barrier impeding wolves to cross but the railway and the power lines redirected wolves movements and were acting as corridors (Paquet and

Callagan, 1996). Similarly, Latch et al. (2011) found that desert tortoise's gene flow was affected by roads but not by power lines.

In addition, species may respond differently to the same type of infrastructure depending on the landscape configuration. For example, Van Buskirk (2012) found that a motorway was limiting gene flow in the alpine newt *Ichthyosaura alpestris* in Switzerland but Prunier et al. (2014) found that a similar motorway did not affect gene flow in the same species in France. Therefore, when trying to understand how a species moves through the landscape, it is crucial to determine the effects of the different types of infrastructures present (Balkenhol, Gugerli, Cushman, Waits, Coulon, Arntzen, Holderegger, Wagner, Arens, Campagne, Dale, Nicieza, Smulders, Tedesco, Wang and Wasserman, 2009).

In the past fifteen years, one of the most powerful tool to estimate landscape connectivity has been landscape genetics (Manel and Holderegger, 2013). This research field integrates population genetics, landscape ecology and spatial statistic tools (Manel et al., 2003; Holderegger and Wagner, 2008; Manel and Holderegger, 2013) in order to elucidate how the genetic variability (at neutral or adaptive markers) is influenced by landscape features. Genetic studies have been widely used in order to address connectivity questions (Storfer et al., 2010) and to estimate the barrier effects of LTIs (Holderegger and Di Giulio, 2010). Therefore, they have wide applications in species management and conservation (Segelbacher et al., 2010). However, one major limitation in genetic studies is the focus on a single species (Balkenhol and Waits, 2009; Segelbacher et al., 2010; Keller et al., 2015). Balkenhol and Waits (2009) reviewed 33 studies that assessed road effects using molecular approaches. Only two of them focused on more than one species. Studies focusing on only one species can hardly be generalised to other species and management and conservation planning can only be applied to that particular species. This considerably limits the reach and relevance for conservation planning (Keller et al., 2015). Multi-specific approaches that go beyond studying related species (e.g. Riley et al., 2006) are required in order to understand how biodiversity is affected by landscape features in general and by LTIs in particular (Keller et al., 2015; Richardson et al., 2016). Those evaluations are particularly requested by local authorities to design mitigation measures (EEA, 2015).

Our main goal in this study was to identify what were the main landscape features affecting gene flow in several sympatric terrestrial species. More precisely, we aimed to understand whether species were affected mostly by natural landscape features or by anthropised elements including LTIs. Accordingly, we monitored four species with contrasted life history traits (two vertebrates and two insects) in a landscape fragmented by six types of LTIs in south-western France: a secondary road network, a country road, a motorway, a railway, a gas pipeline and a power line. We used recent molecular and statistical tools to estimate how landscape features influence gene flow in these four species. We predicted that roads would impede gene flow in most of the studied species but that the motorway, built in 2004, may be too recent for detecting any effect on genetic metrics (Anderson et al., 2010). Finally we hypothesised that the railway, the power line and the gas pipeline would have no effect on gene flow due to low traffic density for the railway and low maintenance perturbations for the two others.

1.2 Material and methods

Study area

The study was carried out in the 'Périgord' region in the south-western France between Brive-La-Gaillarde and Périgueux (45°07'31.8"N; 0°58'56.9"E; Fig. 1.1).

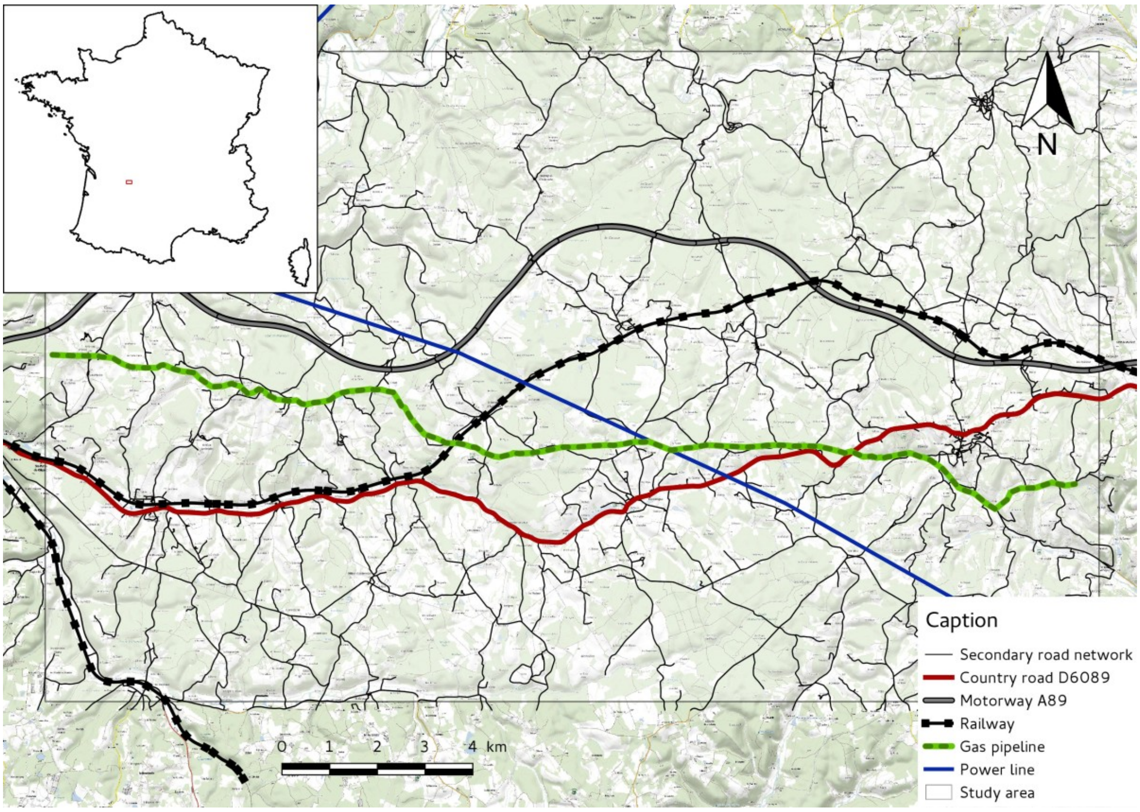

Figure 1.1 – Study area in south-western France

It is a $300 km^2$ rural landscape composed of limestone plateaux including crops, mowed meadows, deciduous forests and small villages. The hydrology is limited to small sized rivers and ponds. Altitude ranges from 91 to 294 m above sea level. Six types of LTIs cross this study area: the fenced motorway “A89”, commissioned in 2004; a low traffic single-track railway built in the 19^{th} century; a high traffic country road historically present since the 18^{th} century; a gas pipeline built in 1955, a power line constructed in 1962 and a 1370 km network of secondary roads (Fig. 1.1).

Biological models

We estimated how this fragmented landscape influences gene flow in four species. The species were selected based on a compromise between abundances on the field (in order to collect large genetic data sets) and the availability of neutral genetic markers. We also chose species with various life history traits. Accordingly, we monitored two vertebrates (a reptile and an amphibian) and two insects (a butterfly and a ground-beetle).

The amphibian studied was the midwife toad *Alytes obstetricans*, a small toad widely distributed in western Europe. This species is characterised by an interesting reproductive strategy with a semi-terrestrial egg development stage. Just after reproduction, males carry the clutches on their back until hatching. This species is of particular conservation interest due to its sensitivity to the chytrid fungus *Batrachochytrium dendrobatidis* (Bosch et al., 2001). Fragmentation is an additional threat because local populations are known to function as relatively independent entities with strong genetic structure detected among populations (Tobler et al., 2013; Maia-Carvalho et al., 2014; Albert et al., 2015). Little is known on the dispersal ability of this species. Trochet et al. (2014) reviewed a maximal dispersal distance of 500 m.

The reptile studied was the grass snake (*Natrix natrix* sensu lato). In this study, we focused on the species *Natrix helvetica* previously considered as the subspecies *Natrix natrix helvetica* (Kindler et al., 2017). Grass snakes are non-venomous and are the most common snake species in Europe with a wide geographical range. Their typical habitats are wetlands and their diet mainly consists in amphibians (Gregory and Isaac, 2004). Mean home-range size is about 40 ha (Wisler et al., 2008) and they are considered as good dispersers with individuals travelling more than 1 km distances in less than a month (Pettersson, 2014). A previous study did not detect any genetic structure in this species in a intensively used agricultural landscape, suggesting a good connectivity ability in fragmented environments (Meister et al., 2012b).

The butterfly studied was the meadow brown *Maniola jurtina*, an univoltine butterfly which is very common in Europe with locally very high densities. The ideal habitat for this species consists in open grasslands. Median life span of adults is 6.55 days (Bubová et al., 2016). Flight period is about 67 days between June and September (Bubová et al., 2016). *Maniola jurtina* has a medium dispersal capacity with mean dispersal distances ranging from about 50 to 300 m (Schneider et al., 2003; Ouin et al., 2008; Stevens et al., 2013).

The ground-beetle studied was *Abax parallelepipedus.* This species is an opportunist carnivorous ground-beetle (Loreau, 1983) that inhabits the upper layer of litter in forest environments (Loreau, 1987). Hedges in agricultural landscapes are also important refuges (Fournier and Loreau, 1999). This species has a low growth rate and a great longevity for a ground-beetle ($>$ 2 years) (Chaabane et al., 1997). A study in a beechwood in Belgium calculated that density was about 2000 individuals per hectare (Loreau and Nolf, 1993) and Keller et al. (2004) estimated density between 632 and 1707 individuals per hectare in a fragmented mixed forest in Switzerland. The mean distance covered per day was estimated between 0.6 and 2.3 m (Brouwers and Newton, 2009) with a home range of approximately 660 m^2 (Loreau and Nolf, 1993). *Abax parallelepipedus* has typically a low dispersal capacity with an avoidance behaviour face to open habitats (Charrier et al., 1997) and high sensitivity to fragmentation due to roads (Keller et al., 2004).

Genetic data sets

For all species, tissues were collected between April and September in 2015 and 2016. For the two vertebrate species (*N. helvetica* and *A. obstetricans*), we followed an individual-based sampling design due to their low abundances in the field. Individual-based sampling design has been proved to be a good alternative method to population-based sampling design as less individuals are required per sampling location (1 to 4). Therefore with a given total number of genetic samples, individual-based sampling design allow to sample more geographical locations over the landscape (Prunier et al., 2013, 2014). Accordingly, the entire study area was prospected at night to collect individuals of *A. obstetricans* (with also opportunistic detection of *N. helvetica*), completed by day surveys to collect individuals of *N. helvetica.* We mainly focused on sampling sites with high suitability of presence such as wetlands, ponds, rivers, woodland edges and small villages. Sites were prospected on foot and headtorches were used during night to locate individuals of both species. Because snakes were hard to detect, 108 artificial shelters were laid across the study area to attract snakes and facilitate data collection. When an individual was detected, it was hand-captured and manipulated directly in the field. A GPS location (Garmin Etrex20, USA) was recorded for each captured individual. See Fig. 1.2 and 1.3 for sampling locations. Each individual was sexed, measured, weighted, marked (to avoid sampling individual twice) and a genetic sample was collected. We used 7x1.35 mm FDX-B Passive Integrated Transponder (PIT) tags (Loligo Systems, Denmark) to mark *A. obstetricans* individuals. For each captured *A. obstetricans*, we collected a non-destructive genetic sample using buccal swab. The mouths of captured individuals were gently opened using a little metal spoon and were then swabbed for about 10 s. For snakes, we individually marked individuals by clipping ventral scales following Brown and Parker (1976) method. The clipped scales were then used for genotyping. We also collected genetic samples of encountered dead snakes and amphibians (road kill or predation) and snake shed skins.

Tissues from the two insect species (*M. jurtina* and *A. parallelepipedus*) were collected within 30 sites using a usual population sampling design. The site locations were obtained by dividing the study area in a 5x6 regular grid leading to 30 sub-areas using QGIS (V. 2.8). In each sub-area, the ultimate sampling site was defined based on the presence of suitable habitats for both species (woodlands for *A. parallelepipedus* and grasslands for *M. jurtina*). In each site, 30 individuals were sampled (15 from each sex as often as possible), resulting in 900 genetic samples per species. See Fig. 1.2 and 1.3 for sampling locations. Butterflies were captured during day time with nets. *A. parallelepipedus* were trap collected using non-lethal pitfalls. At each retained site, we set up 15 dry pitfall traps arranged in circles at regular intervals of 5 m. Traps were 20 cm in diameter and 15 cm in depth. They were emptied every day until 30 individuals were captured. For both insect species, genetic samples were collected by removing a leg. To avoid sampling the same individual twice, we always removed the same leg from all individuals (middle right when seen from above).

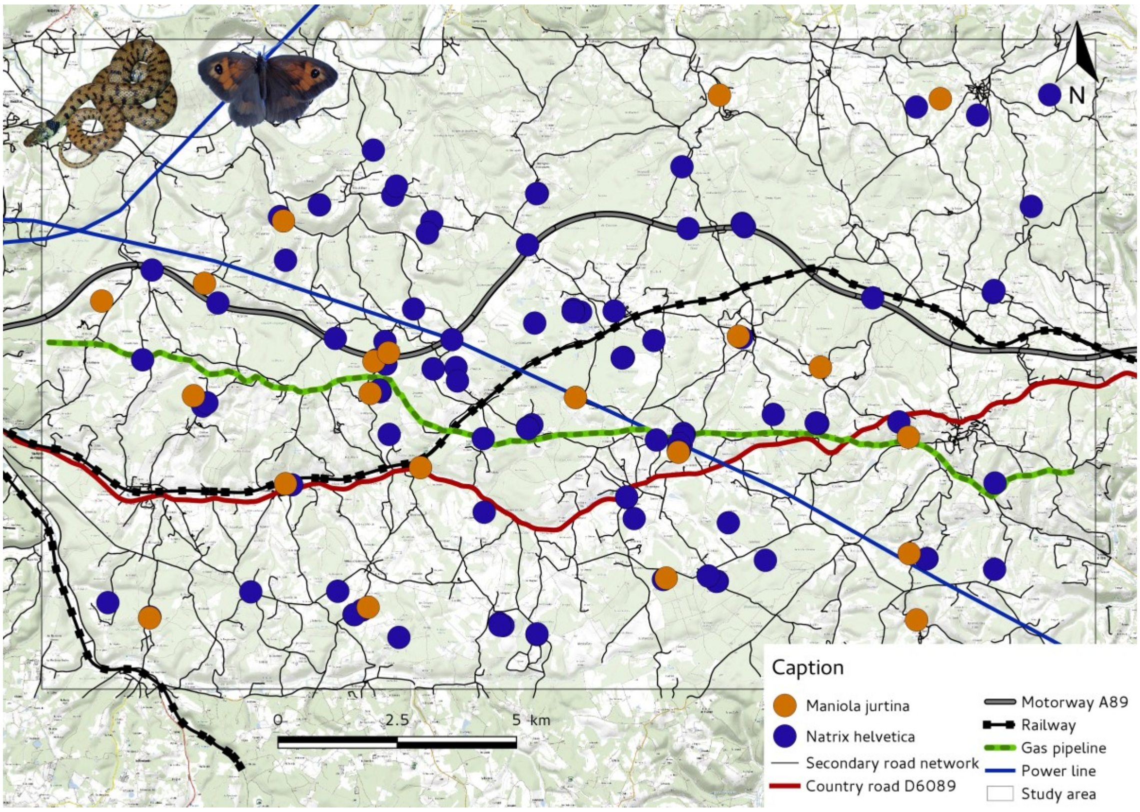

Figure 1.2 – Sampling locations of the species *Natrix helvetica* and *Maniola jurtina* within the study area. Samples were collected in 2015 and 2016. Each *N. helvetica* location represents an individual. Each *M. jurtina* location represents a sampled population (30 individuals per population). For these two species, no genetic structure was identified (see text).

All genetic samples from the four species were stored in 70 % EtoH until DNA extraction. Care was taken to minimise animal handling and stress. All material for marking animals and collecting genetic samples were washed and disinfected using absolute ethanol between each individual. Animals were rapidly released on the place of capture after manipulation.

Laboratory procedures

We amplified 13 (Pokrant et al., 2016), 14 (Tobler et al., 2013; Maia-Carvalho et al., 2014), 15 (Richard et al., 2015) and 14 (Marcus et al., 2013) polymorphic microsatellite loci, for *N. helvetica*, *A. obstetricans*, *M. jurtina* and *A. parallelepipedus*, respectively. For a detailed procedure of DNA extraction, amplification and genotyping, see Appendix A.

We used Genepop 4.2 (Rousset, 2008) to test for linkage disequilibrium among pairs of loci and deviation from Hardy-Weinberg Equilibrium after sequential Bonferroni correction to account for multiple related tests (Rice, 1989). The presence of null alleles was tested using MICROCHECKER 2.2.3 (Van Oosterhout et al., 2004).

Final data sets

The presence of related individuals (siblings for example) in data sets is known to overestimate the number of clusters when assessing population structure (Anderson and Dunham, 2008) and bias subsequent genetic analyses. Therefore, we used COLONY2 (Jones and Wang, 2010) to identify full-sib and parent-offspring groups among our individual-based data sets (*N. helvetica* and *A. obstetricans*). We used the full-likelihood approach based on the individual multilocus genotypes. For both species, we assumed that males and females were polygamous (for the snake, see Meister et al., 2012a). All individuals were considered as potential offspring and no a priori candidate parental genotypes was defined. Allele frequencies were determined directly from genetic datasets. We ran three independent long runs with various seed numbers to test for congruence among results. Only relationships with an associated inclusion probability higher than 95 % were considered as significant. In each group of related individuals, we randomly retained one genotype. Accordingly, 76 genotypes in the *A. obstetricans* data set were discarded. In the *N. helvetica* data set, two genotypes were identical. These two genotypes corresponded to an adult male and a shed skin collected on the same site, 100 m apart, in 2016. Therefore, we discarded the shed skin sample as it probably belonged to the same individual. In addition, because some sites were unevenly sampled for *N. helvetica* and *A. obstetricans*, we only retained a maximum number of three random genotypes per sampling location (Prunier et al., 2013). Some individuals could not be genotyped mainly due to insufficient DNA amount. Therefore, in the population data sets, we only retained populations for which more than 15 genotypes were available. Finally, genotypes with more than 2 loci presenting missing values were discarded to allow robust genetic analyses. The final data sets comprised 848 genotypes (30 populations) in *A. parallelepipedus*, 508 genotypes (21 populations) in *M. jurtina*, 115 genotypes in *N. helvetica* (68 sampling locations) and 132 genotypes in *A. obstetricans* (56 sampling locations).

Hierarchical genetic clustering

For each of the four final data sets (either individual or population based data sets), genetic clustering was investigated using the program STRUCTURE 2.3.4 (Pritchard et al., 2000) with the admixture and the correlated allele frequency models and prior population information when structure in the data was weak. We followed a hierarchical genetic clustering procedure (Coulon et al., 2008). At each hierarchical level, we tested the number K of clusters from 1 to 10 and repeated analyses for each value 5 times. Runs were performed with a burn-in period of 50 000 and the 50 000 subsequent Markov chain Monte Carlo repetitions were retained. We also checked that the alpha value (looking at alpha plots created by STRUCTURE) had stabilised before the end of the burn-in period to ensure convergence. If convergence was not reached, we used a burn-in period of 100 000 and the 100 000 subsequent Markov chain Monte Carlo repetitions were retained. We then used STRUCTURE HARVESTER (Earl and VonHoldt, 2012) to obtain Log-likelihood plots and deltaK statistics to infer the optimal K-value. We used the optimal K-value to performed 20 runs with a burn-in period of 200 000 and the 200 000 subsequent Markov chain Monte Carlo repetitions retained. We compiled the ten best runs using CLUMPP (Jakobsson and Rosenberg, 2007) to obtained the individual or population Q-values. Each individual or population was assigned to the cluster for which its Q-value was higher than 0.6 (Prunier, Colyn, Legendre and Flamand, 2017). We then repeated the analysis for each inferred cluster separately until no more structure was found in the data. For each hierarchical level, we used Q-values to compute pairwise matrices (among individuals or populations depending on the design) of ancestry-based hierarchical genetic distance (HGD) (Balkenhol et al., 2014; Prunier, Colyn, Legendre and Flamand, 2017).

Genetic dependent variables

In each of the four final data sets (one per species), we calculated two types of genetic dependent variables. The first one is a standard genetic distance, calculated from the Bray-Curtis (bc) percentage dissimilarity index (Legendre and Legendre, 1998) for the individual-based data sets (*N. helvetica* and *A. obstetricans*). For the two other species, *M. jurtina* and *A. parallelepipedus*, we calculated inter population genetic distances based on Fst. Classical genetic distances are powerful to detect regional and surface elements affecting gene flow but may be unwilling to detect isolation due to linear elements (Prunier, Colyn, Legendre and Flamand, 2017). Therefore, we used a second type of genetic dependent variable described as hierarchical genetic distances (HGD), which is powerful to detect mainly linear elements affecting gene flow at a more local scale (Prunier, Colyn, Legendre and Flamand, 2017). HGD was calculated only for species where a genetic structure was detected using the STRUCTURE software. When more than one hierarchical level was detected, each hierarchical level (HGD1, HGD2...) was considered.

Landscape predictors

In total, we considered 13 landscape features present in our study area as predictors which were likely to explain the variance in the two types of genetic dependent variables across the four data sets. Six predictors described soil occupancy. They were defined by digitalizing the entire study area on QGIS (V. 2.8) using national maps and aerial photographs (BD Ortho from National Geographic Institute, France). Every elements of the landscape was classified into 49 habitat types of the EUNIS Habitat Classification System (Davies and Moss, 1999). Field botanic expertises were also performed in 2015 to confirm the affiliation of certain habitat types. We combined these features into six main predictors (Table S1): Water (stagnant water bodies, streams and rivers), Crops (intensive and non intensive cultures), Woodlands (all types of forests), Grasslands (open lands that are not cultivated), Urban (villages, agricultural installations, industrial sites, stone quarries, *etc.*) and Roads (all roads excluding small trails). These six spatial classes were rasterised at a 1 m resolution using ARCGIS 10.2.2 and the SPATIAL ANALYST extension. Each spatial class was used to compute a resistance surface based on the spatial density of the corresponding element in the landscape. To do so, we overlaid a 20 m grid on each spatial class and calculated the percentage of the element in each grid (400 m^2) (Balkenhol et al., 2014; Prunier, Colyn, Legendre and Flamand, 2017). For each resistance surface, we rescaled pixel resistance values to range from 1 (null or extremely low densities) to 100 (the element covered the entire pixel). These six resistance surfaces were used in CIRCUITSCAPE 4.0 (McRae, 2006; McRae et al., 2013), implementing a method that determines all possible pathways between two points by analogy to electrical resistance. We obtained electric current values between each pair of locations for our six resistance surfaces. In addition to these six predictors, we included an isolation-by-distance predictor (IBD) and an Altitude predictor using euclidean distances and altitude difference between pairs of locations, respectively. Finally, besides roads, we included five predictors likely to create isolation-by-barriers in our data sets: Motorway A89, Railway, D6089 country road, Gas pipeline and Power line (Fig. 1.1). For each of these five linear elements we computed a binary pairwise matrix between all pairs of locations where 0 indicated that pairs were on the same side of the element and 1 indicated that pairs were on either side of the element.

Spatial scale of analyses

The spatial scale retained in landscape genetic analyses can deeply influence the conclusions of studies (Keller et al., 2013; Schregel et al., 2018). The local influence of landscape elements on genetic distances can remain unnoticed if spatial scale retained is wide in comparison to dispersal capacities of individuals (Anderson et al., 2010). Accordingly, we did not use all possible pairs of populations or individuals in our data sets. For each dataset, we retained a subset of pairwise data by defining a maximum euclidean distance between pairs. The maximum euclidean distance was selected as the neighbouring distance maximizing the R^2 of our full model including all predictors in a classical multiple linear regression. This retained distance was higher than the minimum distance in a neighbouring graph which ensured that no individual was excluded from the network (Jombart et al., 2008). It was estimated using Gabriel graphs with the "adegenet" package (Jombart, 2008) in R 3.3.2 (R Core Team, 2015). Subsequent analyses were only ran with pairwise data associated with Euclidean distances lower than the computed maximum neighbouring distance. This framework corresponded to the comparable approach developed in Schregel et al. (2018).

Multiple linear regression and commonality analysis on genetic distances

For each of the four data subsets and the two types of genetic dependent variables (standard genetic distances or HGD), a complete linear model including the 13 predictors was designed. All predictors were centered. We explored the relationship between the explanatory variable and the predictors using multiple linear regression on vectors (Smouse et al., 1986; Prunier et al., 2015). We used multiple linear regression on vectors instead of matrices because we did not considered complete matrices of pairwise distances but a subset based on the maximum neighbouring distance. The contribution of predictors to the dependent variable was assessed using commonality analyses (CA). Commonality analyses is a procedure of variance partitioning providing decisive support when trying to assess the reliability of model parameters (beta weights and confidence intervals) face to multicollinearity (for more detailed informations on CA, see Prunier et al. (2015)). In commonality analyses, the effect of each predictor can be decomposed into a unique (U) and common (C; shared with other predictors) effect. For a given predictor, the sum of unique and common effects corresponds to the total contribution (T), equal to its squared zero-order correlation with the dependent variable ($U+C=T=r^2$). Therefore, CA represents a good opportunity to assess the reliability of predictors to explain the dependent variable face to collinearity. The magnitude of suppression among predictors is indicated by negative commonalities. Negative commonalities represent the amount of predictive power that would be lost by other predictors if the suppressor variable was not included in the regression model. Accordingly, we can distinguish three specific types of suppressor (Conger, 1974). (i) A classical suppressor corresponds to a predictor whose unique contribution is totally counterbalanced by its negative common contribution ($U+C=0$). (ii) A reciprocal suppressor also described as a partial suppressor is a predictor with a negative common effect but that does not counterbalance its unique contribution to the variance in the dependent variable ($T=U+C>0$). Finally, (iii) cross-over suppressor is similar to a partial suppressor but with reversal sign. Cross-over suppressors are detected by a sign inversion between the structure coefficients and the beta weights (Prunier, Colyn, Legendre and Flamand, 2017). We performed multiple linear regressions and CA using packages ecodist (Goslee and Urban, 2007) and yhat (Nimon et al., 2008) in R 3.3.2 (R Core Team, 2015). To remove classical suppressors, we discarded predictors presenting low univariate squared correlation against the genetic dependent variables (r^2 lower than 0.1). Low correlated predictors are likely to act as classical suppressors leading to the distortion of regression coefficients (Prunier et al., 2015; Prunier, Dubut, Chikhi and Blanchet, 2017). When we discarded those non-informative predictors, we ended up with simplified models containing a reduced number of predictors which were likely to explain the variance in the genetic dependent variables. Predictors that were identified as cross-over and reciprocal suppressors were discarded from our model and subsequent models were ran without these suppressors until no more suppressors could reasonably be discarded from the model (that is, we kept reciprocal suppressors showing a non-negligible unique contribution). We also removed predictors with synergistic (S) association with other predictors, which have a unique contribution to the dependent variable equal to zero but presenting synergistic association with other predictors ($C>0$)(Appendix C).

In the final simplified model, we assessed the linear relationship among our predictors to test for multicollinearity by using Pearson's correlation coefficients r and Variance Inflation Factors VIF (Dormann et al., 2013). Because data are not independent, the p-values were necessary biased and, therefore, were not calculated (Legendre and Legendre, 1998). Yet, we computed 95 % confidence intervals around regression coefficients using a jackknife procedure, with 1000 replicates based on a random removal of 10 % of individuals without replacement (Prunier et al., 2015). These confidence intervals were used to assess the significance of the predictor's contributions to the variance in the genetic dependent variables. We considered that when the confidence intervals did not include 0, the predictor was a robust contributor to the variance in the response.

This framework was repeated for each of the two types of genetic dependent variables and for each data subset. It can be summarised as follow:

1. Define spatial scale between pairs of locations maximizing the R^2
2. Discard predictors with low squared correlations with the genetic dependent variable, likely to act as classical suppressors
3. Perform commonality analyses, discard cross-over and reciprocal suppressors
4. Assess collinearity among final predictors
5. Extract regression coefficients from the final model, as well as unique contributions and confidence intervals for the retained predictors

A predictor with a positive β value was associated with an increase of the genetic distances. It was interpreted as a predictor that impeded gene flow and created barrier to dispersal. A predictor with a negative β was associated to a reduction of genetic distances. It was interpreted as a predictor promoting gene flow and enhancing dispersal (Jacquot et al., 2017).

Output summary

In order to summarise all the results, we built three 100 % stacked barplots showing averaged unique contributions of all retained predictors across the two types of genetic dependent variables. In the first plot, averaged unique contributions were presented per species across predictors merged into three main classes: natural predictors (IBD, Altitude, Woodlands, Grasslands and Water), anthropised predictors (Crops and Urban) and infrastructures (the six types of LTIs). In the second plot, we presented averaged unique contributions per species across infrastructures with their two types of effects (increase or reduction of genetic distances). Finally, in the last plot, we presented averaged unique contribution per type of infrastructure effect (increase or reduction of genetic distances) across all species. Predictors that were absent in the final models were given a unique contribution of 0.

1.3 Results

Genetic data

In the *A. obstetricans* data set, there was no evidence of linkage disequilibrium among loci. We found evidence of null alleles for locus Aly7. Accordingly, we retained 13 loci for subsequent analysis (Aly28, Aly3, Aly4, Aly17, Aly19, Aly20, Aly23, Aly24, Aly25, Aobst14, Aobst15, Aobst16 and Aobst17).

In the *N. helvetica* data set, two loci could not be amplified (Nsμ3 and 3TS) either in multiplex or in standalone PCR. There was no evidence of null alleles, but we found evidence of linkage disequilibrium between loci Natnat05 and μNt8new and between loci Natnat05 and TbuA09. Therefore, we only retained 10 loci for subsequent analysis (Natnat09, μNt8new, μNt3, μNt7, Natnat06, Natnat11, Eobμ 1, Eobμ13, TbuA09 and 30).

In the *M. jurtina* data set, the locus Mj2410 was discarded as it showed sex linkage (Richard et al., 2015; Villemey et al., 2016). As Villemey et al. (2016), we found evidence of frequent null alleles for loci: Mj5522, Mj5287, Mj5647, Mj3956, Mj5563, Mj0272, Mj0283 and Mj3637. Thus, we only retained six loci for subsequent analysis (Mj0008, Mj7132, Mj0247, Mj7232, Mj4870 and Mj5331).

In the *A. parallelepipedus* data set, there was no evidence of linkage disequilibrium among loci. We found evidence of null alleles for loci: apar14, apar44, apar46 and apar50. Then, we retained 10 loci for subsequent analysis (apar20, apar50, apar27, apar34, apar32, apar12, apar23, apar25, apar02, apar46, apar05, apar44, apar14, apar06). Appendix A provides tables summarising markers characteristics in each species.

Genetic structure

STRUCTURE revealed that all individuals from the *N. helvetica* and *M. jurtina* data sets belonged to a single cluster. The log transformed estimates of data probability [ln Pr(X|K)] were maximal for K = 1. Implementing sampling locations as locprior did not permit to find more than one cluster in the two data sets.

In the *A. obstetricans* individual data set, we identified two hierarchical levels (Fig. 1.3). At the first level, one cluster (A) surrounded a second cluster (B) with no clear geographical boundaries explaining this pattern (Fig. 1.3). Ten individuals could not be assigned to any of these two clusters (cross-assigned) suggesting some exchanges between these two clusters. At the second hierarchical level, only cluster A was further divided into three clusters: A1, A2 and A3. These three clusters were not separated by clear geographical patterns. At the second hierarchical level, a high number of individuals (21) could no be assigned to any of these three sub-clusters suggesting frequent exchanges among them. In total, we identified four final clusters (Fig. 1.3).

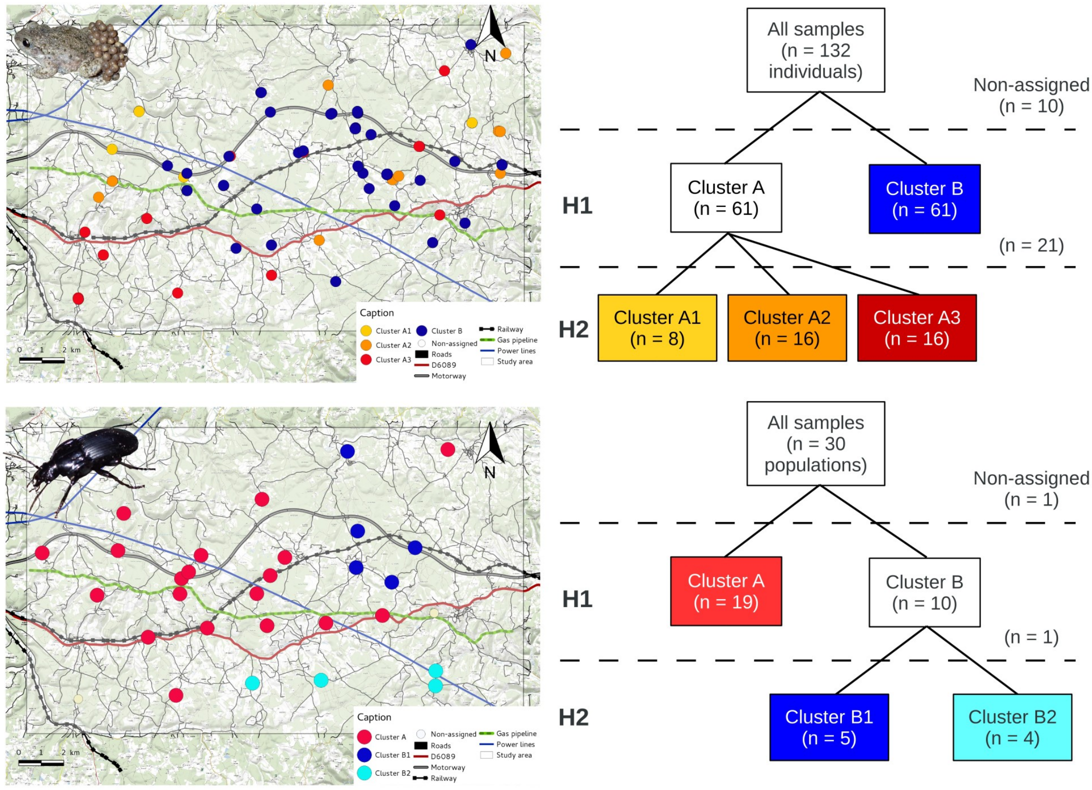

Figure 1.3 – STRUCTURE outputs for the species *A. obstetricans* (132 individuals in 56 sampling locations) and *A. parallelepipedus* (30 populations of 30 individuals) plotted over the study area. Right panels represent the hierarchical splits of clusters inferred with STRUCTURE from the first to the second hierarchical level. n is the number of samples (individuals for *A. obstetricans* and populations for *A. parallelepipedus*) assigned to each cluster. On the right-hand side of panels, we present the number of non-assigned samples at each hierarchical level (Q-values < 0.6).

In the *A. parallelepipedus* population data set, we identified two hierarchical levels (Fig. 1.3). At the first level, 19 populations were assigned to cluster A and ten were assigned to cluster B. Cluster A included populations sampled mostly in the western part of the study area and overall north of the road “D6089” (Fig. 1.3). One population at the extreme south-west could not be assigned to any of these two clusters (cross-assigned). Cluster B, was further divided into two sub-clusters at the second hierarchical level. Cluster B1 comprised five populations north of the “D6089” and the gas pipeline and cluster B2 comprised four populations south of the “D6089” and the gas pipeline. At the second hierarchical level, only one population could not be assigned to any of these two clusters (cross-assigned). This population was located between the road "D6089" and the gas pipeline exactly in-between clusters B1 and B2 suggesting some exchanges between these two clusters. In total, we identified three final clusters (Fig. 1.3).

Spatial scale of analysis

In the four data sets, the minimum neighboring distances detected with the Gabriel graphs were 2400 m, 2700 m, 5100 m and 4500 m for the species *A. obstetricans*, *N. helvetica*, *M.jurtina* and *A. parallelepipedus*, respectively (Appendix B). In the *A. obstetricans* data set, the spatial scales maximizing the R^2 between pairs were 3000 m, 2400 m and 3500 m for the Bray-Curtis genetic distance, HGD1 and HGD2, respectively. In the *N. helvetica* data set, the spatial scale maximizing the R^2 was 2800 m. In the *M. jurtina* data set, the spatial scale maximizing the R^2 was 5500 m. In the *A. parallelepipedus* data set, the spatial scales maximizing the R^2 were 6500 m, 18500 m and 4500 m for the Fst genetic distance, HGD1 and HGD2, respectively (Appendix B).

Correlation among final predictors

Across all data sets and all types of genetic dependent variables, values of Pearson's correlation coefficients among predictors that were retained ranged from -0.303 to 0.489 and Variance Inflation Factors (VIF) ranged from 1.00 to 1.70 (Table S2). These results suggested little collinearity among predictors (Dormann et al., 2013), and thus little distortion in regression outputs (Prunier et al., 2015).

Multiple linear regression and commonality analyses for *A. obstetricans*

Table 1.1 – Outputs of multiple linear regressions and additional parameters from commonality analyses (CA) for each species and for each type of data set. DV represents the dependent variable type: classical genetic distances (GD) calculated either with the Bray-Curtis dissimilarity index (bc) or with Fst and hierarchical genetic distances (HGD1 and HGD2 for first and second level of hierarchy, respectively). For each model, the model fit (Multiple R^2) was estimated with the spatial scale retained between pairs of locations (Distance). For each retained predictor per model, we estimated the structure coefficient (rs), beta weight (β), unique (U), common (C) and total (T) contributions. Significance of the predictor's contribution to the DV was estimated using confidence intervals (CI-inf and CI-sup). A CI that included 0 was considered as a non-informative predictor (indicated in bold). Gray colour indicates predictors with negative relationship to the dependent variable (negative β). They correspond to predictors that are associated with a decrease in genetic distances and may thus be considered as promoting gene flow.

	DV	Species	Multiple R^2	Distance	Predictor	rs	B	CI-inf	CI-sup	U	C	T
	GD(bc)	*A. obstetricans*	11.82%	3000 m	IBD	0.823	0.126	0.066	0.198	0.009	0.071	0.080
					Altitude	0.618	0.098	0.052	0.140	0.008	0.037	0.045
					Woodlands	0.554	0.145	0.091	0.191	0.018	0.018	0.036
					Roads	0.623	0.113	0.062	0.153	0.009	0.037	0.046
					D6089	0.320	0.091	0.043	0.142	0.008	0.004	0.012
	HGD1	A. obstetricans	10.76%	2400 m	Woodlands	0.461	0.100	0.037	0.172	0.010	0.013	0.023
					Crops	0.687	0.185	0.099	0.254	0.032	0.018	0.051
					Roads	0.675	0.159	0.100	0.203	0.024	0.025	0.049
					Railway	0.442	0.108	0.048	0.178	0.011	0.010	0.021
	HGD2	A. obstetricans	19.92%	2500 m	Woodlands	0.538	0.188	0.134	0.240	0.031	0.026	0.058
					Urban	-0.465	-0.241	-0.276	-0.203	0.047	-0.004	0.043
					Roads	0.448	0.184	0.134	0.238	0.033	0.006	0.039
					D6089	0.440	0.196	0.145	0.250	0.037	0.001	0.039
					Motorway	-0.278	-0.120	-0.159	-0.076	0.014	0.002	0.016
	GD(bc)	*N. helvetica*	4.15%	2800 m	Roads	-0.533	-0.125	-0.193	-0.062	0.015	-0.003	0.012
					Motorway	0.616	0.148	0.078	0.221	0.021	-0.005	0.016
					Railway	-0.520	-0.088	-0.155	-0.022	0.008	0.004	0.011
	GD(Fst)	*M. jurtina*	19.91%	5500 m	IBD	0.468	0.264	0.001	0.490	0.066	-0.023	0.044
					Woodlands	0.685	0.315	0.077	0.519	0.089	0.004	0.093
					Power line	-0.595	-0.180	**-0.388**	**0.046**	0.030	0.040	0.071
	GD(Fst)	*A. parallelepipedus*	25.87%	6500 m	Altitude	0.203	0.121	**-0.023**	**0.251**	0.015	-0.004	0.011
					Grasslands	0.971	0.498	0.372	0.610	0.248	-0.004	0.244
	HGD1	*A. parallelepipedus*	17.22%	18500 m	Roads	0.812	0.262	0.170	0.350	0.063	0.051	0.114
					D6089	0.797	0.254	0.159	0.338	0.059	0.051	0.110
	HGD2	*A. parallelepipedus*	26.76%	4500 m	Altitude	0.445	0.223	0.056	0.397	0.049	0.004	0.053
					D6089	0.759	0.350	0.184	0.500	0.114	0.040	0.154
					Motorway	-0.316	-0.114	**-0.273**	**0.041**	0.012	0.015	0.027
					Gas pipeline	0.518	0.225	0.070	0.368	0.049	0.022	0.071

When using the genetic distance based on the Bray-Curtis dissimilarity index (bc), the multiple linear regression explained 11.8 % of the variance (Table 1.1). Five final predictors explained the dependent variable: IBD, Altitude, Woodlands, Roads and the road D6089. All β values were positive, indicating that these predictors were associated with an increase of genetic distance in *A. obstetricans.* Natural predictors (IBD, Altitude and Woodlands) explained most of the dependent variable's variance (67 % of the averaged unique contributions). Woodlands was the landscape element with the highest unique contribution to the genetic distances (U = 0.018). Two infrastructures were associated with an increase of genetic distances in this model: the secondary road network and the country road "D6089". Both explained about 33 % of the averaged unique contribution and had similar unique contributions to the dependent variable (U = 0.009 and 0.008, respectively).

When using the first level of hierarchical genetic distance (HGD1), the linear regression explained 10.76 % of the variance. In the final model, four predictors explained HGD1 and all were associated with an increase of genetic distances (positive β values). Crops was the predictor with the highest contribution to the dependent variable (U = 0.032) followed by Roads (U = 0.024). In this model, Woodlands was also associated with an increase of genetic distances but was the predictor with the lowest unique contribution (U = 0.010). Railway was associated with an increase of genetic distances with a unique contribution of 0.011 to the dependent variable.

With the second level of hierarchical genetic distance (HGD2), a higher portion of the dependent variable's variance was explained by our model: 20 %. The final model comprised five predictors: Woodlands, Urban, Roads, D6089 and Motorway. Woodlands, Roads and the road D6089 were associated with an increase of genetic distances in *A. obstetricans* (positive β values) but urbanization and the motorway had negative β values indicating that these two predictors were associated with a reduction of genetic distances in *A. obstetricans*. The Motorway predictor was the one explaining the lowest part of the dependent variable's variance (U = 0.014). Urbanization was the landscape element affecting the highest part of the dependent variable's variance (U = 0.047). Woodlands, Roads and the road D6089 were all associated with an increase of genetic distances in this model with unique contribution of 0.031, 0.033 and 0.037, respectively.

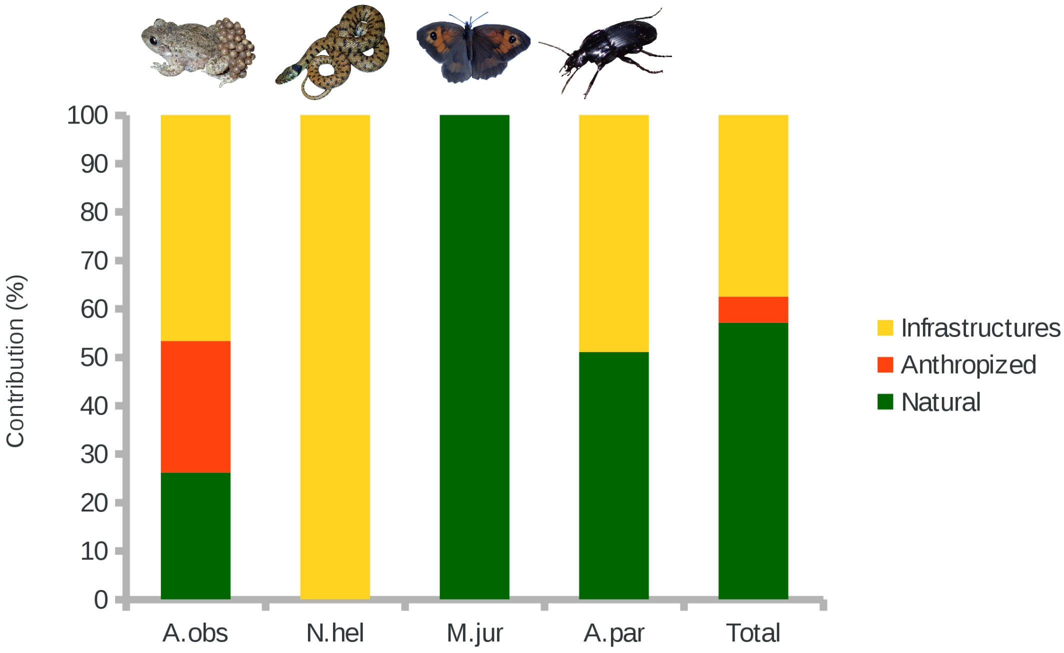

Figure 1.4 – Averaged unique contributions of natural predictors (IBD, Altitude, Woodlands, Water, Grasslands), anthropised predictors (Crops and Urban) and infrastructures (all linear infrastructures) to each species and combined results (Total) across all datasets.

When the unique contribution from the three genetic dependent variables were merged, gene flow of *A. obstetricans* was mostly explained by infrastructures (47 % of the variability, Fig. 1.4). Natural and anthropised predictors explained each about 26 % of the variability. Infrastructures were mostly associated with an increase of genetic distances in *A. obstetricans* with 90 % of the variability in unique contributions explained by barrier effects of infrastructures (Fig. 1.5). The secondary road network and the main road "D6089" were driving most of this pattern (82 % of the unique contributions) and the railway to a smaller extent (8 % of the unique contributions). The 10 % left were associated with a reduction of genetic distances detected across the motorway when using the second level of hierarchical genetic distance (HGD2)(Fig. 1.5).

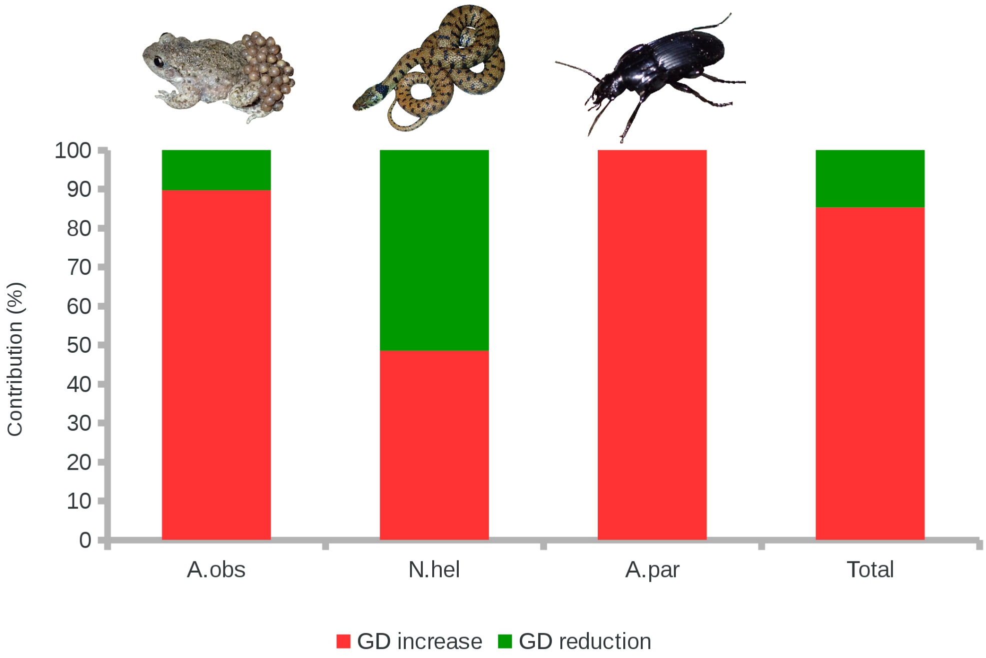

Figure 1.5 – Averaged unique contributions of genetic distances (GD) increase or reduction of the six types of linear infrastructures (Roads, D6089, Motoraway A89, Railway, Gas pipeline and Power line) to each species and combined results (Total) across all datasets. A reduction in GD is associated with a gene flow enhancement and an increase in GD is associated with a barrier effect impeding gene flow.

Multiple linear regression and commonality analyses for *N. helvetica*

With the dependent variable (bc), the multiple linear regression explained a small proportion (4.15 %) of the variance (Table 1.1). The motorway was associated with an increase of genetic distances in *N. helvetica* (positive β value) and explained most of the dependent variable's variance (U = 0.021). The two other types of infrastructures (the secondary road network and the railway) had unique contribution of 0.015 and 0.008, respectively. Both had negative β values, indicating that they were associated with a reduction of genetic distances in the snake.

The entire variability detected in *N. helvetica* was due to infrastructures (Fig. 1.4). When unique contribution of predictors were merged, 50 % of the variability was associated with an increase of genetic distances supported by the motorway and the 50 % left was associated with a reduction of genetic distances (Roads = 34 % and Railway = 17.7

%; Fig. 1.5).

Multiple linear regression and commonality analyses for *M. jurtina*

The butterfly genetic distances were calculated using Fst. The multiple linear regression explained 20 % of the dependent variable's variance (Table 1.1). After non-informative predictors and suppressors were discarded, only IBD, Woodlands and Power line remained in the final model. The 95 % confidence interval of the Power line effect included 0, indicating that this predictor did not significantly contribute to the variance in the dependent variable. Woodlands were associated with an increase of genetic distances (positive β values) in *M. jurtina* and explained most of the variance (U = 0.089). The rest of the explained variance was due to isolation by distance (IBD, U = 0.066). Therefore, the entire variability detected in *M. jurtina* genetic distances was explained by natural predictors (Fig. 1.4).

Multiple linear regression and commonality analyses for *A. parallelepipedus*

When using the genetic distance based on Fst, the multiple linear regression explained 26 % of the dependent variable's variance (Table 1.1). Two final predictors explained the dependent variable: Altitude and Grasslands. Altitude did not significantly explain genetic distances (95 % confidence intervals included 0). Therefore the variance explained by our model was only due to Grasslands associated to an increase of genetic distances in *A. parallelepipedus* (U = 0.248).

When using the first level of hierarchical genetic distance (HGD1), the linear regression explained 17 % of the dependent variable's variance. HGD1 was fully explained by predictors associated with an increase of genetic distances in the ground-beetle (positive β values): the secondary road network (U = 0.063) and the country road D6089 (U = 0.059).

When using the second level of hierarchical genetic distance (HGD2), the linear regression explained 27 % of the dependent variable's variance. Four predictors remained in the final model: the altitude, the road D6089, the motorway and the gas pipeline. The 95 % confidence interval around the β value of the motorway included 0 indicating that the motorway was not significantly explaining HGD2. The three remaining predictors were all associated with an increase of genetic distances (positive β values). The road D6089 was explaining the highest part of the variability (U = 0.114) suggesting a strong barrier effect of this infrastructure on gene flow. The gas pipeline and Altitude had both a unique contribution to the dependent variable of 0.049.

When the unique contribution from the three genetic dependent variables were merged, gene flow of *A. parallelepipedus* was explained by infrastructures (49 %) and natural predictors (51 %) (Fig. 1.4). In this species, infrastructures were all associated with an increase of genetic distances (Fig. 1.5).

Summary of infrastructure effects

In total, 38 % of the genetic variability across all species was due to infrastructures (Fig. 1.4). The secondary road network (12 %) and the country road D6089 (15 %) were the LTIs most affecting genetic distances in the four studies species. The motorway (5 %), the railway (2.5 %) and the gas pipeline (3.5 %) had moderate effects on genetic distances and the power line had no effect on gene flow in any species.

When unique contributions were presented per type of infrastructure and averaged across species, five of the six tested infrastructures were associated with an increase of genetic distances in at least one of the studied species (Fig. 1.6).

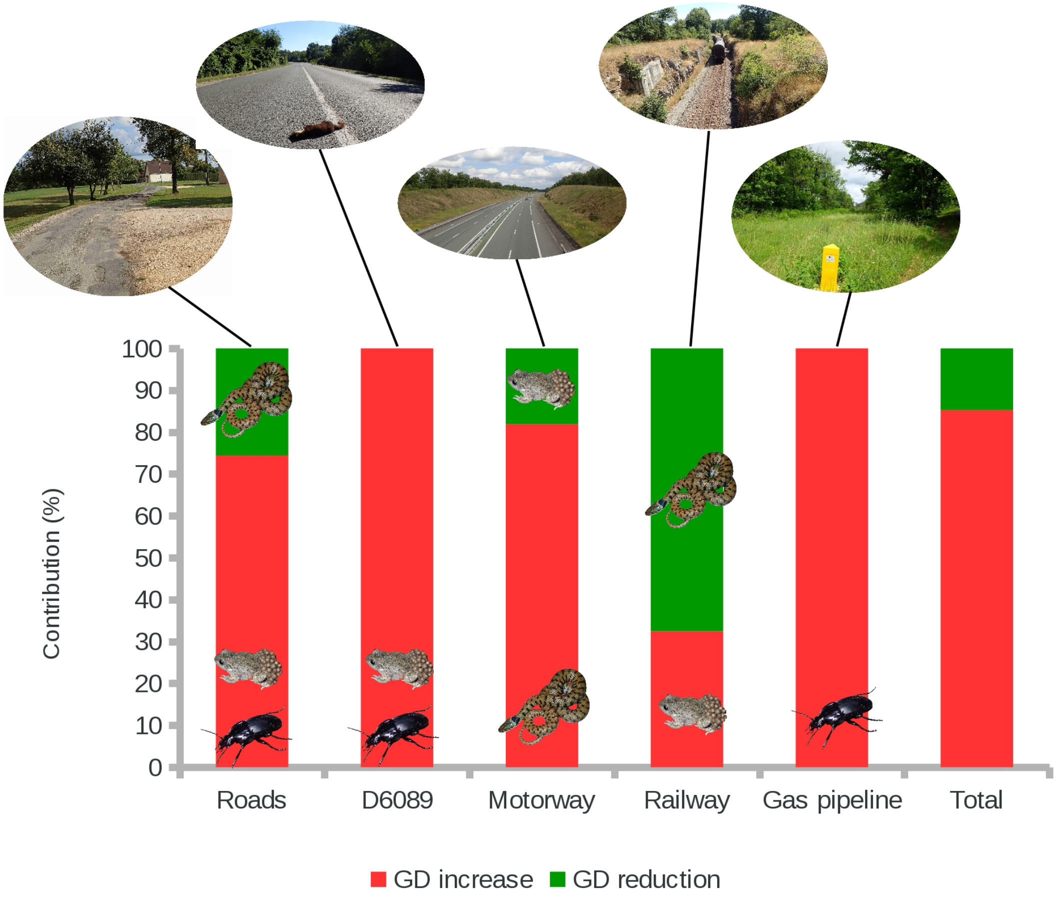

Figure 1.6 – Proportions of the averaged unique contributions of genetic distances (GD) increase or reduction of five linear infrastructure types (Roads, D6089, Motoraway A89, Railway and Gas pipeline) across species. The power line is not represented as no species were affected by this infrastructure (see results). Total represents the combined results across datasets. A reduction in GD is associated with a gene flow enhancement and an increase in GD is associated with a barrier effect impeding gene flow.

The only infrastructure that was not affecting genetic distances across all species was the power line. The secondary road network affected the genetic distances in *N. helvetica*, *A. obstetricans* and *A. parallelepipedus*. 74 % of unique contributions of secondary roads were associated with an increase of genetic distances in *A. obstetricans* and *A. parallelepipedus* (Fig. 1.6). 26 % of unique contributions of secondary roads were associated with a reduction of genetic distances in *N. helvetica*. The country road D6089 was influencing genetic distances in two species (*A. obstetricans* and *A. parallelepipedus*) and 100 % of unique contributions were associated with an increase of genetic distances (Fig. 1.6). The motorway affected genetic distances of the two vertebrate species (*A. obstetricans* and *N. helvetica*). 82 % of unique contributions of the motorway were associated with an increase of genetic distances in *N. helvetica*. The 18 % left corresponded to a reduction of genetic distances in *A. obstetricans*. Similarly, the railway influenced only the two vertebrate species. 32 % of the unique contributions of the railway were associated with an increase of genetic distances in *A. obstetricans* and 68 % of the unique contributions were associated with a reduction of genetic distances in the snake. The gas pipeline was only affecting genetic distances in the ground-beetle *A. parallelepipedus* and was associated with an increase of genetic distances. In total, infrastructures were mostly associated with an increase in genetic distances (85 %).

1.4 Discussion

In this study we assessed landscape connectivity in four species in a fragmented environment in south-western France. We were particularly interested in the convergent effects of six types of large-scale transportation infrastructures. We used individual and population based analyses, restricted spatial scale and regression commonality analyses to evaluate the relative contribution of various landscape predictors to the variance in both, classical and hierarchical genetic distances.

Analytical framework

Individual-based sampling scheme is a recent promising tool in landscape genetics. Because less individuals are needed per sampling location (3-4 individuals), more sampling locations can be covered. It allows to capture a wide amount of genetic variation and provide an broad representation of the landscape heterogeneity (Prunier et al., 2013). In our study, we used individual-based analyses for the snake *N. helvetica* and the toad *A. obstetricans*, as a population-based sampling scheme would require between 20 to 30 individuals per population (Prunier et al., 2013). Considering the ecology of these two species, an individual-based sampling scheme is optimal. The grass snake has a diffuse distribution in the landscape with low detectability, which makes the use of a population-based sampling scheme almost impossible. The midwife toad has a clumped distribution in the landscape but population sizes are small. Sampling 20 to 30 individuals would require both a huge time investment in the field and an optimal landscape configuration with large populations. By using individual-based analyses with the Bray-Curtis dissimilarity index, we were able to explain 4 and 12 % of the genetic variability in *N. helvetica* and *A. obstetricans*, respectively. These amounts were lower than the variance explained for the two other species studied using population-based analyses (20 % in *M. jurtina* and 26 % in *A. parallelepipedus*). Prunier et al. (2013) argue that individual-based methods should outperform population methods based on allelic frequencies but a direct comparison between individual and population based-methods on the same biological model is still required to test this hypothesis (Luximon et al., 2014).

By using restricted spatial scales in our analyses, we were able to optimize the detection of landscape features which were likely to explain the variability in genetic distances (Keller et al., 2013). Some local influences of landscape elements on genetic distances can remain unnoticed if all pairs of genetic distances are retained. This is especially true for pairs separated by important distances where isolation by distance is likely to cover up the variability explain by isolation by barriers or isolation by resistance (Anderson et al., 2010). For example, if all pairs were retained in the *A. obstetricans* data set with classical (bc) genetic distances, the variability explained would be reduced to 5 %, which corresponds to a diminution of more than 50 % compared to the variability explained by the restricted spatial scale (Appendix B).

The use of hierarchical genetic distances (HGD) in addition to classical distances is a great improvement in landscape genetic analyses (Balkenhol et al., 2014; Prunier, Colyn, Legendre and Flamand, 2017). HGD allows the detection of sharp genetic variations caused by linear elements, whereas classical genetic distances considered the sampled area as a single continuous genetic unit and inform on the regional landscape permeability. The use of both metrics give a deep understanding of the landscape features affecting gene flow at different geographical scales (Prunier, Colyn, Legendre and Flamand, 2017). In our study, this was particularly true for *A. parallelepipedus*. When using the classical genetic distances we found that only the feature Grasslands was identify as affecting gene flow in this ground-beetle. However, linear elements affecting gene flow in this species were detected when using HGD. In the first level (HGD1), the secondary road network and the country road D6089 explained the genetic variability, indicating that these two features were impeding dispersal at the regional scale (Prunier, Colyn, Legendre and Flamand, 2017). In the second level (HGD2), the country road and the gas pipeline were explaining most of the genetic distances variability, indicating that these features limited dispersal at the local scale (Prunier, Colyn, Legendre and Flamand, 2017). Among the four studied species, we could calculate HGD only for *A. obstetricans* and *A. parallelepipedus*. STRUCTURE was not able to find clusters for the two other species. In *A. obstetricans*, the informations provided by HGD (HGD1 and HGD2) were redundant with the informations from the classical genetic distances. For example, Woodlands and roads were elements affecting gene flow when using the Bray-Curtis dissimilarity index, HGD1 and HGD2. However, the use of HGD, revealed that the railway and the motorway were two linear elements affecting HGD1 (regional) and HGD2 (local), respectively.

Commonality analyses has been used in previous landscape genetic studies (e.g. Gouskov et al., 2016; Prunier, Colyn, Legendre and Flamand, 2017; Renner et al., 2016; Seeholzer and Brumfield, 2018; Prunier et al., 2018) and is a powerful framework to identify synergistic association among predictors and suppressors likely to bias the interpretation of genetic results (Prunier et al., 2015). In our study, the use of commonality analyses was a great tool to end up with a reduced number of predictors with little collinearity among them (Dormann et al., 2013), and thus little distortion in regression outputs (Prunier et al., 2015).

Effects of the road network and the country road D6089 on gene flow

The secondary road network and the country road D6089 were affecting gene flow in three of the four studied species (all except the butterfly). They were mostly acting as barriers to gene flow (Fig. 1.6) and corresponded to the LTIs with the strongest effects on gene flow across species. Together, the secondary road network and the country roads were responsible for about 27 % of the total explained variability in genetic distances across species.

Among vertebrates, amphibians are one of the groups mostly affected by LTIs (Fahrig and Rytwinski, 2009). This statement was confirmed in this study. Across the four studied species, *A. obstetricans* was the most impacted by LTIs, with three of the six studied LTIs impeding gene flow (Table 1.1). The secondary road network and the country road D6089 were the main barriers to dispersal in *A. obstetricans* as they were affecting both, the classical genetic distance (bc) and the second hierarchical level (HGD2). In addition, the secondary road network also impeded gene flow in the first hierarchical level (HGD1). Our results are similar to Garcia-Gonzalez et al. (2012) who found that all roads, including small secondary roads, are obstacles for gene flow in *A. obstetricans* in northern Spain. Roads are creating barriers to gene flow mostly because of road kills (Forman and Alexander, 1998; Hels and Buchwald, 2001; Beebee, 2013), which obviously limit gene exchanges across roads. Amphibians are particularly vulnerable to road kills because of their seasonal migration between breeding water bodies and shelters. In addition, they have slow moving capacities (Trochet et al., 2014) with limiting ability to escape an approaching vehicle. This study is an additional clue revealing that roads have a tremendous negative effect on amphibian dispersal and that mitigation measures are crucial in order to limit road kill (Beebee, 2013).

Roads are also responsible of a tremendous number of killing in snakes (Rosen and Lowe, 1994). Snakes are known to bask on road surfaces to absorb radiant heat; this behaviour increases the likelihood of collisions (Rosen and Lowe, 1994) and results in a reduction of gene flow across roads (Clark et al., 2010). However, our results suggest the exact reverse pattern. We found that the secondary road network present in our study area enhanced gene flow in *N. helvetica*. This conflicting result could be explained by an attractive effect of roads that provides basking surfaces coupled with a low risk of roadkill. Low roadkill probability can be explained by the small width of secondary roads and the weak traffic volume. In addition, this result could be linked to the particular life-history traits of this species. Grass snakes' distribution is strongly dependent on wetlands because of their diet. Secondary roads are often alongside water-filled ditches providing interesting alternative habitats full of amphibian preys (Matos et al., 2012). This could result in a local increase of abundance of grass snakes along roads, favouring road crossings and gene flow. A similar explanation was proposed by Johansson et al. (2005) who found a positive effect of gravel roads (with ditches surrounding them) on genetic distances in the common frog (*Rana arvalis*).

Gene flow in *A. parallelepipedus* was impeded by these two types of LTIs. The country road D6089 and the secondary road network explained the whole variance at the first hierarchical level (HGD1) resulting in clusters A and B (Fig. 1.3). At the second hierarchical level (HGD2) the country road D6089 (but also the gas pipeline) was a barrier to gene flow and explained the separation of cluster B in two sub-clusters (Fig. 1.3). Our results are congruent with Keller et al. (2004) who found that roads are barriers to dispersal in *A. parallelepipedus* but also in other ground-beetle species (e.g. Keller and Largiader, 2003). Roads may act as barrier to gene flow because of road kills but also because ground-beetles may be reluctant to cross roads due to behavioural changes (Holderegger and Di Giulio, 2010).

Effects of the motorway A89 on gene flow

The motorway A89 was affecting gene flow in the two vertebrate species (positively for the toad and negatively for the snake). 5 % of the total explained variability in genetic distances across species was due to the motorway.

Motorways are usually known to impede gene flow in amphibians. For example, Van Buskirk (2012) found that a Switzerland motorway reduced gene flow in the alpine newt (*Ichthyosaura alpestris*) and the frog *Rana temporaria*. Yet, in our study, *A. obstetricans* gene flow was promoted by the motorway at the second hierarchical level (Table 1.1). This counter-intuitive genetic pattern could be explained by the alternative open habitats provided by right-of-ways. For instance, adults and tadpoles of *A. obstetricans* were detected in eight out of the ten retention ponds present along the studied motorway (data not shown). These retention ponds may provide interesting breeding water bodies free of predatory fish and with sand or gravel in close vicinity (ideal substrates to build their burrows). Besides interesting alternative habitats, the motorway is crossed by underneath culverts and tracks which are good dispersal ways for amphibians, especially when their are filled with water (Veenbaas and Brandjes, 1999; Lesbarrères et al., 2004). This is not the first study showing a potential positive effect of a motorway on amphibian gene flow. Prunier et al. (2014) revealed that a 40-years old motorway was not a barrier for the alpine newt (*Ichthyosaura alpestris*) and could even serve as a longitudinal dispersal corridor due to recent landscape changes. Interestingly, they even found negative beta values indicating that gene flow across the motorway was enhanced. But because they analysed the data using one-tailed Mantel test, their method was not designed to reveal such effect (Prunier et al., 2014). Even if 10-years old LTI can affect gene flow (Yu et al., 2017), our results must be interpreted with caution due to the recent age of the motorway ($<$ 15 years old). This genetic pattern could be explained by ancestral landscape configurations before the building of the motorway such as high proportion of wetlands and optimal habitats for this species. Direct approaches such as Mark-Release-Recapture surveys will be necessary to confirm this pattern.

Genetic studies estimating gene flow of reptiles across LTI are lacking (Holderegger and Di Giulio, 2010) (but see Clark et al., 2010). Here, we revealed that the motorway A89 impeded gene flow in *N. helvetica* and accounted for half of the explained variance. Because the motorway is fenced with fine mesh, snakes can only reach the other side by using crossing structures (bridges, underpasses, culverts, roads). These crossing structures may be seldom use by grass snakes due to inadequate placement, architectural design and snakes' behaviour (Woltz et al., 2008). Thermoregulatory behaviour of reptiles is probably the main reason why individuals would not use underpasses (Rodriguez et al., 1996), as a 50 m-length underpass under the motorway would provide inadequate thermal conditions due to the absence of sunlight. In addition, Baxter-Gilbert, Riley, Lesbarrères and Litzgus (2015) evaluated the effectiveness of different mitigation measures implemented to reduce reptile road mortality (including underneath culverts) and found that these structures were seldom used by reptiles.

Effects of the railway on gene flow

The railway was explaining a low proportion (2.5 %) of the total explained variability in genetic distances across species. However, the railway was significantly affecting gene flow of the two vertebrate species (negatively for the toad and positively for the snake).

In the first hierarchical level, *A. obstetricans* gene flow was impeded by the railway (Table 1.1) although cluster A and cluster B were not clearly separated by this LTI (Fig. 1.3), suggesting a modest effect of the railway. Railways are known to restrict gene flow in some amphibian species such as frogs or salamanders (e.g. Reh et al., 1990; Bartoszek and Greenwald, 2009) and many studies on train collision with wildlife reported a high abundance of amphibian killed (Borda-de Agua et al., 2017) representing up to 47 % of all vertebrate records (Heske, 2015). However, the railway in our study area has a low traffic density with approximately 10 trains/day. It seems not plausible that train collisions alone drive the gene flow limitation in *A. obstetricans*. The physical features of the railway are more likely to explain this pattern. Amphibians have a high probability to be trapped between the rails, depending on their agility to overcome the rails and be more vulnerable to railway mortality than other vertebrates (Budzik and Budzik, 2014). The age of the railway may also be an important driver of the detected effect. A recent study on the alpine newt (*Ichthyosaura alpestris*) revealed that a high-speed railway was not a barrier for gene flow in this species (Prunier et al., 2014). However, the authors argue that the railway was too recent (29 years old) to detect any genetic isolation. In our study area, the railway was older than 150 years, which seems a reasonable time length to detect a barrier effect (Cushman and Landguth, 2010; Epps and Keyghobadi, 2015). We revealed that even a low traffic secondary railway may be an important driver of genetic isolation in amphibians.

Finally, we found that the railway promoted dispersal in the snake species. Reptiles are among the vertebrates species with the lowest probability to be impacted by railways (Borda-de Agua et al., 2017). Railways embankments provide important alternative habitats for reptiles with optimal thermal conditions for basking (Graitson, 2006; Stoll, 2013). Even active lines with optimal sunny areas have particularly high richness of reptiles (Graitson, 2006). The absence of human presence along the rails provides a peaceful environment with many shelters (Borda-de Agua et al., 2017). Railways may even contribute to gene flow by creating dispersal corridors (Graitson, 2006). Snakes may avoid collision with trains thanks to their developed perceptions. When trains are approaching, the vibration transmitted through the rails and the ballast can be felt by snakes. This warning message might help snakes to reach a shelter before collision (Borda-de Agua et al., 2017). Similar to the secondary road network, the railway in the study area probably has an attractive effect on snakes and explain the detected gene flow enhancement across the railway.

Effects of the gas pipeline and the power line on gene flow

The gas pipeline was affecting negatively gene flow only in the ground-beetle *A. parallelepipedus*. It accounted for about 3.5 % of the total explained variability in genetic distances across species. A previous study that used telemetry to follow *A. parallelepipedus* found that when individuals entered open fields such as meadows, they shifted their walking behaviour from a random walk to directed displacements (Charrier et al., 1997). This behaviour is indicative of unsuitable area that can lead to a diminution in survival probability (Charrier et al., 1997). Linear rights-of-ways such as the one provided by the gas pipeline easement can be considered as open fields. The barrier effect detected for *A. parallelepipedus* could be because individuals are unwilling to cross open fields such as the one present above the gas pipeline. Interestingly, this pattern was not detected for the power line, potentially because the vegetation cover maintenance differs between the two LTI. The maintenance is greater for the gas pipeline to avoid vegetation interferences with nozzles. The vegetation cover under the electric lines could be dense enough to enable ground-beetle crossings.

The power line was not affecting any of the four studied species. It confirms previous studies showing the limited impact of power lines on wildlife dispersal (Latch et al., 2011; Bartzke et al., 2015; Jahner et al., 2016). Although, some taxa not studied in this work might be negatively affected by power lines such as birds (Loss et al., 2014, 2015).

Non-linear elements affecting gene flow

Infrastructures accounted for about 38 % of the total explained variability in genetic distances across species. The 62 % left were explained by natural (57 %) and anthropised (5 %) features (Fig. 1.4).

The non-linear features influencing gene flow in *A. obstetricans* were isolation by distance (IBD), altitude differences, crops, woodlands and urban areas (Table 1.1). Despite classical knowledge on amphibians (Van Buskirk, 2012), we revealed that woodland is a strong driver and is a major barrier to gene flow because it affected the classical genetic distances (bc), the first and second hierarchical level (HGD1 and HGD2). Several hypotheses can be suggested to explain this observation. Individuals may be reluctant to move through woodlands because of inadequate soil characteristics, higher predation level, mitigation of their calling calls due to dense vegetation or absence of optimal breeding water bodies. We were able to detect IBD in this study area that was not detected for the same species in Spain (Garcia-Gonzalez et al., 2012) probably because they used mitochondrial DNA instead of microsatellites which are less variable at narrow geographical scale (Kohn et al., 2006). Individuals separated by high altitude differences were more genetically distant than individuals sampled at similar altitude level. This result could be linked to a hydrology gradient with individuals sampled in the same water catchment more prone to be close genetically. Crops impeded gene flow at the first hierarchical level (HGD1). A similar result was found for the frog *Rana temporaria* in Germany (Lenhardt et al., 2017). Individuals may be unwilling to cross this landscape feature or be killed while crossing crops because of pesticide exposures (Brühl et al., 2013) or dehydration risk. Finally, urban areas are landscape elements promoting gene flow in *A. obstetricans*. Urban areas are usually considered as inappropriate habitats, limiting gene flow in amphibians (Goldberg and Waits, 2010; Van Buskirk, 2012). Our result could be explained by the habitat requirements of this species. Old farmhouses are ideal habitats because they combine permanent water bodies (watering trough, cattle ponds, wells, *etc.*), open areas and shelters (stone walls, rubble piles, sand piles, tarps, *etc.*). In the rural landscape studied, old farmhouses are the main urbanised features with few small villages. It is likely that in more intensive landscapes with large towns, this genetic pattern would differ.

In our study area, the genetic structure of *N. helvetica* was weak. The software STRUCTURE detected only one cluster (interpreted as a single population) indicating that gene flow through this landscape was important. This result may explain the low proportion of the genetic variance explained by landscape features (4 % of the variance). In a comparable landscape in Switzerland, Meister et al. (2010) also found that grass snakes belong to a single population. In this study, we found that *N. helvetica* gene flow was affecting only by infrastructures (roads, motorway A89 and the railway). In seems that, at the local scale, grass snake dispersal is not affected by intensively used landscape features such as crops or urban areas (Wisler et al., 2008; Meister et al., 2010, 2012b). Isolation by distance explains the genetic variance at the regional level (Meister et al., 2012b) and genetic structuring can probably only be detected at the biogeographical level (Kindler et al., 2013; Pokrant et al., 2016; Kindler et al., 2017, 2018).

Compared to a previous individual-based study that explained less than 5 % of the genetic variance in three sites across France in the butterfly *M. jurtina* (Villemey et al., 2016), we were able to explain about 20 % of the variance when using a population-based method and a restricted spatial scale (maximum neighbouring distance = 5500 m). STRUCTURE was not able to find any genetic structure in the data, probably because of high abundance, low specialisation and great dispersal capacity in this butterfly (Villemey et al., 2016). Interestingly, we were able to detect an isolation-by-distance effect. This IBD effect was not detected in Villemey et al. (2016) with pairwise distances up to 60 km apart. We found that woodlands were impeding gene flow in *M. jurtina*, a result similar to Villemey et al. (2016). The absence of sunlight and the dense vegetation may limit the movements through woodlands. None of the six LTI types was influencing gene flow in this species despite evidence of previous studies showing that roads (Polic et al., 2014) and motorways (Remon et al. accepted) can hinder crossing events of butterfly. Remon et al. (accepted) were using direct Mark-Release-Recapture surveys in the same landscape on *M. jurtina* and found that crossing events through the motorway were fivefold reduced compare to adjacent habitats. In this study, we used indirect method based on genetic, which is subject to population sizes bias (Prunier, Dubut, Chikhi and Blanchet, 2017) and time lag bias due to the recent construction of the motorway (Anderson et al., 2010). However, even with very wide infrastructures such as motorways, some butterflies are able to reach the other side (Remon et al. accepted) and may sustain gene flow at landscape level (Munguira and Thomas, 1992). This confirms that genetic tools should not be used alone (Safner et al., 2011). A combination of Mark-Release-Recapture studies coupled with landscape genetic can inform precisely how animals move through landscapes.

Unlike Marcus et al. (2015), we found a strong genetic structure in the ground-beetle *A. parallelepipedus* within the studied landscape. The explained proportion of the classical Fst genetic distance was due to grasslands acting as barrier to gene flow. This result is linked to previous studies showing that this species intentionally avoids open fields such as grasslands (Charrier et al., 1997; Petit and Burel, 1998). This encourages the maintenance of hedges in agricultural environments to favour landscape connectivity among woodland patches (Charrier et al., 1997; Petit and Burel, 1998; Fournier and Loreau, 1999). Altitude affected gene flow at the second hierarchical level (HGD2), but its effect was modest (Table 1.1). In any case, the fragmentation of woodlands due to land conversion, roads or other kind of LTIs could lead to strong isolation of ground-beetles populations. Population abundance are high in this species (Loreau and Nolf, 1993; Keller et al., 2004) but its dispersal capacity is very limited (Charrier et al., 1997; Brouwers and Newton, 2009). Therefore, populations which are not linked by dispersal may suffer from geographical isolation (Fahrig and Rytwinski, 2009; Beyer et al., 2016).

Conclusion

In fragmented landscape such as the one we studied, the accumulation of many LTIs is likely to isolate more strongly populations than single LTI. For instance, we found that the combination of roads and the railway reduced *A. obstetricans* dispersal. Similarly, roads and the gas pipeline constrained dispersal of the ground-beetle *A. parallelepipedus*. This cumulation effect might represent complete barriers for wildlife (Bélisle and St. Clair, 2001; Connelly, 2011). We highlight the fact that species-specific mitigation measures are required (Glista et al., 2009). Future LTIs building or reclassification of old LTIs need to diversify crossing structure types which would benefit to the widest range of species. According to our expectations, roads were the most detrimental studied LTIs in this study which confirms current knowledge on their negative impacts on a wide range of species (Holderegger and Di Giulio, 2010). The construction of passages under rural roads (Woltz et al., 2008) or traffic calming (Jaarsma and Willems, 2002) could contribute to road kill limitation and favour landscape connectivity.

In addition, our results reveal the high variability of infrastructure effects depending on the species under study. Therefore in future landscape studies, we encourage multi-specific approaches. Building large data bases which link infrastructure effects and life history traits of species (such as dispersal ability) would help to implement appropriate conservation planning in fragmented landscapes.

Authors' contributions

JR, JGP, MB and SM contributed to the conception and design of the study. JR, SM and JHC collected the data. LG digitalised the soil occupancy of the study area. JR and MR performed laboratory analyses and genotyping. JR and JGP performed data analyses. JR wrote the manuscript. All authors participated in critical revisions of the manuscript.

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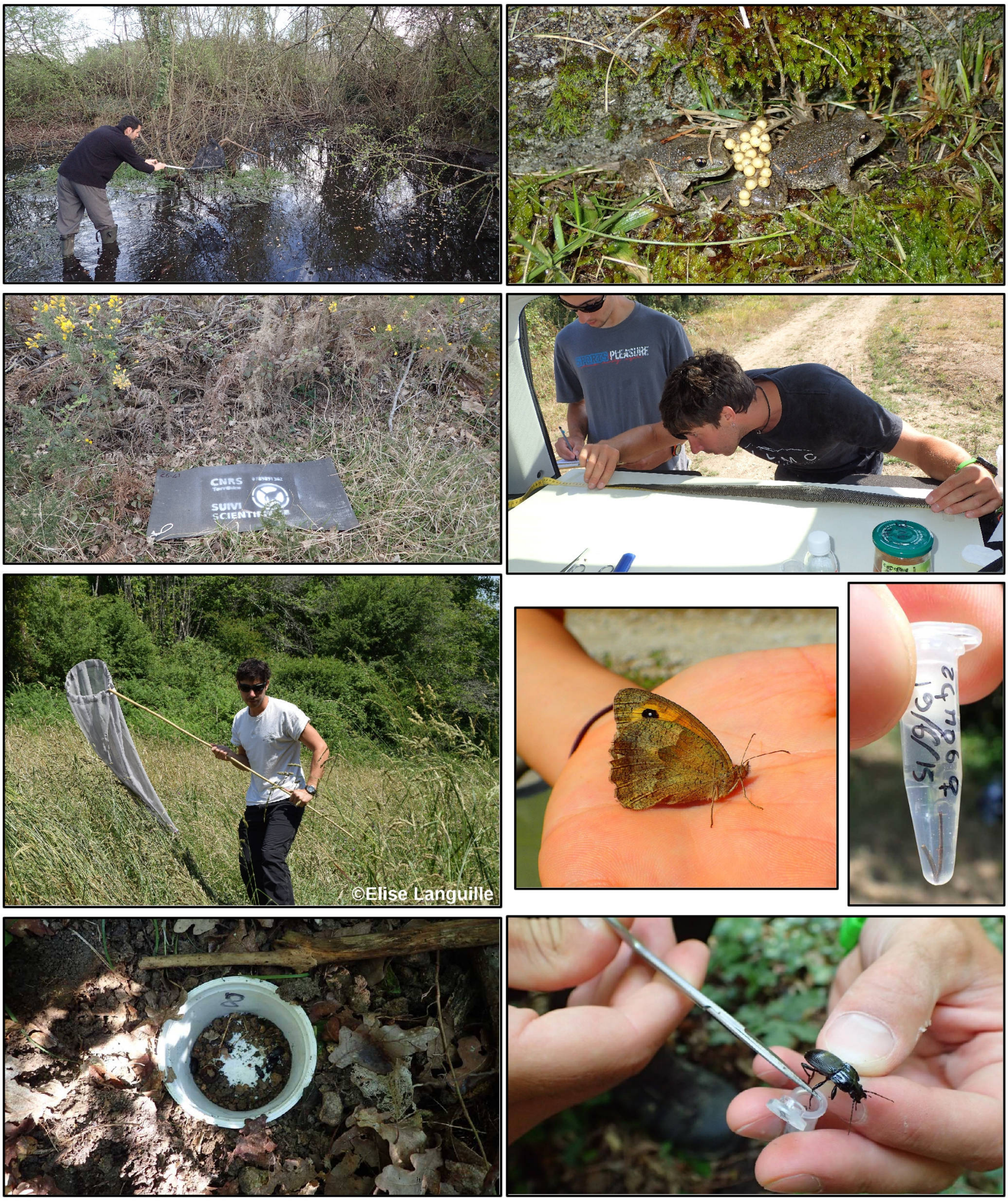

Planche 1. De gauche à droite et de haut en bas : repérage de la présence d'Alyte accoucheur en identifiant les larves d'amphibiens dans une mare ; couple d'Alytes accoucheurs juste après accouplement ; plaque à serpent pour faciliter la capture de couleuvre ; mesure SVL d'une Couleuvre helvétique ; capture de Myrtil au filet à papillon ; Myrtil femelle ; patte de Myrtil préservée dans l'éthanol ; pitfall installé dans un bois ; prélèvement d'une patte de Féronie noire.

Chapitre 2

Estimating the permeability of linear infrastructures using recapture data

Avant-propos

Les effets barrières des Infrastructures Linéaires de Transport (ILT) participent fortement à la fragmentation des habitats en limitant la connectivité des espèces dans les paysages. Pour estimer ces effets barrières, des méthodes permettant de mesurer la connectivité sont nécessaires. La génétique du paysage est un outil pertinent pour répondre à ce type de problématique mais inadapté dans certains cas de figure. Alternativement, les méthodes basées sur les suivis directs comme par Capture-Marquage-Recapture permettent d'étudier le mouvement des individus à travers les ILT. Cependant des méthodes fiables et robustes visant à estimer les effets barrières des ILT restent à développer.

Dans ce chapitre, nous présentons une méthode permettant de calculer, pour une espèce donnée, la probabilité attendue de franchissement d'une infrastructure linéaire en l'absence d'effet barrière de cette dernière. Par la suite, cette probabilité attendue est comparée au nombre de franchissements réels observés par des suivis de CMR, afin d'estimer l'effet barrière de l'infrastructure. Nous avons testé la fiabilité de cette méthode à l'aide de simulations puis nous l'avons appliquée au Myrtil à travers deux types d'ILT.

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Estimating the permeability of linear infrastructures using recapture data

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Abstract

Barrier effects of Large-scale Transportation Infrastructures (LTIs) are among the main factors contributing to the fragmentation of habitats. The reduction of dispersal across LTIs can drive small, local populations to extinction. To understand how LTIs modify dispersal, efficient and workable evaluation methods are required.

We developed a method based on Mark-Release-Recapture surveys to estimate barrier effects of LTIs that could be easily applied in various landscape contexts and on any mobile species.

Our method uses dispersal kernels of animal movements to calculate an expected probability of crossing any particular linear feature. This probability is then compared to observed crossing events to estimate the barrier effect. We used simulations to test the reliability of our method and applied this framework on the butterfly *Maniola jurtina* in a landscape fragmented by a motorway and a railway.

Simulations showed that our method was able to detect efficiently even weak barrier effects given that enough data are available. When sample size was reduced, our method was able to detect barrier effects only when the infrastructure width was small in comparison to the average movement capacity of organisms. In our case study, both infrastructures acted as significant barriers.

The power of our method is to use MRR data which are more representative of population processes than telemetry monitoring and are not limited by time-lag involved

in genetic studies. This framework is of particular interest for conservation studies in order to assess how individual movements are modified by linear infrastructures.

Key-words: barrier effects; butterfly; habitat fragmentation; crossing probability; Mark-Release-Recapture; dispersal kernels

2.1 Introduction

Large-scale Transportation Infrastructures (LTIs) are any kind of linear infrastructures allowing the transportation of goods, vehicles or energy. They are expending considerably, creating dense transportation networks in growing anthropogenic landscapes (Dulac, 2013; Laurance et al., 2014). Despite their high impacts on natural ecosystems and their contribution to habitat fragmentation (Forman and Alexander, 1998; Trombulak and Frissell, 2000; Balkenhol and Waits, 2009), methods are lacking to properly evaluate their barrier effects in landscapes.

Large-scale Transportation Infrastructures affect mobile organisms by direct vehicular collisions (Trombulak and Frissell, 2000). They also induce behavioural modifications of organisms, leading to infrastructure avoidance (Ascensao et al., 2016). Individuals may avoid LTIs because of traffic noise, modification of their natural habitat, perturbation of their reproductive success and perturbation of their physiological state (Trombulak and Frissell, 2000). All these perturbations may lead to barrier effects that limit dispersal (the movement of individuals that sustains gene flow within landscapes (Ronce, 2007)). Populations which are not linked by dispersal may suffer from geographical isolation (Fahrig and Rytwinski, 2009; Beyer et al., 2016). Isolated and small populations exhibit higher rates of inbreeding and genetic drift. It results in the decrease in heterozygosity and increases the risk of population extinction (McCauley, 1991; Fagan and Holmes, 2006).

In practice, LTIs effects are not always negative and are context dependent. The most common LTIs are roads, motorways, railways, power lines, pipelines and canals. Roads (including motorways) are the most studied infrastructures and are considered as strong barriers for a large range of animal species. Roads tend to have more negative than neutral or positive effects (Fahrig and Rytwinski, 2009). Railways can be barriers for certain species (Whittington et al., 2004; Bartoszek and Greenwald, 2009; Breyne et al., 2014), be neutral to movement (Vandevelde et al., 2012), increase species richness and abundance near infrastructures (Li et al., 2010) or create corridors (Penone et al., 2012).

Power lines sometimes lead to avoidance behaviour (e.g. prairie grouse; Pruett et al., 2009), but few studies revealed effects of these infrastructures on animal movements (Latch et al., 2011; Bartzke et al., 2015; Jahner et al., 2016). Power lines are even attractive to some birds by providing perches for hunting activities (Morelli et al., 2014). The other types of LTIs (gas pipelines, canals, *etc.*) have been less studied and require more investigations (but see Dyer et al., 2002; Coulon et al., 2006; Breyne et al., 2014; Kaya Özdemirel et al., 2016).

For a given species, a particular type of infrastructure may act as a strong barrier to movements while an other type might not. For example, in Norway, moose avoid crossing roads but power lines do not impede their movements (Bartzke et al., 2015). Similarly, gene flow of desert tortoises is affected by roads but not by power lines (Latch et al., 2011). Even with the same infrastructure type, effects can be landscape-specific. For example, Van Buskirk (2012) found that a motorway reduces gene flow in the alpine newt in Switzerland but Prunier et al. (2014) found that a similar motorway did not affect gene flow in the same species in France.

Therefore, when trying to understand how a species travels through the landscape, it is crucial to determine the effects of the different infrastructure types present (Balkenhol and Waits, 2009). Those evaluations are particularly requested by local authorities to design mitigation measures (EEA, 2015).

In the past fifteen years, one of the most powerful tool to estimate landscape connectivity has been landscape genetics (Manel and Holderegger, 2013). Genetic studies have been widely used in order to estimate the effects of LTIs (Holderegger and Di Giulio, 2010). However, one major limit is the time-lag before detection of a barrier effect (Epps and Keyghobadi, 2015). Recent infrastructures may not have been in place for long enough to allow detecting effects on genetic metrics (e.g. Prunier et al., 2014). Furthermore, genetic methods can be expensive and deterrent for small local studies. Direct monitoring using telemetry or Mark-Release-Recapture (MRR) data provides an interesting alternative to follow individual movements within a landscape. Telemetry framework have been previously developed to assess barrier effects of infrastructures (e.g. Shepard et al., 2008; Colchero et al., 2011; Beyer et al., 2016). However, telemetry data might be tricky to obtain for small organisms, they require costly equipment and generally concern a small fraction of the population. Alternatively, MRR data are cost effective, a large portion of the populations can be monitored and they can be applied to small species for which other monitoring techniques are inappropriate (e.g. small butterflies). MRR data are used to estimate population sizes and demographic parameters of populations (Lebreton et al., 1992) but provide additional information about individuals' mobility. They are an easy way to obtain dispersal kernels (the shape of the distribution of dispersal distances (Baguette et al., 2013)). Dispersal kernels can be used in modelling frameworks in order to predict the movement of individuals across specific barriers. The comparison between the predicted number of individuals crossing the barrier and direct crossing observations can be achieved using MRR data. So far, such modelling frameworks have been used only in one dimension environments (rivers) to estimate barrier effects of infrastructures (Pépino et al., 2012, 2016). Specifically, Pépino et al. (2012) used dispersal kernels and observation data to estimate the permeability of motorway-crossing structures for fishes. However, stream environments only host a portion of the global biodiversity and similar methods are lacking to study terrestrial organisms.

We aimed at developing a modelling framework where the dispersal kernels of organisms can be used to assess barrier effects in two-dimension landscapes. This would allow the application of this framework to a wide number of species in various landscape configurations.

A majority of studies estimating barrier effects of LTIs focus on large animals. Insects are dramatically under-represented (Fahrig and Rytwinski, 2009) despite their huge mortality due to collision with vehicles (Baxter-Gilbert, Riley, Neufeld, Litzgus and Lesbarrères, 2015; Skórka et al., 2015) and their drastic decline in Europe (Hallmann et al., 2017). Insects also make it easy to collect large data sets that are useful to investigate new methods such as the one we developed here. Therefore, as an example of the method deployment, we applied our framework to study a butterfly species within a landscape crossed by a motorway and a railway. We predicted that the motorway would limit, at least to some extent, crossing events of butterflies due to vehicular collisions (Baxter-Gilbert, Riley, Neufeld, Litzgus and Lesbarrères, 2015) but that the railway would be neutral to movements (Vandevelde et al., 2012).

2.2 Material and methods

Method framework

The first step of the method consists in measuring the distribution of dispersal distances (dispersal kernel) of the species under study. The dispersal kernel is a dispersal index calculated as the inverse cumulative proportion of individuals moving certain distances. Dispersal kernels are obtained by fitting mathematical curves to the empirical data. They are commonly used to compare dispersal abilities of species (e.g. Stevens et al., 2010). In our framework, the dispersal kernel is a proxy to estimate movement capacity of individuals. Movement distances are obtained using Mark-Release-Recapture surveys. Because kernels might vary due to landscape settings (e.g. Baguette and Van Dyck, 2007), their shapes might be biased by infrastructures. Therefore, dispersal kernels should be estimated on a control site with no LTI (or LTIs known as neutral) but with similar habitat configuration and similar time frame to the site under study. In addition, in order to cover the entire range of distances travelled by the model species, the study site must be large enough to detect long distance dispersal events.

The second step of our method consists in obtaining data of individuals crossing or not crossing a LTI using Mark-Release-Recapture surveys on the study site. Ideally, the LTI is located in the middle of the study site and individuals monitored all around. Capture sessions must be close enough in time to obtain a relatively high number of recapture distances. During the surveys, each side of the LTI should be equally sampled for marked individuals that either crossed the LTI or stayed on the same side.

The third step consists in fitting the dispersal kernel (obtained at the first step on a control site) to a theoretical distribution and to estimate the expected crossing probability across the LTI on the study site. Dispersal kernels are usually fitted to a large range of theoretical distributions, including log-normal (Skarpaas et al., 2005), leptokurtic (Pépino et al., 2012), negative exponential and inverse power distributions (Hill et al., 1996), among others. Once the best theoretical distribution is fitted to the data, the parameters derived from the theoretical distribution are used to calculate the expected crossing probability P_{cross} (probability for an individual to reach the other side of the LTI) as well as the expected non-crossing probability P_{stay}. P_{cross} and P_{stay} are calculated for each recaptured individual under the hypothesis that the LTI is completely permeable to individual movements (neutral model). Expected probabilities are based only on recaptured individuals as these values are later compared to crossing observations which are available only for recaptured individuals. Expected probabilities are computed as a function of the orthogonal distance between an individual capture location and the infrastructure (insuring that this individual was later recaptured). The longer the distance to the LTI, the lower the probability that the individual may cross the infrastructure. Figure 2.1 provides a three-dimensional representation of the conceptual framework used to calculate expected probabilities of crossing a LTI. The probability $P(x)$ for an individual captured at location C to be recaptured at a distance x is integrated on the geometry of the field site. A recaptured individual can be recaptured either in area $A3$ with a certain probability (P_{cross}), or in $A1$ with the probability P_{stay}. $A2$ is the area corresponding to the probability to be on the LTI (P_{LTI}) and is usually inaccessible during MRR surveys (e.g. fenced motorways and railways).

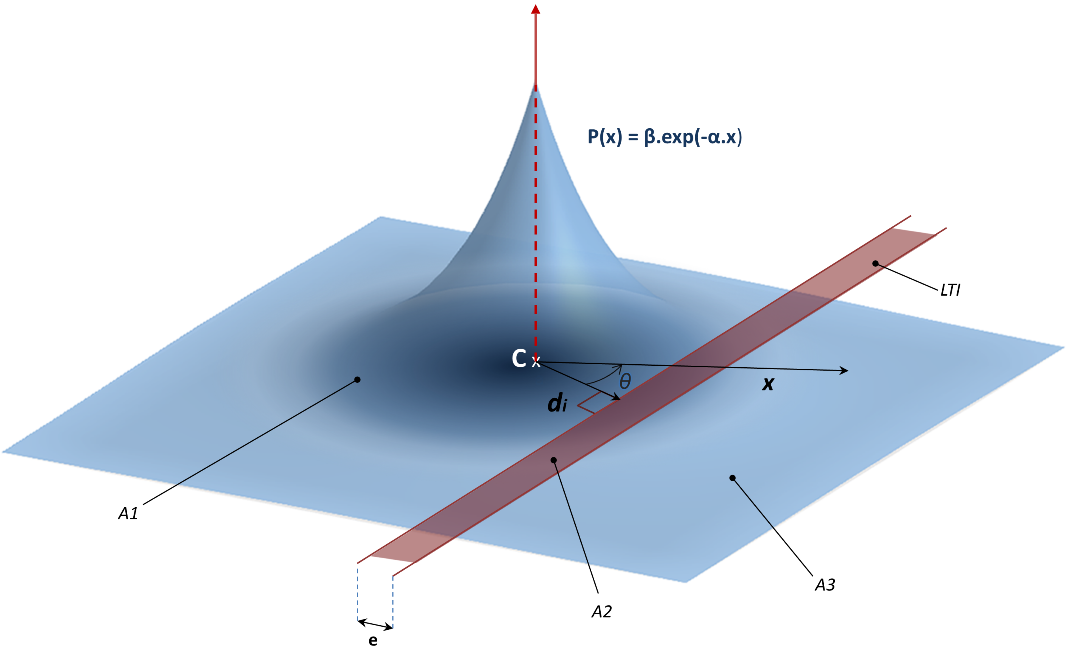

Figure 2.1 – Three-dimensional representation of the conceptual framework used to calculate expected probabilities of crossing a Large-scale Transportation Infrastructure (LTI)(see text). Empirical data on movement are used to fit the negative exponential function $P(x) = \beta e^{-\alpha x}$ (dispersal kernel). The longer the distance between the capture location (C) and the infrastructure (d_i) and the width of the infrastructure (e), the lower the probability that the individual may cross the infrastructure. The distance x and the angle θ are used to estimate the area A1 (staying) and A3 (crossing).

The last step consists in investigating the barrier effect of the LTI on individual movements. To do so, P_{cross} is compared with empirical data obtained in step 2. Empirical data provide the proportion of individuals that either successfully crossed the LTI or stayed on the same side. The probability of crossing (success) or staying (fail) follows a Bernoulli trial with a number of trials corresponding to the number of individuals recaptured on the study site. The observed ratio between the number of successes and the number of trials is compared to the average expected probability of crossing (P_{cross})

using an exact binomial test. In addition, OddsRatios are used to compute the magnitude and the precision of effect sizes, comparable among studies and organisms.

Simulations

In order to test the reliability of the method, we designed a simulation study using personal R-scripts. We simulated a study site with a linear infrastructure of 1000 m in length. As in real study design, we adapted the sampling area to the movement abilities of the studied species: on each side of the infrastructure, the width of the studied area was set as 95% of the dispersal kernel maximum distance. We simulated two specific cases with 100 or 500 points randomly distributed on the study site, respectively. These points represented the capture locations of individuals that we defined as being recaptured in our framework. We choose 100 points as it corresponded to the number of recapture events available in our empirical case and 500 to represent a scenario with a larger data set. In both cases, each individual was then assigned a random direction and a random movement distance sampled from a Negative Exponential Function (NEF: $P(x) = \beta e^{-\alpha x}$) kernel distribution, obtained from an inverse transform sampling method (Devroye, 1986). We used NEF as it fits the distribution kernels of a wide range of organisms (e.g. Palomares et al., 2001; Byrne et al., 2014) and has been widely used for butterflies (Hill et al., 1996; Fric and Konvicka, 2007). In NEF, α is a synthetic descriptor of the kernel and $1/\alpha$ corresponds to the average distance travelled by the butterfly (Stevens et al., 2010).

We recorded the final destination coordinates of each individual. If the final destination of an individual was located outside the study site or on the infrastructure, this sample was discarded from the data set. In such cases, additional simulations were performed to insure to the targeted number of data was obtained (100 or 500 individuals). We recorded whether an individual stayed or crossed the structure and applied our method to calculate the average expected probability of crossing among all individuals.

We generated three scenarios depending on the barrier intensity of the infrastructure; strong barrier effect, weak barrier effect or no effect. The strong barrier effect was generated by applying a crossing cost equal to four times the average movement capacity ($4 \times 1/\alpha$). For example, with an average kernel movement ($1/\alpha$) of 20 m, the final movement distance of an individual that was initially supposed to move over 100 m and to cross the infrastructure was reduced of 80 m. Thus, the final movement distance shrinks to 20 m, possibly preventing that individual from actually crossing the infrastructure. The weak barrier was defined with a cost of ($1 \times 1/\alpha$) and the neutral model with no cost.

We generated 5000 simulations per scenario. For each simulation, we randomly generated (i) the average movement distance $1/\alpha$, (ii) the corresponding kernel distribution and the subsequent width of the study area on each side of the barrier (95% of the kernel distribution maximum distance), (iii) the 100 or 500 capture locations of individuals, respectively and (iv) the width of the infrastructure. Alpha was picked from a uniform distribution ranging from 0.002 (average movement distance of 500 m) to 0.1 (average movement distance of 10 m). Infrastructure width was picked from a uniform distribution ranging from 5 to 50 m, so that the ratio between the infrastructure width (W) and the average movement distance $1/\alpha$ (D) was lower than 1.5 (W/D ratio).

For each simulation, we compared the average expected probability of crossing and the actual number of crossing events to compute the magnitude (effect size) and the precision (95% confidence interval) of the barrier effect. Here, effect sizes were computed in the form of logOddsRatios, following Borenstein et al. (2009) (equations 5.8 and 5.9).

Odd-ratios were computed as the ratio of observed to theoretical odds of crossing events. With N the total number of recaptured individuals, obs the number of observed crossing events and P_{cross} the average expected probability of crossing, observed odd was computed as the ratio of observed crossing events (obs) to observed non-crossing events ($N - obs$), whereas theoretical odd was computed as the ratio of theoretical crossing events ($N \times P_{cross}$) to theoretical non-crossing events ($N - N \times P_{cross}$). Hence:

$$OR = \frac{obs}{N - obs} \times \frac{N - N \times P_{cross}}{N \times P_{cross}} \tag{1}$$

And

$$logOR = \ln(OR) \tag{2}$$

The approximate variance V and 95% confidence interval CI of logOddsRatio were then respectively computed as follows (Borenstein et al., 2009) (equations 5.10 and 5.11):

$$V = \frac{1}{obs} + \frac{1}{N - obs} + \frac{1}{N \times P_{cross}} + \frac{1}{N - N \times P_{cross}} \tag{3}$$

And

$$CI = logOR \pm 1.96 \times \sqrt{V} \tag{4}$$

LogOddsRatios range from $-\infty$ to $+\infty$. A null logOddsRatio indicates that the observed odd of crossing is equal to the theoretical one. A barrier effect would thus be detected when the upper bound of the 95% CI is strictly negative, indicating that observed crossing events are way scarcer than expected.

Application of the method to the butterfly *Maniola jurtina*

Study site and biological model

The study area was located in the 'Périgord' region in South-Western France, between Brive-La-Gaillarde and Périgueux (45°07'31.8"N; 0°58'56.9"E; Fig. 2.2). The studied LTIs crossed a rural landscape composed of limestone plateaux with low human density. The landscape included crops, mowed meadows, deciduous forests and small villages. We monitored two sites: a control site and a study site (Fig. 2.2). The control site (9.7 ha) was used to estimate the dispersal kernel of the studied organism. The study site (11.9 ha) was crossed by a motorway (50.6 m wide) and a low traffic single-track railway located within a trench (8.2 m wide and 4 m deep). The shapes of the control and the study sites were constrained by inadequate landscape features surrounding meadows and forest edges where sampling took place. Inadequate landscape features were mostly non-habitat annual crops impracticable for experimenters (Delattre et al., 2010), in addition to hosting low *M. jurtina* densities (Ouin et al., 2008). The two sites were separated by approximately 6.7 km (Fig. 2.2) and comprised similar landscape elements. On the control site, a power line and a gas pipeline crossed the area but they were considered as having no effects on butterflies' movements (buried gas nozzles and aerial electric lines; see Appendix D for a detailed rationale behind this statement).

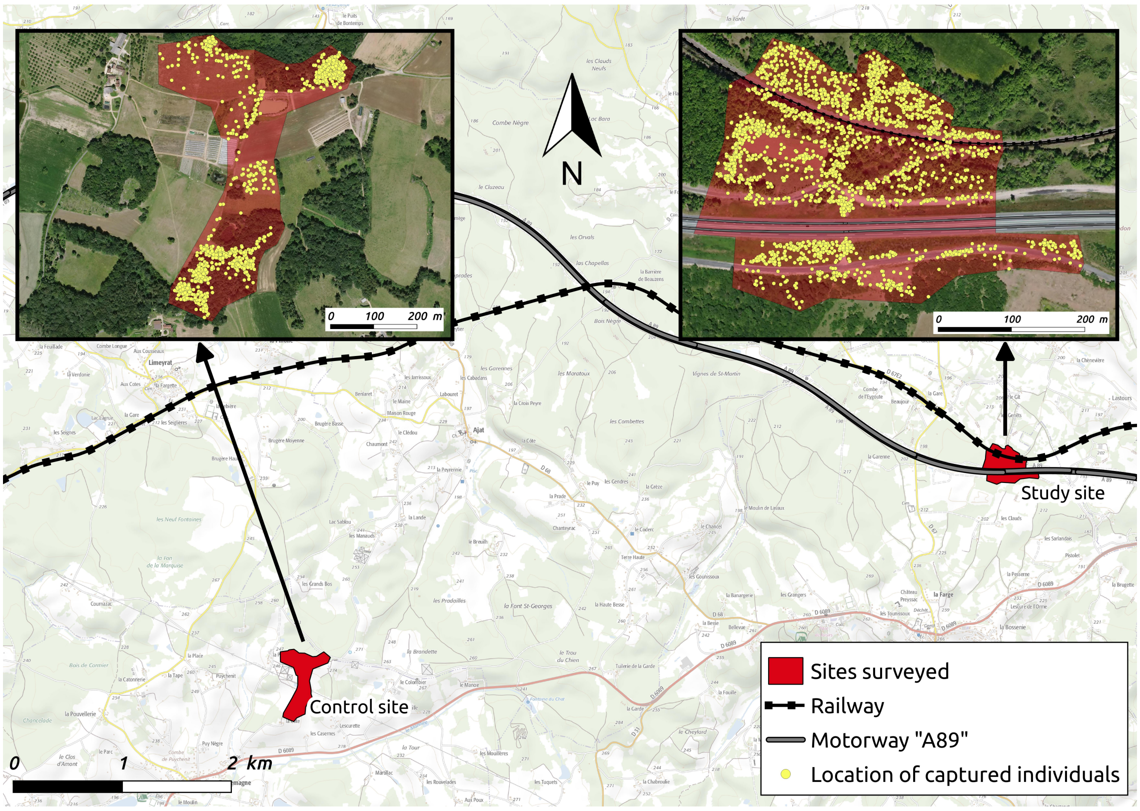

Figure 2.2 – Study area in the 'Périgord' region in the South-West of France. The control site was surveyed in 2015 and the study site in 2016. On the study site, two infrastructures were studied for their barrier effects: a railway and a motorway.

We chose to test the method on a mobile and generalist species with large demographic densities. These conditions were fulfilled by the meadow brown, *Maniola jurtina*, a common and widespread butterfly species in Europe. The ideal habitat for this species consists in open grasslands with medium to high vegetation cover. Based on MRR data, a median residence time of adults of 6.55 days was reported in Bubová et al. (2016) but under specific conditions, residence time can reach much higher values (Grill et al., 2013; Haeler et al., 2014). Flight period lasts in average 67 days (Bubová et al., 2016) but vary considerably between mid-May to October depending on geographic location, altitude and climate (Grill et al., 2013). Caterpillars feed on a wide range of grass species with some preferences for *Poa spp.*, *Agrostis spp.* and *Lolium spp.* (Brakefield, 1982; Thomas and Lewington, 1991).

Data collection

The mobility of *M. jurtina* was investigated with MRR surveys in summer 2015 on the control site (from 13 July to 26 August) and in summer 2016 on the study site (from

04 July to 16 August). Each site was surveyed for a time length of 44 and 43 days, respectively. We applied a similar sampling scheme on both sites: we randomly walked through each entire site during day time (9am to 6pm) and captured the maximum number of *M. jurtina* individuals following a robust sampling design (Pollock, 1982). Sites were surveyed for three consecutive days (secondary sampling events) every two weeks (primary sampling events). This protocol is similar to a previous MRR study performed on the same species in Switzerland (Lörtscher et al., 1997). The protocol was standardised and performed in the same way on both sites to insure that dispersal kernel obtained on the control site could be applied to the movements of butterflies on the study site. The variation of dispersal kernels in time is plausible (Schtickzelle et al., 2012) but because weather conditions, landscape settings and sex-ratio were similar on both sites (see results), there was no indication that movements of butterflies in 2015 should differ from 2016.

Butterflies were captured with nets, sexed and individually marked with fine-tipped permanent ink pen on the underside of the left hind-wing. Date of (re)capture and GPS locations were recorded (Garmin Etrex20, USA). See Fig. 2.2 for the sampling effort on each site. Care was taken to minimise butterflies handling and wing injuries. On the study site, we sampled equally each side of the two infrastructures for new individuals and recaptured individuals. To compare weather conditions between the two sites, we retrieved climatic data (temperatures and wind speed) for the periods July-August 2015 and 2016 from the nearest weather station at Gourdon (ca. 52 km from the study site, Météo-France).

Data analysis

When butterflies were recaptured, we measured both the euclidean distance and the direction of the observed trajectories from capture to recapture locations. To determine whether the average direction of observed trajectories were random or showed a direction trend, we performed Rayleigh tests at the site level (pooling all recapture events from a given site). On the study site, we also determined the shortest orthogonal distances between capture location and both LTIs. Recapture events were classified either as 0 when butterflies remained on the same side of the LTI or as 1 when they crossed the LTI. Individuals recaptured within the same day were excluded from analyses to avoid any bias due to butterflies' altered behaviours short after capture events.

The recapture events on the control site were used to generate the dispersal kernel of

M. jurtina. The dispersal kernel was fitted using a negative exponential function (NEF : $P(x) = \beta e^{-\alpha x}$) and an inverse power function (IPF: $P(x) = \alpha x^{\beta}$), the two most commonly used theoretical distributions for butterflies' dispersal kernels (Hill et al., 1996). In both distributions, the probability to travel a certain distance $P(x)$ depends on the distance x and the constants β and α. Preliminary results showed that NEF gave a better fit than IPF ($R^2 = 0.84$ (IPF) and 0.91 (NEF)). Therefore, we used NEF to model *M. jurtina* dispersal kernel. The value of α was used to calculate P_{cross}. As illustrated in Fig. 2.1, P_{cross} corresponded to the probability of recapturing an individual captured at C in the $A3$ area (volume occupied by the dispersal kernel behind the LTI and covering $A3$). Hence:

$$P_{cross} = \gamma \int_{\frac{-\Pi}{2}}^{\frac{\Pi}{2}} \int_{d_i+e}^{\infty} P(x)dx.d\theta \tag{5}$$

With d_i the shortest orthogonal distance between the initial capture location (C) and the LTI, θ the angle between d_i and the intersection between the radius and the LTI, and e the LTI's width (Fig. 2.1). P_{cross} is bounded between 0 and 1 while NEF is defined on R^*. Thus, γ corresponds to the adjustment parameter insuring that probability ranges from 0 to 1. γ was estimated by considering the specific case where $d_i + e = 0$, then P_{cross} $= 0.5$ leading to $\gamma = \frac{\alpha}{2\beta\Pi}$.

Consequently:

$$P_{cross} = \frac{1}{2\Pi} \int_{\frac{-\Pi}{2}}^{\frac{\Pi}{2}} e^{-\alpha \frac{d_i+e}{cos\theta}} d\theta \tag{5'}$$

In situations where the area $A2$ cannot be sampled (individuals on the infrastructure), the probability of crossing (P_{cross}) is corrected (CP_{cross}) with the inaccessibility of the LTI. Therefore, we estimated (P_{LTI}), the probability that an individual is located on the infrastructure area:

$$P_{LTI} = 1 - (P_{cross} + P_{stay}) \tag{6}$$

Where P_{stay} corresponds to the probability of recapturing an individual captured at C in the $A1$ area (volume occupied by the dispersal kernel before the LTI and covering $A1$). It can be estimated as follow:

$$P_{stay} = 1 - \gamma \int_{\frac{-\Pi}{2}}^{\frac{\Pi}{2}} \int_{d_i}^{\infty} P(x)dx.d\theta \tag{7}$$

Leading to:

$$P_{stay} = 1 - \frac{1}{2\Pi}\int_{\frac{-\Pi}{2}}^{\frac{\Pi}{2}} e^{-\alpha \frac{d_i}{cos\theta}} d\theta \tag{7'}$$

Finally, the corrected probability of crossing is calculated as follow:

$$CP_{cross} = \frac{P_{cross}}{1 - P_{LTI}} \tag{8}$$

Comparison between CP_{cross} and empirical data were made using binomial tests and effect sizes were computed using logOddsRatios. We provided a R-script with the function that we developed (NEFbarrDetect) which enables the calculation of these probabilities and the barrier effect statistics and effect sizes based on a data fame of recapture events (Supplementary file). All analyses including simulations were performed in R 3.2.3 (R Core Team, 2015) and QGIS (V. 2.8). Results were given with standard errors unless specified.

2.3 Results

Simulations

The ability of our method to detect barrier effects depended on the W/D ratio. Small W/D ratios reflect a narrow infrastructure width in comparison to the average movement capacity of the studied organism. A W/D ratio of 1 corresponds to an infrastructure width equal to the averaged distance moved by the studied organism.

When the infrastructure was permeable to movements, our method did not detect any artefactual barrier effect in the N = 100 or N = 500 scenario whatever the W/D ratio (less than 5% of detection errors, Fig. 2.3). For N = 100, simulated data revealed that our method was able to detect barrier effects when W/D ratios were small (Fig. 2.3). Based on the 95% confidence intervals, we found that when the infrastructure had a strong barrier effect, we were able to detect the effect only for W/D ratios smaller than 0.2. With a 50 m-wide LTI, this means that we can always detect the effect if the average distance moved by the studied organism is larger than 250 m. The barrier effect could be detected up to W/D ratios of 0.5, but in such cases, the proportion of detection failures was high (Fig. 2.3). For weak barriers, our method lacked power to detect the barrier effect for the N = 100 scenario.

Our method was much more powerful when the sample size increased (N = 500 scenario). In the strong barrier case, our method was able to detect efficiently the barrier effect whatever the W/D ratio. In the weak barrier case, our method was still powerful enough to detect the barrier for W/D ratios lower than 0.5. With a barrier of 50 m, this corresponded to an average distance moved by the studied organism larger than 100 m.

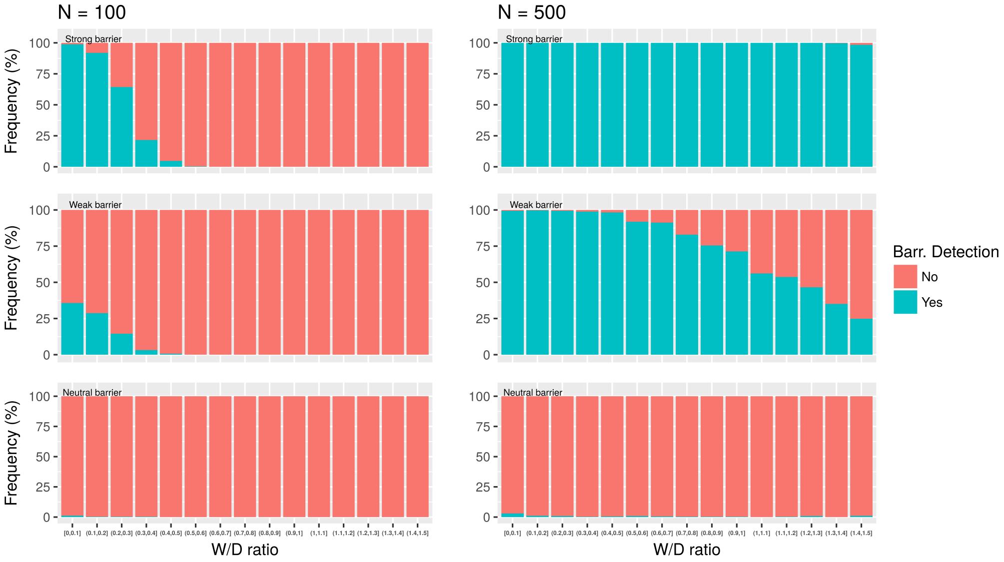

Figure 2.3 – Method application on 5000 simulated data per scenario type. We simulated two specific study cases with either 100 or 500 recaptured individuals. For each case, three scenarios were simulated: a strong barrier, a weak barrier and a neutral barrier. Various barrier sizes (from 5 to 50 m) and various movement capacities (mean distance capacity from 10 to 500 m) were also simulated. These two components were synthesised into a single ratio (W/D ratio = barrier width divided by average distance capacity). A W/D ratio of 1 corresponds to a barrier width equal to the average distance capacity of the organism. Barrplots represent the frequency of simulations that either detect a barrier effect or not according to logOddsRatios 95% CI.

Survey on the butterfly *Maniola jurtina*

A total of 2182 *Maniola jurtina* butterflies were captured and marked, 1035 on the control site of which 92 were recaptured at least once (8.9%), and 1147 on the study site of which 77 were recaptured at least once (6.7%).

The temperatures and wind speed between the sampling periods in 2015 and 2016 were similar (Temperatures: 2015 = 26.0 ± 0.3°C; 2016 = 25.5 ± 0.3°C; t(487) = 1.02; $p = 0.31$; Wind speed: 2015 = $2.43 \pm 0.07 m.s^{-1}$; 2016 = $2.30 \pm 0.05 m.s^{-1}$; t(470) = 1.47; $p = 0.14$).

The largest measured distance between two capture sessions was 504 m within a 14 days interval but a 409 m distance was recorded in a single day interval (control site) showing that some individuals were able to cover large distances rapidly. Butterflies were recaptured on average after 4.12 ± 0.45 days on the control site and 4.47 ± 0.89 days on the study site. Longest recapture intervals were 29 days and 42 days on control and study site, respectively, and both individuals were females.

We recaptured more females than males on both the control and the study sites (Control site: 58 females as against 34 males, $\chi^2(1) = 6.26$, $p = 0.012$; Study site: 51 females as against 26 males, $\chi^2(1) = 8.12$, $p = 0.0044$). On both sites, the movement of butterflies did not deviate from a uniform (random) directionality (Control site: Rayleigh test = 0.054, $p = 0.74$; Study site: Rayleigh test = 0.164, $p = 0.11$).

Based on the kernel estimated on the control site, we found an average movement distance ($1/\alpha$) of 116 m. We found that males were more mobile than females with an average movement distance ($1/\alpha$) of 166 m for males and 104 m for females. Because, the sample size was already limited on the study site and because sex ratio was similar on both sites, we decided to analyse male and female data sets simultaneously and to use the value of $1/\alpha = 116$ m to build the dispersal kernel. When applying our method on this case study, we found that the W/D ratios ranged from 0.07 for the railway (8.2/116) to 0.44 for the motorway (50.6/116).

On the study site, two butterflies crossed the motorway as against 12 expected crossing events, and 7 butterflies crossed the railway as against 15 expected crossing events. The motorway was identified as a strong barrier (logOddsRatio -2.02 [95% CI -3.55– -0.48]; binomial test $p = 0.0007$; Fig. 2.4) with a sixfold diminution of crossing events. In the same way, the railway was identified as a barrier to butterflies movements (logOddsRatio -1.02 [95% CI -1.97– -0.06]; binomial test $p = 0.015$; Fig. 2.4) with a twofold reduction in crossing events. None of the butterfly crossed both infrastructures.

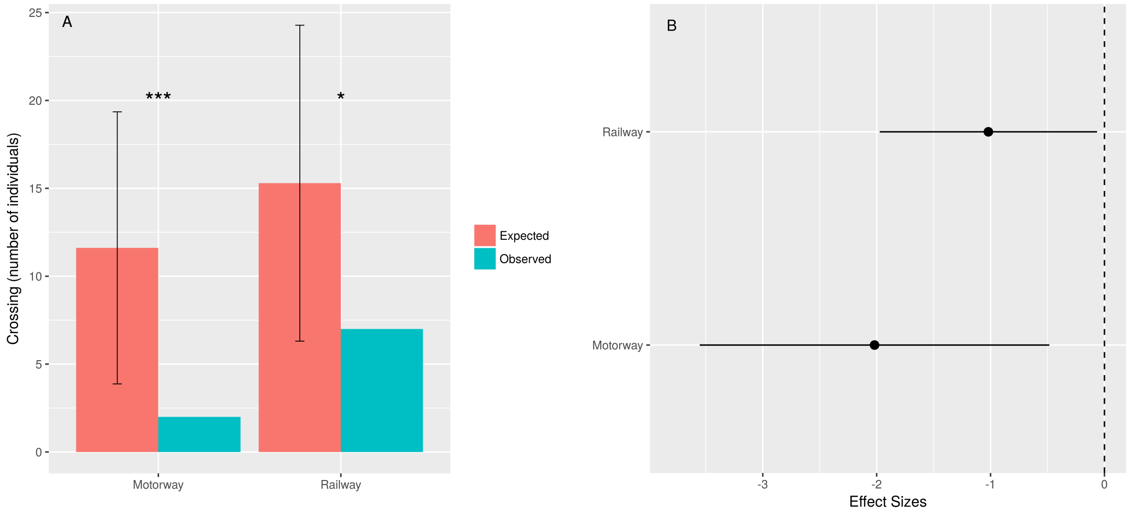

Figure 2.4 – Comparison between expected and observed probability that *Maniola jurtina* individuals cross two types of LTIs on the study site. Expected probabilities were calculated from a theoretical distribution fitted to a dispersal kernel as if LTIs were completely permeable. Panel A shows the comparison between expected and observed number of crossing events. Error bars represent mean ± SD. Significance was based on binomial tests. * : $p \leq 0.05$, *** : $p \leq 0.001$. Panel B shows effect sizes (logOddsRatio) ± 95% confidence intervals.

2.4 Discussion

Understanding how animal movements are affected by LTIs is a key issue in applied ecology. Dispersal kernels based on MRR data has been used to estimate barrier effects of infrastructures in one-dimensional environments (Pépino et al., 2012, 2016). But so far, a method applicable to two-dimensional landscape was lacking. Our framework proposes a simple way of estimating the permeability of linear LTIs on a wide range of terrestrial species. Compared to Pépino et al. (2012) whose framework relies on the use of both observation data and dispersal kernels corrected for the expected barrier permeability, our modelling framework is only based on dispersal kernels. It is therefore analogous to Rodríguez (2010) and does not require any a-priori information on the barrier effect of the studied infrastructure.

We found that our method performed well in detecting barrier effects as soon as an important data set is available (N = 500 scenario). For smaller sample sizes (N = 100 scenario), our method proved to detect barrier effects when the width of the infrastructure is small in comparison to the average movement capacity of the studied organism (small W/D ratio) and/or the effect of the barrier is strong.

Considering these results, we believe that our method is particularly suitable for organisms with good mobile capacities such as mammals, birds or flying insects. If the barrier effect is weak and the sample size reduced, our method might be unsuitable for organisms with low mobility or low locomotor capacities such as ground insects, amphibians (Trochet et al., 2014) or reptiles (Grimm et al., 2014), except when the considered infrastructure is narrow enough to counterbalance the lack of power associated with low average movement distances. With an only 5 m-wide barrier and a sample size of 500 individuals, the method will still be able to detect weak barrier effects as soon as the studied organism shows an average movement capacity of 10 meters or more. This will be the case for most organisms including small insects, amphibians or reptiles. Detecting barrier effects of wide infrastructures such as motorways would be complicated for animals with reduced movement capacities and small data sets. However, for such structures, ecologists and managers are usually more interested in the connectivity of large animals such as wolves or deer (Fahrig and Rytwinski, 2009). For example, the average movement distance capacity of a badger is 1.7 km (based on 474 movement records) (Byrne et al., 2014). With a wide infrastructure of 50 m like a motorway, the corresponding W/D ratio would be 0.03, providing great power to detect even weak barrier effects (Fig. 2.3).

In this study, data on the butterfly *M. jurtina* along two types of LTIs were used to illustrate the method. The estimated kernel calculated with butterflies from the control site (average movement capacity = 116 m) was very similar to the kernel estimated in a previous MRR study performed on the same species in western France (average movement capacity on three sites = 100 m) (Ouin et al., 2008).

The W/D ratio was high for the motorway (0.44) suggesting that a barrier effect, if present, would have been hard to detect considering the reduced sample size in our study. Yet, we found that the number of crossing through the motorway was sixfold reduced. We were able to detect this effect probably because the motorway had a strong barrier effect that would have not been detected if the barrier effect was weaker. Concerning the railway, the W/D ratio was small (< 0.1) and therefore, our method can be considered powerful enough to detect a strong barrier effect if present (Fig. 2.3). We detected an effect of this infrastructure although we were expecting a neutral effect because the studied railway is a small single rail structure with low traffic density. Our results differ from Vandevelde et al. (2012) who found a neutral effect of a high speed railway on a butterfly with life history similar to *M. jurtina*.

The barrier effects detected can arise from two causes. Butterflies might avoid crossing the structures or be killed while trying. Avoidance behaviour due to LTIs has been demonstrated in previous studies (Munguira and Thomas, 1992; Polic et al., 2014). Butterflies might be able to perceive the danger of flying over the motorway or the railway. Danger perception to fly over inadequate features suggests that movements are not random and that butterfly behaviours are influenced by landscape structures (Dover and Settele, 2009). Avoidance might be due to the physical characteristics of these two LTIs preventing butterflies to cross. These characteristics may include aerial turbulences due to traffic, changes in thermal conditions, edge configuration, and noise generated by traffic. In our study, avoidance behaviour was supported by field observations, with individuals observed heading back when reaching the motorway. Alternatively, butterfly might be killed while trying to cross these LTIs due to collision with vehicles. Given the low traffic density on the railway, mortality due to collision is supposed to be of limited intensity. It is more likely that edge configuration and/or changes in thermal conditions explain the barrier effect of the railway. For instance, the steep change in slope characterising the railway trench might act as an edge barrier to dispersal, although further investigation are now needed to confirm this hypothesis. However, mortality due to collision on the motorway may be substantial as road-kill is known to affect tremendously butterflies (Baxter-Gilbert, Riley, Neufeld, Litzgus and Lesbarrères, 2015; Skórka et al., 2015) and to participate greatly to the large-scale decline of insects (Hallmann et al., 2017). Both causes (avoidance and mortality) might drive together the detected barrier effect of the motorway. In order to disentangle the two causes, behaviour monitoring of butterflies along the infrastructure could help understand which cause is the most influential in driving the barrier effect.

Seasonal variation in the movements of butterflies (and any type of organism in general) is likely to occur (Schtickzelle et al., 2012). For example, butterflies tend to be less active during the hottest month of summer with reduced travelled distances than earlier or later in the season (Grill et al., 2013). As a consequence, the dispersal kernel estimated might vary depending on the sampling period on the control site. This implies that, besides similar landscape characteristics, similar sampling time periods are to be considered between the control and the study site: the species dispersal kernel might otherwise be under- or overestimated, with possible spurious conclusions as to the barrier effects of studied infrastructure (see Appendix E for details). For the same two reasons, we discourage the use of data from the literature to compute the dispersal kernel. Our method is also limited by sample size. We believe that data sets with 500 recapture events or more are optimal to apply our method. Depending on the species, this number might be difficult to achieve but would provide solid conclusions. Our method also implies that the LTI under study is linear across the study site as it considerably simplifies the equations. A potential improvement of our method would be to broaden the equations to account for non-linear LTIs. Yet, linear LTIs are most often encountered in landscapes due to obvious cost reasons and our method should be applicable in most cases. Although our method may be used to assess the cumulative barrier effect of several contiguous LTIs, our empirical dataset did not allow us to test for this as no butterfly crossed both the railway and the motorway (at least one crossing event is necessary to calculate logOddRatios).

Conclusion

We developed a method that allows estimating barrier effects due to linear infrastructures on a wide range of terrestrial species. We showed that this method is powerful to detect barrier effects, especially for organisms with good mobile capacities. We encourage managers to adapt this framework when investigating the connectivity of populations within landscapes fragmented by LTIs, notably when landscape genetic approaches are not worth considering. This could be used to set up mitigation programs on existing infrastructures and to propose conservation management strategies for species particularly at risk. We recommend to collect large data sets (ideally 500 recapture events) with similar time frame and landscape characteristics between the study and the control sites in order to build solid conclusions when applying this framework. Finally, while flying insects, such as *Maniola jurtina*, already suffer drastic declines, we revealed that motorways and railways can constrained organism home ranges and represent an additional threat to small wildlife.

Authors' contributions

JR, EC, SM and MB contributed to the conception and design of the study. EC and JR collected the data. EC, JR and JGP performed data analysis. JGP designed the simulation study, ran simulations and analysed simulated data. JR wrote the manuscript. All authors participated in critical revisions of the manuscript.

Data accessibility

Butterfly empirical data (motorway.csv and railway.csv) and R-scipts are uploaded as online supporting information. We provided a standalone R function (NEFbarrDetect.R) that estimate the barrier effect of any linear feature based on our method. Supplementary material (Appendix D and E) is uploaded as online supporting information.

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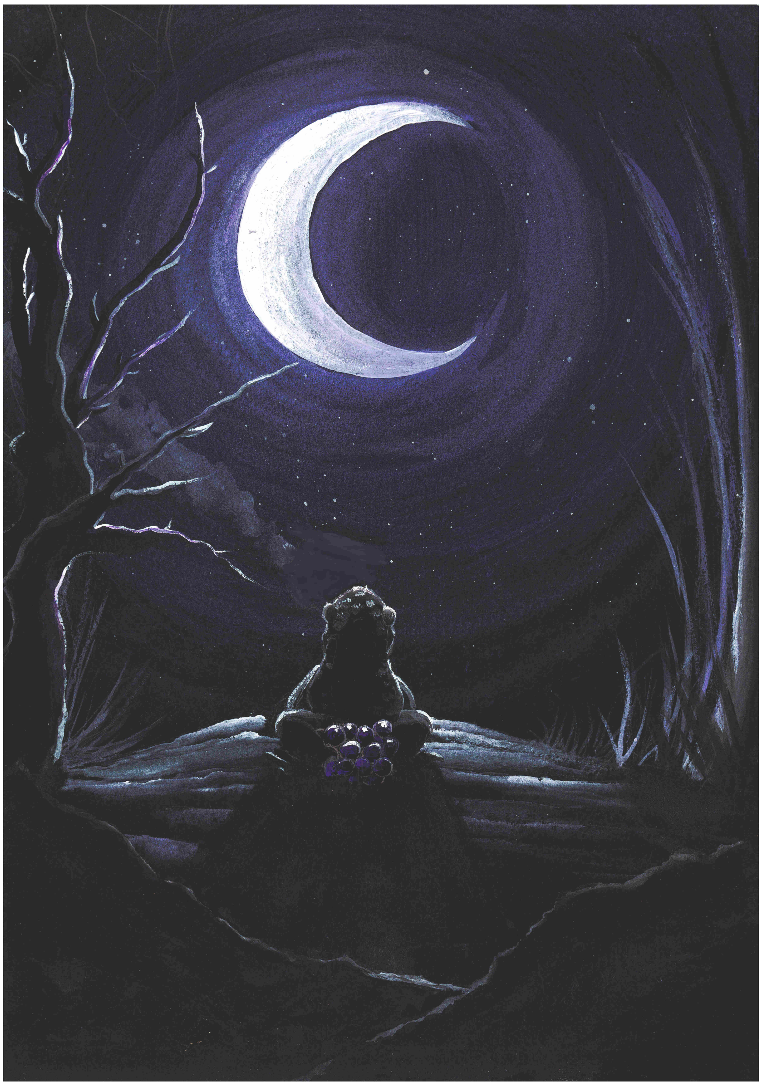
Planche 2. De gauche à droite et de haut en bas : équipe de choc en 2015 sur le site témoin ; femelle et mâle Myrtil recapturés ; capture de Myrtil sur le site témoin sous la ligne électrique ; capture de Myrtil le long de la voie ferrée ; capture de Myrtil dans un champ au Nord de l'A89.

Chapter 3

Low genetic diversity associated with fitness cost because of road proximity: an amphibian case study



Avant-propos

La diversité génétique des populations peut être fortement affectée par la fragmentation des habitats. Par ailleurs, il existe une corrélation importante entre la perte de diversité génétique et la réduction de la fitness moyenne des populations. L'étude simultanée de la variabilité génétique et de la fitness est susceptible d'apporter une meilleure compréhension de la viabilité des populations face à la fragmentation due aux routes.

Par conséquent, nous avons exploré le lien existant entre la diversité génétique et la fitness chez l'Alyte accoucheur dans un paysage fragmenté par plusieurs types d'ILT. Plusieurs indices de variabilité génétique ainsi que deux paramètres de fitness (fécondité via le nombre d'oeufs par chapelet et condition corporelle) ont été mesurés dans neuf populations. De plus, parmi ces neuf populations, trois ont été suivies intensivement par Capture-Marquage-Recapture (CMR) en 2015, 2016 et 2017 afin d'estimer deux paramètres démographiques additionnels (survie des adultes et taille des populations). L'ensemble de ces informations a été relié à deux paramètres environnementaux : la densité de routes secondaires entourant les populations et la proximité des populations aux infrastructures majeures (la route D6089 et l'autoroute A89).

Un premier article est en cours de préparation en vue d'une publication dans *Conservation Genetics* et compose ce chapitre. Dans cet article, le lien entre fitness, diversité génétique et fragmentation due aux routes est exploré.

De plus, le suivi CMR sur trois populations d'Alyte accoucheur pendant trois ans, a permis l'obtention de nombreuses informations chez les adultes de cette espèce : variabilité morphologique entre populations, vitesse de croissance, différence de fécondité entre populations et déplacements intra-population. Ces résultats ne sont pas valorisés dans ce chapitre mais sont détaillés explicitement dans l'Annexe F. Ces derniers pourront faire l'objet d'une publication ultérieure dans une revue spécialisée.

Low genetic diversity associated with fitness cost because of road proximity: an amphibian case study

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Manuscript in preparation for *Conservation Genetics*

Abstract

Environmental conditions such as habitat fragmentation due to roads are known to affect genetic diversity of populations. The loss of genetic diversity is usually correlated with a fitness cost in terms of survival, fecundity and/or growth. But few studies have tried to investigate the synergistic association among genetic diversity, fitness and roads.

We tested whether roads impacted genetic diversity and whether this impact was associated with a fitness cost in the midwife toad *Alytes obstetricans*. This hypothesis was tested in an environment fragmented by a secondary road network and two main roads (a country road and a motorway). We used microsatellite loci and Mark-Release-Recapture (MRR) surveys in order to obtain genetic diversity and fitness parameters in nine populations. We explored and summarised the relationship between genetic diversity and fitness using multivariate analyses. The principal components provided by multivariate analyses were tested against secondary road density surrounding populations and proximity to main roads.

We found that populations with high genetic diversity had bigger clutch sizes, better population growth rate and higher adult survival. The density of secondary roads surrounding populations was not driving this pattern. However, this pattern was explained by the proximity to main roads. Populations that were closer to the main country road or the motorway had lower genetic diversity and lower fitness than populations further apart.

This pattern might be driven by road kill associated with physical perturbations such as traffic noises. Our findings encourage the development of mitigation measures to counterbalance the negative effects of roads. In addition, our results indicate that combining MRR and genetic surveys on wild populations allows a better understanding of the mechanisms of fragmentation due to roads.

Key-words: genetic diversity; inbreeding; fitness; roads; fragmentation; amphibians

3.1 Introduction

Since the first development of modern roads by the Scottish engineer John Loudon McAdam in 1820, about 36 million kilometres of roads were built on Earth (CIA The World Factbook, retrieved 16 January 2018). That is enough to make 100 times the trip to the Moon. Roads allow human mobility and participate greatly to countries' development. But on the other hand, they are among the main driver of habitat fragmentation and have deep impacts on natural ecosystems (Dulac, 2013; Laurance et al., 2014).

Roads affect all types of organisms. Their impacts are broad, from demographic to genetic consequences (Forman and Alexander, 1998). Road mortality due to collision with vehicles probably is the most visible effect of roads. Though, indirect effects such as behavioral avoidance, pollution or alteration of the physical environment can limit individual movements in landscapes and impede successful road crossings (Trombulak and Frissell, 2000). This limits dispersal among populations which is essential to sustains gene flow within landscapes (Ronce, 2007). Populations that are not linked by dispersal events, suffer genetic isolation (Fahrig and Rytwinski, 2009; Beyer et al., 2016). When populations are isolated and small, they exhibit higher rates of inbreeding and genetic drift, which shrink the genetic diversity (Rowe and Beebee, 2003), resulting in a decrease in heterozygosity (McCauley, 1991). When populations have a low proportion of heterozygotes, they are more likely to accumulate deleterious alleles which impedes population fitness (Allentoft and O'Brien, 2010) and increases the risk of population extinction (McCauley, 1991; Fagan and Holmes, 2006). High genetic variability is a good insurance against changing environmental conditions, likely to bring stochastic and catastrophic events.

Among researchers, there is a good consensus that genetic diversity of populations is linked to fitness (Reed and Frankham, 2003; Allentoft and O'Brien, 2010; Markert et al., 2010). However, few studies have tried to link these two components to habitat disturbances such as fragmentation (but see Hitchings and Beebee, 1998; Ficetola et al., 2007; Rogell et al., 2010). Most of the literature focuses either on negative impacts of roads on genetic diversity (Keller and Largiader, 2003; Clark et al., 2010) or effects of roads on fitness (Barrass, 1985; Caorsi et al., 2017). There is a lack of evidence that genetic diversity associated with fitness cost decreases because of roads (Rogell et al., 2010). There is an urgent need to combine demographic and genetic approaches when trying to evaluate the conservation status of populations facing changing environments (Balkenhol and Waits, 2009; Lowe and Allendorf, 2010; Safner et al., 2011; Richardson et al., 2016).

Amphibian, is the most threatened group on Earth with more than 30 % of species likely to become extinct (Houlahan et al., 2000; Stuart et al., 2004; Hussain and Pandit, 2012). They are suffering overall decline due to habitat loss and fragmentation, pollution and diseases (Bower et al., 2017). Amphibians have typically low dispersal abilities and a particular breeding strategy which requires water bodies. Therefore, the loss of genetic diversity is likely to be greater than in many other taxa (Allentoft and O'Brien, 2010). A review that examined the link between genetic diversity and fitness in 34 amphibian studies found that populations with reduced genetic diversity had also lower fitness in most cases (Allentoft and O'Brien, 2010). For example, the genetic diversity (heterozygosity) of the frog *Rana perezi* was correlated with its survival, with higher heterozygosity leading to adults living older (Schmeller et al., 2007).

Amphibians are particularly affected by roads because of seasonal migrations between reproductive (water bodies) and overwintering places (Fahrig et al., 1995). Migration distances may occur up to a few kilometres (Sinsch, 2014) and cross several roads. Thus, road kill can decrease the overall population fitness by killing individuals likely to participate in the reproductive success of populations (Forman and Alexander, 1998; Hels and Buchwald, 2001; Beebee, 2013). In addition, road kill of dispersing individuals (individuals that leave a population to reproduce in an other one) is likely to genetically isolate populations by impeding gene exchanges among populations.

Roads can be classified in two distinct categories: secondary roads and large main roads. The secondary road network corresponds to small (< 5 m) single-lane rural roads with usually reduced traffic density but with a dense coverage in landscapes (high road density). Secondary roads have been associated with detrimental effects on amphibians. For example, the minor rural roads' density was explaining the genetic isolation of a newt and an anuran in northern Spain (Garcia-Gonzalez et al., 2012). Similarly, Carr and Fahrig (2001) found that population densities of the leopard frog were negatively affected by secondary roads within a radius of 1.5 km surrounding populations. On the other hand, main roads (including motorways) are wide (> 5 m) with high traffic density. They are likely to kill a large proportion of amphibians (Hels and Buchwald, 2001) and lead to genetic isolation to a greater extent than smaller roads (Marsh et al., 2008). The extent of their biological effects (called "road-effect zone") can reach several kilometres (Eigenbrod et al., 2009). Lesbarrères et al. (2006) found that *Rana dalmatina* populations had lower genetic diversity in ponds sampled on either side of a 20 years old motorway than populations further kilometres away. In a study completed along a motorway in Canada, Eigenbrod et al. (2009) found that the best predictor to explain the abundances of seven amphibian species was the proximity to a large motorway. Populations near the motorway had lower abundances than populations further apart with biological effects detected up to 1400 m from the motorway (Eigenbrod et al., 2009).

In landscapes fragmented by both secondary and main roads, amphibian populations may be strongly isolated with reduced population fitness and negative demographic consequences. Many studies have shown the relationship between the density of roads surrounding populations (or the proximity to roads) and the loss of genetic diversity (e.g. Lesbarrères et al., 2006; Garcia-Gonzalez et al., 2012; McKee et al., 2017) but the link with demographic characteristics of populations requires more investigations (Balkenhol and Waits, 2009; Safner et al., 2011).

In this study, we examined the relationship between genetic diversity and fitness of midwife toad (*Alytes obstetricans*) populations in a landscape fragmented by a secondary road network and two main Large-scale Transportation Infrastructures (LTIs, a country road and a motorway). More precisely, we aimed to determine whether (i) the genetic diversity of midwife toad populations was linked to fitness metrics (fecundity, body condition, growth rate and survival) and whether (ii) the genetic diversity and fitness were affected by both the density of secondary roads surrounding populations and the proximity to large infrastructures. We expected that populations with reduced genetic diversity would have lower fitness. In addition, we predicted that the density of secondary roads and the proximity to main roads would negatively affect genetic diversity and fitness of midwife toad populations.

3.2 Material and methods

Study area and biological model

The study was carried out in a fragmented area in the 'Périgord' region in south-western France between Brive-La-Gaillarde and Périgueux (45°07'31.8"N; 0°58'56.9"E). It is a 300 km^2 rural landscape composed of limestone plateaus including crops, mowed meadows, deciduous forests and small villages. The hydrology is limited to small sized rivers and ponds. Altitude ranges from 91 to 294 m above sea level. This landscape is fragmented by a network of 1370 km of secondary roads (Fig. 3.1). In addition, two large-scale transportation infrastructures related to roads cross the landscape: the motorway "A89", built in 2004 and a high traffic country road (D6089) historically present since the 18th century.

Twelve amphibian species were inventoried within this landscape (personal observations). Among them, we studied the midwife toad *Alytes obstetricans*, a small toad widely distributed in western Europe. This species is characterised by an interesting reproductive strategy, with a semi-terrestrial egg development stage. Just after reproduction, males carry the clutches on their back until hatching. This species is of particular conservation interest due to its sensitivity to the chytrid fungus *Batrachochytrium dendrobatidis* (Bosch et al., 2001). Fragmentation is an additional threat because local populations function as relative independent entities with strong genetic structure detected among populations (Tobler et al., 2013; Maia-Carvalho et al., 2014; Albert et al., 2015). The midwife toad is also vulnerable to roads and has reduced genetic diversity when the density of rural roads increases (Garcia-Gonzalez et al., 2012). Little is known on the dispersal ability of this species. Trochet et al. (2014) reviewed a maximum dispersal distance of 500 m.

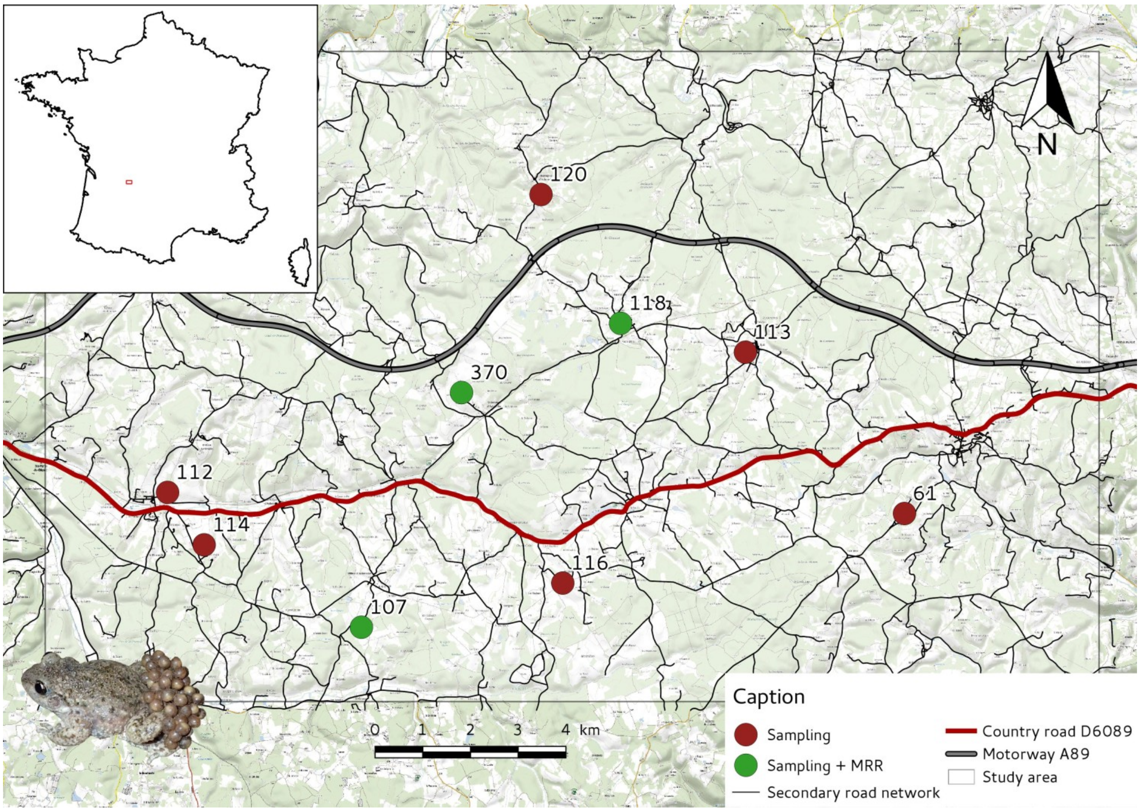


Figure 3.1 – Study area in south-western France. Nine populations of *A. obstetricans* were genetically sampled with additional fitness parameters recorded (fecundity and body condition). In three of them (Sampling + MRR), we performed Mark-Release-Recapture surveys and obtained additional parameters on population growth rates and survivals. ID numbers of the studied populations are indicated on the figure.

Data collection

Data were collected on adults *A. obstetricans* in nine populations across this landscape (Fig. 3.1). We were able to acquire genetic material as well as estimates of fecundity and body conditions within these nine populations. However two other fitness metrics (population growth rate and survival) were obtained only for three populations (Fig. 3.1).

The nine study sites were covered by foot using head torches during nights in 2015 and 2016 and we actively searched for *A. obstetricans* adults. Populations were visited only once or several times to collect a sufficient number of individuals per population ($>$ 15, ideally). Adults were captured by hands, measured (snout-vent length; SVL), weighted, sexed based on external morphological features (size, colour, ovocytes visible through the skin, eggs on the back) and GPS locations were recorded (Garmin Etrex20, USA). Each new individual was marked with a 7x1.35 mm FDX-B Passive Integrated Transponder (PIT) tag (Loligo Systems, Denmark)(Fig. 3.2). Tags provided individual

Figure 3.2 – Genetic sample collection (left figure) and PIT-tag introduction under the skin (right figure).

codes readable with a microtracker. When a male carrying a clutch was caught, we estimated the clutch size by counting the number of eggs present on its back without removing the clutch. In addition, when a new individual of *A. obstetricans* was captured, we collected a non-destructive genetic sample using buccal swab (Fig. 3.2). The mouths of captured individuals were gently opened using a little metal spoon and swabbed for about 10 s. Genetic samples were stored in 70 % EtoH until DNA extraction. Care was taken to minimize animal handling and stress. All materials for marking animals and collecting genetic samples were washed and disinfected using absolute ethanol between each individual. Animals were rapidly released at the place of capture after manipulation (less than 3 hours between capture and release).

Among the nine populations, three (**107**, **118** and **370**) were intensively monitored with Mark-Release-Recapture (MRR) surveys to estimate two additional fitness parameters: population growth rate and survival (Fig. 3.1).

MRR sessions were performed following a robust sampling design (Pollock, 1982). Robust design models incorporate sampling at two temporal scales, that are, primary and secondary sampling events. Secondary sampling events assumed that the sampling site is demographically closed (no gains by birth or immigration and no looses by death or emigration). Primary sampling events (longer intervals between surveys) consider the sampling site open and allow for gains and losses. Secondary sampling events were used to estimate the probability of capture (c), the probability of recapture (p) and the number of missing individuals ($f0$). Between primary sampling events, this type of model estimates apparent survival (probability to survive to $t+1$, given alive at t) and the seniority parameter (probability that an animal present at time $t+1$ was also present at time t, (Pradel, 1996)). The power of such study design is to use closed and open population estimates simultaneously, which gives more robust analyses than if estimates were calculated separately (Kendall, 2001). Accordingly, the three sites were monitored during nine secondary sampling events from April to August throughout three primary sampling events (2015, 2016 and 2017), resulting in a total of 27 sampling events per site. Each event consisted of approximately two hours survey per site where we randomly walked and actively searched for adults using head torches. The three sites were usually surveyed during the same night. Individuals were sexed, measured, weighted, marked, swabbed and their GPS coordinates were recorded following the above description. We built an encounter history for each captured individual with a 0 when the individual was not recaptured during any MRR session and a 1 when the individual was recaptured.

Laboratory procedures and genetic analyses

We extracted total DNA from swabs using the DNeasy Blood and Tissue kit (Qiagen, Valencia, CA). We amplified 14 polymorphic microsatellite loci (Tobler et al., 2013; Maia-Carvalho et al., 2014)(Appendix A). Loci were amplified with a Qiagen Type-it Microsatellite kit in 10 μl reaction volumes containing 2 μl multiplex PCR Master Mix, 1.2 to 1.6 μl of primer mix (between 0.13 and 0.25 μM of each primer), 5.4 to 5.8 μl of purified water and 1 μl of template DNA (10-20 ng μl^{-1}).

Polymerase Chain Reaction (PCR) conditions were set on an Applied Biosystems thermal cycler. Conditions were set as follows: initial denaturation 10 min at 95°C; 30 cycles of 30 s at 95°C, 90 s at 51 to 60°C (depending on the multiplex, see Appendix A for details) and 30 s at 72°C; final elongation of 5 min at 72°C.

All PCR products were ten times diluted and were run on an ABI 3730 DNA Analyser (Applied Biosystems) with the GeneScan-600 LIZ size standard. Genotyping was performed with GENEMAPPER 5.0 (Applied Biosystems) and all peaks were manually confirmed.

We used Genepop 4.2 (Rousset, 2008) to test for linkage disequilibrium among pairs of loci and deviation from Hardy-Weinberg Equilibrium after sequential Bonferroni correction to account for multiple related tests (Rice, 1989). The presence of null alleles was tested using MICROCHECKER 2.2.3 (Van Oosterhout et al., 2004).

The presence of related individuals (siblings for example) in data sets is known to over-estimate population structure (Anderson and Dunham, 2008) and bias subsequent genetic analyses. Therefore, we used COLONY2 (Jones and Wang, 2010) to identify full-sib and parent-offspring groups. We applied the full-likelihood approach based on the individual multilocus genotypes. We assumed that males and females were polygamous.

All individuals were considered as potential offspring and no a priori candidate parental genotypes were defined. Allele frequencies were determined directly from the genetic dataset. We ran three independent long runs with various seed numbers to test for congruent results. Only relationships with an associated inclusion probability higher than 95 % were considered as significant. In each group of related individuals, we randomly retained one genotype. Accordingly, 76 out of 445 genotypes of *A. obstetricans* were discarded.

To compare genetic attributes among the nine studied populations, we computed five genetic metrics. We calculated the first three ones using Genepop 4.2 (Rousset, 2008): observed heterozygosity (Ho), expected heterozygosity (He) and the inbreeding coefficient (Fis). We estimated the standardised allelic richness (AR) and the standardised private allelic richness (PA) using the rarefaction procedure implemented in ADZE 1.0 (Szpiech et al., 2008). We choose 13 as the standardised sample size across the three populations (i.e. the lowest number of genotypes available on site **120**).

We tested for genetic structure across the nine populations using the program STRUCTURE 2.3.4 (Pritchard et al., 2000) with the admixture and the correlated allele frequency models. We tested the number K of clusters from 1 to 9 and repeated analyses for each value five times. Runs were performed with a burn-in period of 50 000 and the 50 000 subsequent Markov chain Monte Carlo repetitions were retained. We also checked that the alpha value (looking at alpha plots created by STRUCTURE) had stabilised before the end of the burn-in period to ensure convergence. We used STRUCTURE HARVESTER (Earl and VonHoldt, 2012) to obtain Log-likelihood plots and deltaK statistics to infer the optimal K-value. We used the optimal K-value to performed 20 runs with a burn-in period of 200 000 and the 200 000 subsequent Markov chain Monte Carlo repetitions were retained. We compiled the ten best runs using CLUMPP (Jakobsson and Rosenberg, 2007) to obtained the population Q-values. Each population was assigned to the cluster for which its Q-value was higher than 0.6 (Prunier, Colyn, Legendre and Flamand, 2017). We identified first generation migrants with GeneClass2 (Piry et al., 2004). This method estimates the probability that any individual belong to predefined populations. We used the partial Bayesian approach (Rannala and Mountain, 1997) and the Monte Carlo method of Paetkau et al. (2004) was used for exclusion analysis with 10,000 simulated individuals and $\alpha = 0.01$.

Fitness metrics

Within the nine studied populations, we estimated two fitness metrics. The first one is an index of body condition calculated as the residuals from a regression of body mass on the body size of individuals (SVL) (Schulte-Hostedde et al., 2005). The second one was a proxy for population fecundity, assessed using clutch sizes (number of eggs per clutch) carried by all encountered males. For each site, we averaged the body conditions across all individuals but males carrying clutches because their weight could not be measured without the eggs. Clutch size was averaged per site across all males carrying a clutch. We ended up with an unique value of body condition and clutch size per site.

Additional demographic parameters were obtained within the three populations followed with MRR encounter histories based on Pradel seniority robust design (Pradel, 1996) with Huggins' estimates. We assumed that capture of midwife toads did not affect their probability of recapture. Therefore in all tested models we settled $c = p$ (same probability of capture and recapture). Sex was not put as a covariate due to the impossibility to sex all individuals. We implemented all models in program MARK (White and Burnham, 1999). Within the candidate model set, models were compared using the Akaike's Information Criterion adjusted for sample size AICc (Burnham and Anderson, 2002). The model with the lowest AICc value was considered as the most supported by the data (Appendix G). A difference of ΔAICc greater than 2 between two models supported good differences (Burnham and Anderson, 2002). To incorporate model uncertainty, we used model averaging of parameter estimates across candidate models with AICc weight > 0 (White et al., 2001). To test for heterogeneity and Goodness Of Fit (GOF) for which there are no standard tests for robust design, we ran prior analyses using Cormack-Jolly-Seber (CJS) models. We then tested GOF using Test 2 + 3 of Program RELEASE (Burnham et al., 1987) and global test of program U-CARE (Choquet et al., 2009). Parameter estimates were presented with 95 % confidence intervals unless specified. By using this model, we were able to estimate annual population sizes (N) derived from missing individuals (f0) and apparent survival between primary sessions (2015-2016 and 2016-2017). The index for population growth rate (r) was defined as the slope of the linear regression of population sizes across the three studied years. A stable, growing or declining population would be indicated by a r $= 0$, > 0 or < 0, respectively. The index for annual survival (S) was computed by averaging the annual survivals for the periods 2015-2016 and 2016-2017. We ended up with an unique value of r and S for three sites.

Principal component analyses and fragmentation predictors

We performed two principal component analyses (PCA1 and PCA2) to explore the relationships among genetic and fitness metrics with package FactoMineR (Lê et al., 2008). The first one (PCA1) included only the five genetic metrics (Ho, He, Fis, AR, PA) and the second one (PCA2) included the five genetic metrics and the four fitness parameters (body condition (BC), clutch size (CS), growth rate (r) and survival (S)). Results from the second PCA including both genetic and fitness metrics were compared to results from the first PCA to assess the possible influence of missing data (r and S available for only three populations). To handle missing values in the fitness parameters, we used the package missMDA (Josse and Husson, 2016). Coordinates of sites on the two first axes of PCA1 and on the three first axes of PCA2 were used as dependent variables in subsequent analyses (see results).

We used two types of predictors to estimate the relative isolation of populations due to roads. The first one is the cumulated road length in a 500 m radius area surrounding each population. We chose a 500 m radius, because it was the maximum reported dispersal value in this species (Trochet et al., 2014). We also considered two other radius sizes: 250 m and 1000 m. Because the secondary road network may have a limited effect compared to large main roads (Fahrig et al., 1995; Hels and Buchwald, 2001) or motorways (Reh et al., 1990; Eigenbrod et al., 2009; Van Buskirk, 2012), we calculated a second type of predictor: the euclidean distance between each population and the nearest large infrastructure (the motorway A89 or the country road D6089). We used simple linear regressions to test whether the effect of road density or distance to the nearest LTI were associated with the axes of PCA1 (two axes) and PCA2 (three axes). All analyses were performed with QGIS (V. 2.8) and R 3.3.2 (R Core Team, 2015).

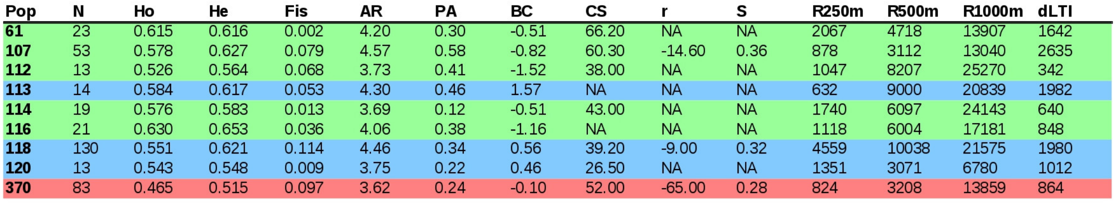
3.3 Results

Population genetic attributes

A total of 369 *A. obstetricans* genotypes were available across the nine studied populations ranging from 13 genotypes on site **120** to 130 genotypes on site **118**. There was no evidence of linkage disequilibrium between pairs of loci. We found evidence of null alleles for locus Aly7. Accordingly, we retained 13 loci for subsequent analysis (Aly28, Aly3, Aly4, Aly17, Aly19, Aly20, Aly23, Aly24, Aly25, Aobst14, Aobst15, Aobst16 and

Aobst17)(Appendix A). The five genetic metrics (Ho, He, Fis, AR and PA) were calculated within each population and summarised in Table 3.1.

Table 3.1 – Genetic and fitness metrics of nine populations of *A. obstetricans* in south-western France: (Pop) represents the population ID and (N) is the number of individuals per population. Genetic diversity is represented by the following metrics: expected heterozygosity (He), observed heterozygosity (Ho), allelic richness (AR) and number of private alleles (PA). Fis is the inbreeding coefficient. Body condition (BC) and clutch size (CS) were calculated within the nine populations (data were lacking within two populations for CS as no males carrying clutches were encountered). Population growth rate (r) and annual survival (S) were calculated within three populations followed with Mark-Release-Recapture surveys. We tested whether those metrics varied with the road density present in 250, 500 and 1000 m radius surrounding populations (R250m, R500m and R1000m) and the distance to the nearest large-scale infrastructure dLTI (motorway A89 or country road D6089). The latter results are presented in meters. Note that colours are similar to Fig. 3.3 and 3.5.



Pop	N	Ho	He	Fis	AR	PA	BC	CS	r	S	R250m	R500m	R1000m	dLTI
61	23	0.615	0.616	0.002	4.20	0.30	-0.51	66.20	NA	NA	2067	4718	13907	1642
107	53	0.578	0.627	0.079	4.57	0.58	-0.82	60.30	-14.60	0.36	878	3112	13040	2635
112	13	0.526	0.564	0.068	3.73	0.41	-1.52	38.00	NA	NA	1047	8207	25270	342
113	14	0.584	0.617	0.053	4.30	0.46	1.57	NA	NA	NA	632	9000	20839	1982
114	19	0.576	0.583	0.013	3.69	0.12	-0.51	43.00	NA	NA	1740	6097	24143	640
116	21	0.630	0.653	0.036	4.06	0.38	-1.16	NA	NA	NA	1118	6004	17181	848
118	130	0.551	0.621	0.114	4.46	0.34	0.56	39.20	-9.00	0.32	4559	10038	21575	1980
120	13	0.543	0.548	0.009	3.75	0.22	0.46	26.50	NA	NA	1351	3071	6780	1012
370	83	0.465	0.515	0.097	3.62	0.24	-0.10	52.00	-65.00	0.28	824	3208	13859	864

STRUCTURE identified 3 final clusters (Fig. 3.3). The cluster A comprised only the population **370**. The cluster B included the populations **120**, **118** and **113**, all encountered in the north of the study area. The motorway A89 separated the population **120** from the populations **118** and **113**. The five remaining populations composed the cluster C (**112**, **114**, **107**, **116** and **61**) which were all located in the south of the study area. All but population **112** were separated from the two other clusters by the country road D6089. No population was cross-assigned between several clusters. When using GeneClass2, we identified 13 first generation migrants (Fig. 3.3).

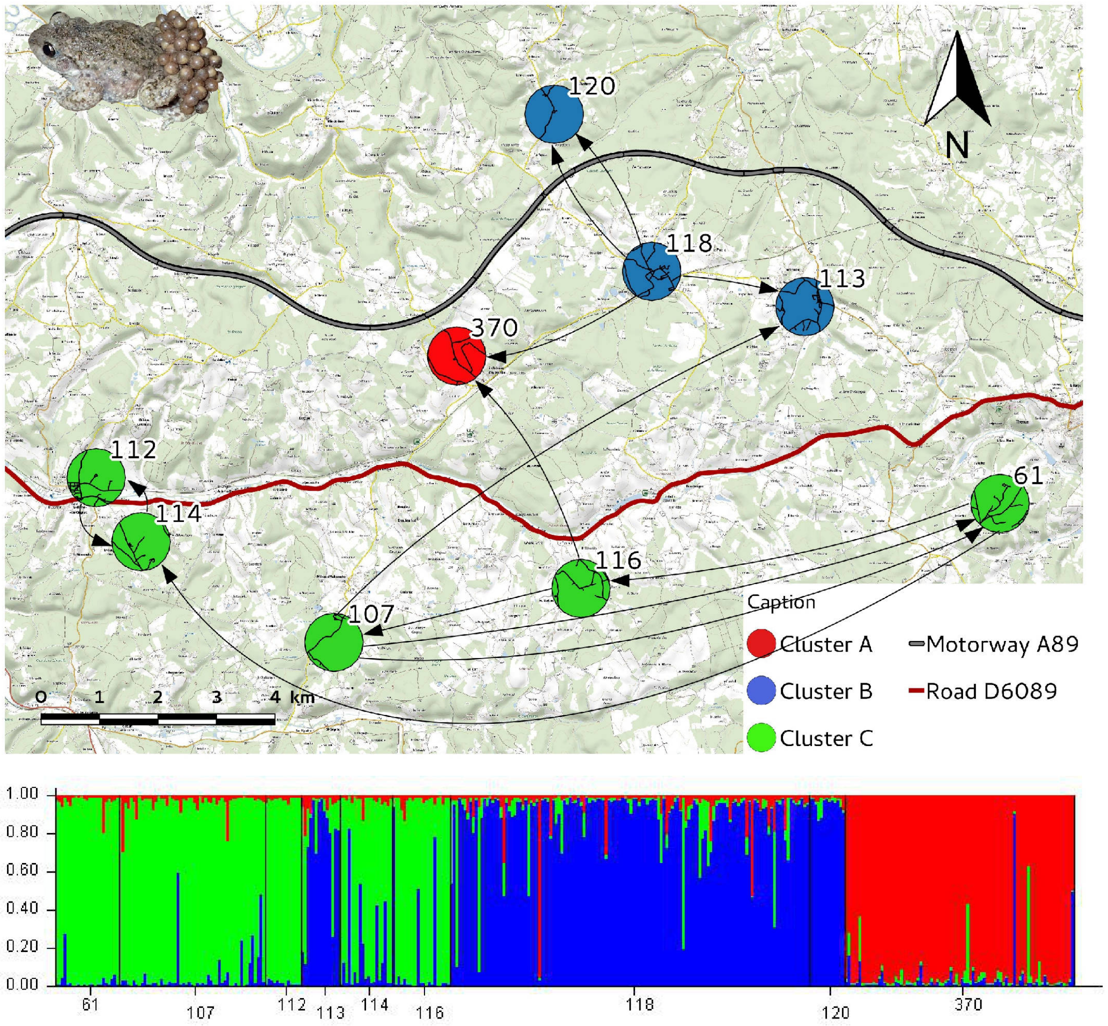


Figure 3.3 – Coloured 500 m radius surrounding the nine studied populations confidently assigned to their STRUCTURE clusters (Q-values < 0.6). Inside each radius, the linear road network is represented. In the lower panel, each bar represents one individual from the different populations, with colours referring to the assignment probability of individuals to any of the three genetic clusters. First generation migrants estimated with GeneClass2 are represented by black arrows. Each arrow represents one individual and its migration path.

Fitness parameters

Body conditions varied between -1.52 on site **112** to 1.57 on site **113** (Table 3.1). The averaged clutch size per population varied between 26.5 on site **120** to 66.2 eggs on site **61**. In two populations (**113** and **116**) no males carrying clutches were encountered (Table 3.1).

On the three sites where MRR surveys were performed, a total of 442 adults were marked (92 on site **107**, 208 on site **118** and 142 on site **370**). 161 were females, 177 were males and 104 could not be sexed. There was no consistent departure from CJS model assumptions among secondary sampling occasions in any of the three sites (RELEASE Test 2 + 3 and U-Care global test: all $p > 0.94$). This indicated that our model fits were good and that demographic parameters were reliable. For site **107** and **370**, the top four models encompassed more than 99% of the weight based on the AICc scores. On site **118**, the top three models encompassed 100% of the weight (Table S4).

We were able to estimate population sizes within the three studied sites for each year. The largest population size recorded was in 2015 on site **370** with 160 ± 15 adults (Fig. 3.4). On the same site, the population size collapsed to 67 ± 7 individuals in 2016 and to 29 ± 4 individuals in 2017. Therefore, the population growth rate (r) was the lowest among the three populations (r = -65.1), indicating a strong population decline on this site. We also recorded an important population size decline on site **107** from 64 ± 9 adults in 2015 to 34 ± 4 individuals in 2017, with a r of -14.6. On site **118**, the population size remained overall constant with 110 to 128 individuals depending on the year (r = -9.0; Fig. 3.4).

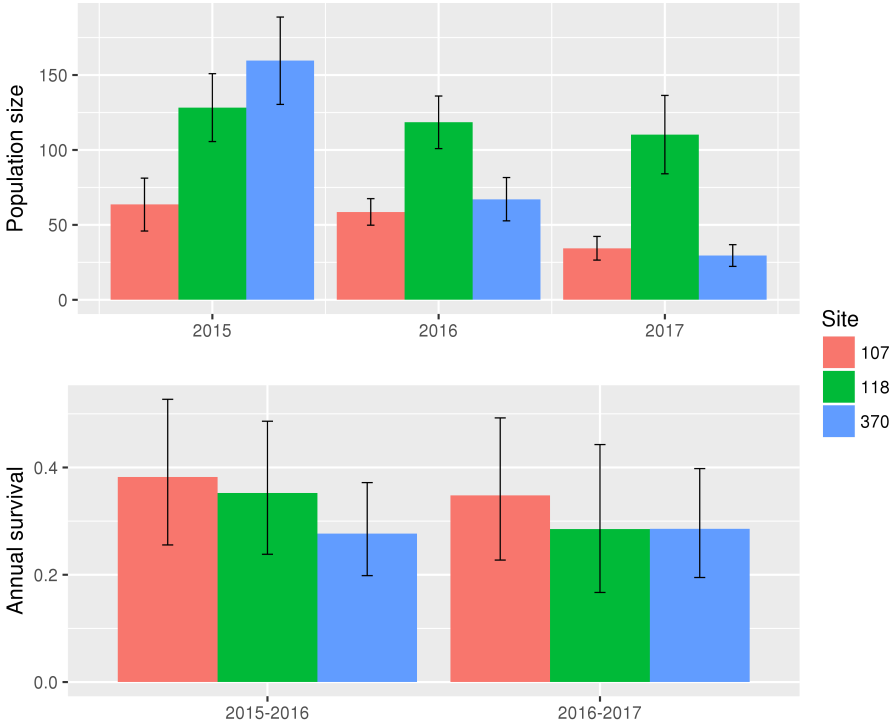


Figure 3.4 – Annual estimates of population sizes and annual survival with 95% confidence interval in three populations of the species *A. obstetricans* in south-western France.

Annual apparent survivals of midwife toads were low on the three sites ranging from 0.28 ± 0.04 to 0.38 ± 0.06. There was no strong pattern of survival variation among sites and years (Fig. 3.4). The lowest averaged survival across years (S) was on site **370** (S

= 0.28). Population **118** had a S of 0.32 and population **107** had the highest S (0.36) (Table 3.1).

Relationships among genetic diversity, fitness and isolation due to roads

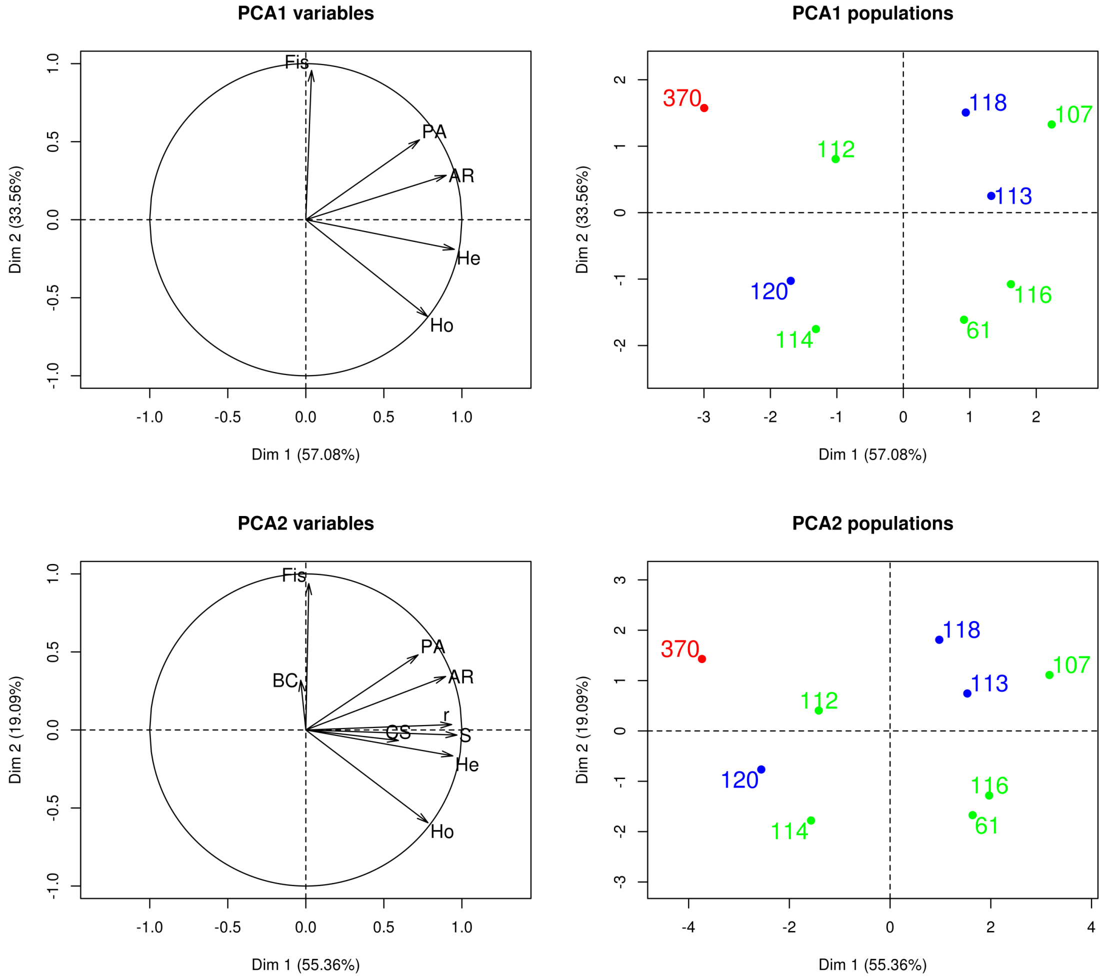


Figure 3.5 – First and second axis of the principal component analyses. PCA1 was performed only with genetic metrics. In PCA2, fitness parameters were included in the analysis. Left and right panels represent the coordinates of variables and populations on the two first axes, respectively. Population ID are coloured the same way as in Fig. 3.3. In PCA1, the first component summarised genetic diversity (expected heterozygosity (He), observed heterozygosity (Ho), allelic richness (AR) and number of private allele (PA)). In PCA2, the first component summarised genetic diversity and three fitness parameters (clutch size (CS), annual survival (S) and population growth rate (r)). Thus, this component can be describe as a 'viability' axis. The second axis summarised the inbreeding coefficient (Fis) in both PCA. The body condition (BC) is a fitness parameter unrelated to the two first components but was strongly correlated with the third one (see Table S5 for additional informations).

PCA1 and PCA2 were highly similar in terms of projection of variables and interpretation of axes (Fig. 3.5). With PCA1, the first principal component explained 57.1 % of the variability, and was strongly and positively correlated with Ho, He, AR, and PA (all scores > 0.73)(Fig. 3.5 and Table S5). This first component was thus an effective statistic summary for genetic diversity. The second principal component explained 33.6 % of the variability, and was strongly and positively correlated with the inbreeding coefficient (Fis; score $= 0.96$). The third axis was not relevant in PCA1 (Table S5) and not used in subsequent analyses.

With PCA2, the first principal component explained 55.4 % of the variability, and was strongly and positively correlated with Ho, He, AR, PA, r and S (all scores > 0.72). Clutch size (CS) was also positively correlated with this axis but to a lesser degree of magnitude (score $= 0.59$) (Fig. 3.5 and Table S5). This first component was an effective statistic summary for population viability (genetic diversity and three fitness parameters). Similarly to PCA1, the second principal component was strongly and positively correlated with the inbreeding coefficient (Fis; score $= 0.94$) and explained 19.1 % of the variability. The third axis was strongly and positively correlated with the body condition (BC; score $= 0.87$) and explained 12.7 % of the variability (Table S5). Thus, we kept these three axes as new variables to test the effect of isolation due to roads.

Population **370** had a particularly great inbreeding coefficient and the lowest viability (lowest genetic diversity and fitness) among all populations (Fig. 3.5). This population was also identified as a specific cluster by the STRUCTURE software (Fig. 3.3), indicating that genetic characteristics of this population was strongly different from the two other clusters.

The road densities surrounding populations are summarised in Table 3.1. With a 500 m radius, the population **120** was surrounded by 3000 m of roads, which represented the lowest density of roads across populations. In comparison, the population **118** was the one with the highest density of roads with about 10 000 m of roads in a 500 m radius. This represented about a three fold difference in road density among populations. This range of differences was comparable with radius of 250 m or 1000 m (Table 3.1).

Road densities were unrelated to the two first axes of PCA1 and to the three first axes of PCA2 either considering a radius of 250, 500 or 1000 m (all $p > 0.29$, Table S6). This suggested that secondary road density was not a good predictor of the variability in genetic diversity, inbreeding or fitness of *A. obstetricans* populations.

The second predictor tested was the distance to the nearest LTI represented by the motorway A89 or the country road D6089. These distances ranged between 342 m for the population **112** to 2635 m for the population **107** (Table 3.1).

The first axes of PCA1 (genetic diversity) and PCA2 (population viability) were significantly explained by the distance to the nearest LTI (PCA1: $F_{1,7} = 7.04$, $p = 0.033$; PCA2: $F_{1,7} = 7.44$, $p = 0.029$) (Fig. 3.6). However, the second component (inbreeding) was not explained by the distance to the nearest LTI either considering PCA1 ($F_{1,7} = 0.95$, $p = 0.36$) or PCA2 ($F_{1,7} = 1.55$, $p = 0.25$). Finally, the body condition that was synthesised on the third axis of PCA2 was unrelated to the distance to the nearest LTI ($F_{1,7} = 0.50$, $p = 0.50$).

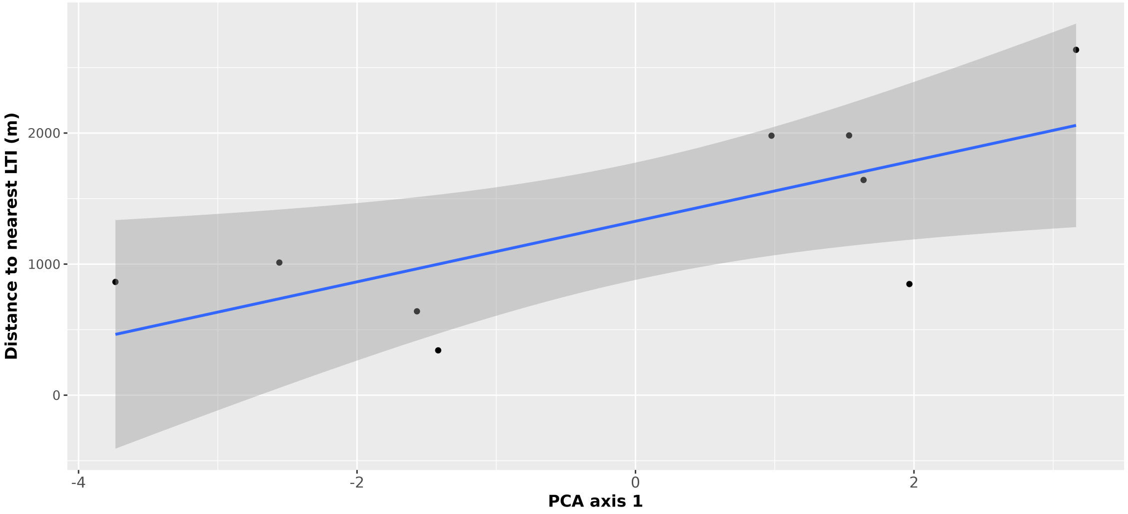


Figure 3.6 – Relationship with 95% confidence intervals between distance to the nearest large-scale transportation infrastructure (motorway A89 or country road D6089) and the first axis of PCA2 (principal component analysis). The first axis of PCA2 represents a summary of genetic diversity and population fitness described as population viability.

3.4 Discussion

In this study we were interested in the genetic and fitness attributes of nine *A. obstetricans* populations in a landscape fragmented by roads in south-western France. We obtained these metrics by performing genetic and field-based (MRR) monitoring on adults. Combining field-based and molecular approaches can increase the understanding of the consequences of road effects and help to define appropriate mitigation measures (Balkenhol and Waits, 2009).

Conducting MRR surveys on *A. obstetricans* is challenging due to its cryptic behaviour. To our knowledge, our study describes the first MRR survey on adults of this species. As a consequence, we reported the first estimation of adult annual apparent survival of *A. obstetricans*, which ranged from 0.28 ± 0.04 to 0.38 ± 0.06 with an average value of 0.32 across the three sites and years. This value was lower than values reported in *Bufo bufo* (0.60), *Bombina variegata* (0.80) and *Pelophylax sp.* (0.70), but similar to values reported in *Pelobates fuscus* (0.34) (Trochet et al., 2014).

The three populations followed by MRR were experiencing population size decrease. One of them was almost stable (**118**) but the two others experienced strong collapse, especially the population on site **370**. On this latter site, no juveniles were detected across the three studied years. The only potential breeding pond detected on this site was filled by predatory fish. Some tadpoles were detected in this pond in 2015, but none in the subsequent years. We suggest that the rapid population collapse could be due to the absence of recruitment in this population. This explanation is reinforced by the analysis of first generation migrants (Fig. 3.3), that showed that in our data set, no individual was dispersing from population **370**. The absence of recruitment in this population is maybe due to the recent introduction of fish in the breeding pond.

Relationship between genetic diversity and fitness

Using PCA, we created orthogonal variables synthesizing genetic and fitness information. Because fitness parameters could not be estimated for all populations, we built two PCA, the first one including only genetic metrics. We showed that these two PCA were highly similar (Fig. 3.5). In both cases, the first axis summarised genetic diversity (He, Ho, AR and PA) and the second axis was strongly and positively correlated with inbreeding (Fis). These two PCA supported similar conclusions regarding fragmentation due to roads.

In PCA2, axis 1 synthesised both measures of genetic diversity and fitness (viability axis) and the second axis was a good indicator of inbreeding. We found evidences that genetic diversity was positively correlated with three fitness parameters. Populations with higher genetic diversity had also greater adult survival, higher population growth rate and greater clutch sizes. This is in light with previous studies showing that higher genetic diversity is associated with better fitness in amphibian populations (Allentoft and O'Brien, 2010).

Interestingly, the second axis of the PCA which corresponded to inbreeding (Fis) was not correlated to any fitness parameters (Fig. 3.5). This result differs from Andersen et al. (2004) who found a negative correlation between Fis and fitness (larvae survival) in the tree frog *Hyla arborea*.

The body condition of *A. obstetricans* was the only fitness metric not correlated with any of the two first axes of PCA2, but it was correlated with the third one. This suggests that body condition was unrelated to any genetic metrics and other fitness parameters (Fig. 3.5 and Table S5). McAlpine (1993) found a similar result by looking at body size instead of body condition of green treefrog (*Hyla cinerea*). They revealed that body size was not associated with genetic diversity (heterozygosity). In addition, one can expect that body condition should be correlated with other fitness parameters such as clutch size. In the midwife toad, this pattern was previously demonstrated by Márquez (1997). These authors found that larger males were carrying larger clutch sizes than small males. We were not able to identify this pattern in our study. This could be explained by a lack of power in our analysis because our comparison was made among a small number of populations in comparison to Márquez (1997) who did it on individuals.

Relationship between genetic diversity associated with fitness and roads

Previous studies have revealed that high density of small secondary roads affected fitness in amphibians. For instance, Carr and Fahrig (2001) found that population densities of the leopard frog were negatively affected by secondary roads within a radius of 1.5 km surrounding populations. Besides effect on fitness, secondary roads are also responsible for a loss of genetic diversity in amphibian populations. This was demonstrated for many species, including the common frog (*Rana temporaria*) (Johansson et al., 2005) and *R. latastei* (Ficetola et al., 2007). These two species presented a negative relationship between genetic diversity and road length calculated in 500 and 1500 m buffers surrounding populations, respectively. For the toad *A. obstetricans*, a previous study in northern Spain found that the number of secondary roads between sampling locations was an important driver of genetic isolation (Garcia-Gonzalez et al., 2012).

Yet, in our study, the genetic diversity alone (PCA1) or the genetic diversity associated with fitness cost (PCA2) were not affected by the density of secondary roads surrounding populations. This pattern was verified whatever the size of the radius considered (250, 500 or 1000 m).

However, our study revealed that large-scale infrastructures (the country road and the motorway) were affecting the genetic diversity alone (PCA1) and the genetic diversity associated with fitness cost (population viability, PCA2). The greater the distance to the country road or the motorway, the higher the population viability (Fig. 3.6). This pattern was not detected when the two infrastructures were considered independently (data not shown). This suggests that both infrastructures have a non-negligible impact on population viability.

In this study, we could not clearly identify why the motorway and the main country road had a negative effect on genetic diversity and fitness of *A. obstetricans* populations. A potential way that could help to clarify the involved mechanism would be to design a multi-states MRR design (Lowe and Allendorf, 2010) in a meta-population of this toad with populations on either side of the LTIs. This would allow the estimation of demographic connectivity (the effect of dispersal on demographic parameters and viability of populations) (Lowe and Allendorf, 2010). Such a design would requires a high field investment but would be very valuable in terms of conservation issues.

Nevertheless, results similar to ours were found in previous studies and may help to highlight potential explanations to this pattern. A comparable result was found by Lesbarrères et al. (2006) who found that populations of the frog *Rana dalmatina* had lower genetic diversity in populations sampled close to a 20 years old motorway than populations further kilometres away. Reh et al. (1990) also found that motorways reduced genetic diversity in the frog *Rana temporaria*. This supports the hypothesis that large roads may be more detrimental than smaller secondary roads (Fahrig et al., 1995; Hels and Buchwald, 2001). For instance, a large motorway was responsible for genetic isolation in the red-backed salamander, but smaller roads were not (Marsh et al., 2008). The effect of main roads called “road-effect zone” can reach several kilometres (Eigenbrod et al., 2009). In our study, the distance between the nearest large infrastructure and amphibian populations ranged from 340 to 2600 m. These were distances comparable to Eigenbrod et al. (2009) who found an effect of a large motorway on seven amphibian species.

Main roads can affect the viability of amphibians for several reasons. Physical and chemical perturbations due to roads can be strong (Trombulak and Frissell, 2000). Such disturbances are likely to affect amphibian populations’ fitness living near main roads. Night-time noises due to traffic can interfere with calling activities of amphibians and have overall negative effects on immune responses and fitness (Troïanowski et al., 2017). For instance, Barrass (1985) found that two amphibian species populations located close to a noisy motorway showed a reduction in egg mass. Traffic noises may also disrupt females’ ability to move toward calling males (Bee and Swanson, 2007). Amphibians can also waste more energy to change acoustic parameters of their calls in response to traffic noises, such as elevating call intensities (Bee and Swanson, 2007; Lukanov et al., 2014; Caorsi et al., 2017). This energy could have been used for reproductive investment, maintaining populations with better breeding success and larger sizes over time (Parris et al., 2009).

Traffic mortality on main roads may also play a role (Fahrig et al., 1995; Beebee, 2013). In the study led by Eigenbrod et al. (2009), two amphibian species were not avoiding sites near a motorway for breeding but their low abundances were due to road mortality. In our study area, the main road D6089 is likely to kill a high proportion of amphibians. However, the motorway is fenced with narrow mesh preventing amphibians to reach the motorway pavement and be killed. Therefore, because both infrastructures are affecting genetic diversity and fitness, traffic mortality alone is unlikely to drive this pattern.

Previous studies have shown that barriers to migration caused by main infrastructures (potentiality due to traffic mortality) can explain the genetic diversity reduction (e.g. Lesbarrères et al., 2006). Yet, we suggest that this was not the case in our study and that gene flow limitation and loss of genetic diversity associated with fitness cost might result from two independent processes. Two elements supported this idea.

First, the inferred genetic structure (Fig. 3.3) revealed that in the blue cluster, the motorway was not separating the population **120** from the two other populations. This result should be interpreted with care as the motorway is a recent infrastructure and a time lag exists between the creation of a barrier and the observable genetic structure (Epps and Keyghobadi, 2015). However, in the green cluster, the country road D6089 (several hundred years old) was not separating the population **112** from the four other populations located south of the road. If these two infrastructures were acting as strong barriers, we would have expected a strong genetic differentiation between each side of infrastructures (or at least for the country road D6089). Therefore, these infrastructures are not totally impermeable to gene flow as proved by GeneClass2 analysis showing that some first generation migrants crossed the country road and the motorway (Fig. 3.3). This indicates that there is at least some gene flow exchanges among populations separated by these infrastructures preventing genetic isolation and loss of genetic diversity.

Secondly, our results differ from Remon et al. (in prep.) who studied *A. obstetricans* gene flow within the same landscape. They found that the secondary road network was impeding dispersal of *A. obstetricans*. One could expect that a gene flow limitation across secondary roads should necessary lead to a genetic erosion and a loss of genetic diversity in populations isolated by a high density of secondary roads. Yet, in our study, we did not detect an effect of the secondary road network on genetic diversity of populations (associated with fitness or not). Our study was not able to link the loss of genetic diversity with a reduction of gene flow. Previous studies have already described that gene flow could be reduced through infrastructures without having any effect on genetic diversity (Hitchings and Beebee, 1997; Keller et al., 2004). This could be a consequence of the inverse relationship between genetic diversity and effective population sizes. Larger populations may remain undifferentiated in terms of genetic diversity even if they are strongly isolated because large effective population sizes act as a buffer against genetic drift (Richardson et al., 2016).

We suggest that low genetic diversity near main infrastructures are not necessary driven by reduction of gene flow. Therefore, future studies which assess the effects of main infrastructures should try to disentangle connectivity effect (gene flow limitation) from other perturbations leading to a loss of genetic diversity potentiality affecting population fitness by using several approaches such as mark-release-recapture and genetic surveys across infrastructures (Lowe and Allendorf, 2010).

Limitations and conservation perspectives

We do acknowledge that we are drawing conclusions from a limited number of nine populations. A greater number of populations could reinforce our understanding of the mechanisms driving genetic diversity and fitness. However, similar conclusions have been established with even fewer populations than in our study (e.g. Ficetola et al., 2007) allowing us to be confident in the detected effect of the main country road and the motorway on genetic diversity associated with fitness.

At least 65 % of the total records of road mortality consist in anurans (Beebee, 2013). This high rate of road kill can affect strongly the viability of populations near roads even if gene flow is not entirely impeded (Fahrig et al., 1995). Therefore, traffic calming on main roads could be a possible measure to enhance the conservation of populations. In addition to limit road kill, this could mitigate traffic noises which are known to affect amphibians' fitness. This measure seemed efficient for newt but not for species with high sensitivity to fragmentation such as *A. obstetricans* (Garcia-Gonzalez et al., 2012). Although, warning signs or seasonal road closings are possible mitigation measures, they are unlikely to be effective (Glista et al., 2009; Beebee, 2013). Alternatively, wildlife underpasses seems a promising tool to offset the detrimental effects of roads. They have been used for more than 50 years (Beebee, 2013). They have worked well for some species (Woltz et al., 2008; Pagnucco et al., 2012) and were effective to rescue populations in some cases (Jolivet et al., 2008; Beebee, 2013).

To conclude, we revealed that the proximity to main roads was linked to a decrease of the genetic diversity in an European anuran species. Populations with reduced genetic diversity were also the one with low fitness in terms of survival, population growth rate and fecundity. Our results suggest that the limitation of genetic connectivity (gene flow) alone is not driving this pattern but that roads might have more insidious effects related to demographic connectivity. This implies that even if gene flow is maintained among populations, it doesn't mean that populations are viable. A combination of genetic and MRR studies are required if one wants to estimate the long term persistence of populations.

Authors' contributions

JR, and SM contributed to the conception and design of the study. JR, SM and JC collected the data. JR performed laboratory analyses and genotyping. JR, AB and JGP performed data analysis. JR wrote the manuscript. All authors participated in critical revisions of the manuscript.

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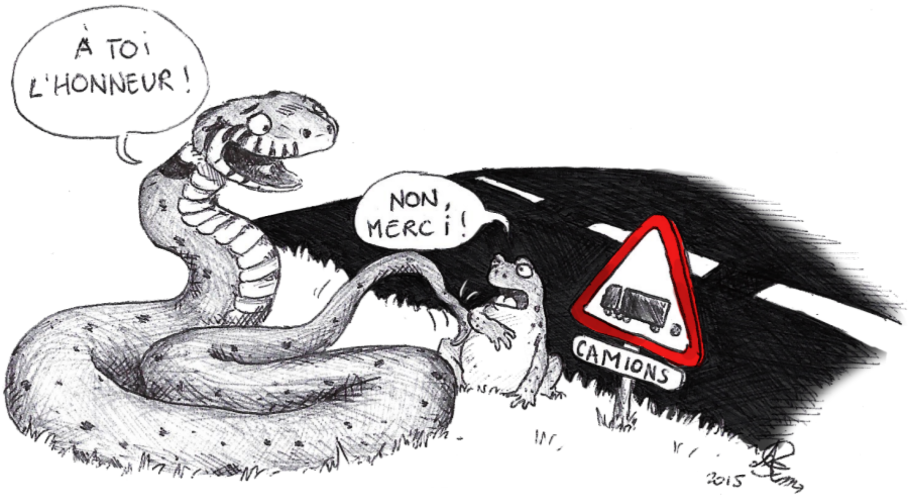
Planche 3. De gauche à droite et de haut en bas : vue sur le site 107 (terrain de moto-cross) ; manipulation nocturne à l'arrière du véhicule de terrain sur le site 118 ; Alyte accoucheur adulte et transpondeur (PIT-tag) ; quelques exemples de variabilité des chapelets d'oeufs transportés par des mâles.

Discussion et perspectives



La prise en considération de la connectivité fonctionnelle dans les outils d'aménagement du territoire est de plus en plus d'actualité. Pour répondre à cette demande, de nombreux outils de modélisation de la "résistance" des milieux permettent d'appréhender la connectivité fonctionnelle potentielle (e.g. chemins de moindre coût, Adriaensen et al., 2003). Toutefois, la validation empirique de ces modèles reste essentielle pour dresser des conclusions robustes en terme de conservation (Spear et al., 2010 ; Zeller et al., 2012). Les données empiriques permettant de valider ces modèles peuvent être de nature très variée. Dans cette thèse, nous nous sommes concentrés sur deux types de données empiriques : les suivis génétiques d'une part (Baguette et al., 2013) et les suivis par Capture-Marquage-Recapture d'autre part (Lebreton et al., 1992). La première approche permet de renseigner la **connectivité génétique** via l'étude des flux de gènes, tandis que la seconde met en évidence la **connectivité démographique** (Lowe and Allendorf, 2010). Utilisées indépendamment, ces deux approches traduisent la connectivité fonctionnelle réelle. Utilisées conjointement, elles permettent de mieux comprendre les effets de la fragmentation sur la viabilité des populations (Lowe and Allendorf, 2010).

Cette thèse s'est focalisée sur la connectivité fonctionnelle réelle de deux espèces d'invertébrés (Féronie noire et Myrtil) et deux vertébrés (Couleuvre helvétique et Alyte accoucheur) dans un paysage fragmenté par six types d'infrastructures en Dordogne (l'autoroute A89, une voie ferrée, la départementale D6089, un gazoduc, une ligne moyenne tension et le réseau routier secondaire).

Dans un premier temps, les outils de génétique du paysage ont été appliqués dans cette zone d'étude afin de comprendre les effets d'un cumul d'ILT (Infrastructures Linéaires de Transport) sur la connectivité génétique (flux de gènes) de cet ensemble d'espèces. Dans un second temps, nous avons développé une méthode permettant de mettre en évidence les effets barrières des ILT en se basant sur des données de mouvements obtenus par CMR. Enfin, nous avons étudié le lien existant entre les données génétiques et démographiques chez plusieurs populations d'Alyte accoucheur fragmentées par le réseau routier.

Apport des données génétiques pour estimer la connectivité fonctionnelle

La génétique du paysage est une discipline en constante évolution avec des avancées statistiques et méthodologiques en perpétuel développement (Balkenhol, Waits and Dezzani, 2009 ; Guillot et al., 2009 ; Richardson et al., 2016). Elle offre une méthode idéale pour estimer la connectivité fonctionnelle réelle via l'étude des flux de gènes dans les paysages. L'une des grandes questions de cette thèse a été de savoir si les ILT perturbent les flux de gènes des espèces étudiées. A l'issue de ce travail, on peut assurément répondre par l'affirmative car les flux de gènes de toutes les espèces, hormis le Myrtil, sont impactés par au moins un type d'ILT. Environ 38 % de la variabilité génétique expliquée entre les quatre espèces dans ce paysage est due aux ILT.

Certains résultats originaux se dégagent de cette thèse. Il s'avère que les effets des infrastructures sont plus complexes qu'attendus. Toutes ne jouent pas un rôle de barrière aux flux de gènes. Certaines participent à favoriser la connectivité génétique et ces résultats sont fortement dépendants de l'espèce étudiée. Par exemple l'A89 a été identifiée comme une forte barrière pour les flux de gènes de la Couleuvre helvétique alors que le réseau routier secondaire participe à améliorer la connectivité génétique chez cette espèce. Ce même réseau secondaire représente une barrière aux flux de gènes pour l'Alyte accoucheur et pour la Féronie noire. De la même manière, la voie ferrée étudiée apparait comme une barrière à la connectivité génétique de l'Alyte accoucheur mais est favorable aux flux de gènes de la Couleuvre helvétique. Ces résultats ont donc de profondes implications pour la gestion de ces infrastructures et la conservation de ces espèces. Comment favoriser la dispersion de l'Alyte accoucheur à travers la voie ferrée tout en maintenant son effet positif pour la dispersion de la couleuvre ? Comment limiter la fragmentation des forêts qui isole la Féronie noire sans impacter les flux de gènes du Myrtil eux-mêmes limités par les forêts ? Doit-on se focaliser sur la conservation spécifique d'une espèce, au risque d'en défavoriser une autre ? Doit-on favoriser la connectivité d'un maximum d'espèce ou se concentrer sur certaines espèces clefs dans les écosystèmes comme les prédateurs supérieurs ?

Il n'existe pas de réponses claires à ces questions. On ne peut qu'encourager les approches globales visant à appréhender les effets de la fragmentation sur la connectivité fonctionnelle du plus grand nombre d'espèces possibles (Baguette et al., 2013). Balkenhol and Waits (2009) invitent à évaluer les effets des infrastructures sur des groupes fonctionnels voire des guildes d'espèces entières.

L'approche multi-spécifique

L'approche multi-spécifique a justement été explorée dans cette thèse. Dans les nombreuses études qui s'intéressent à la connectivité à l'aide de marqueurs moléculaires, la majeure partie se focalise sur une seule espèce (Keller et al., 2015 ; Richardson et al., 2016). Dans une revue de la littérature de Balkenhol and Waits (2009) identifiant l'effet des routes à l'aide de méthodes génétiques, les auteurs montrent que 94 % des études recensées sont mono-spécifiques. Cela s'explique facilement par l'investissement nécessaire sur le terrain qui est démultiplié lorsqu'il s'agit d'échantillonner plusieurs espèces. De même, les coûts humains et financiers liés aux analyses moléculaires en laboratoire sont conséquents, spécialement s'il s'agit de développer les marqueurs moléculaires généralement spécifiques à chaque espèce. A ce titre, il est peu étonnant qu'un aussi faible nombre d'études se soit intéressé à plusieurs espèces simultanément. Cette approche mono-spécifique est critiquée, car elle apporte peu d'informations transposables en terme de mesures de conservation (Segelbacher et al., 2010 ; Keller et al., 2015 ; Richardson et al., 2016). Les rares études s'intéressant à plusieurs organismes simultanément trouvent généralement des effets du paysage qui varient d'une espèce à l'autre (Keller et al., 2015). Certaines, cependant, identifient des éléments paysagers influençant de la même manière plusieurs espèces. Parmi elles, Goldberg and Waits (2010) trouvent que le développement urbain et rural affecte de la même manière plusieurs espèces d'amphibiens. De façon similaire, Delaney et al. (2010) démontrent que l'urbanisation a un rôle négatif sur les flux de gènes de trois espèces de lézards et un oiseau.

Les études comparatives étant rares, la compréhension de la manière dont répondent plusieurs espèces dans un même paysage est limitée. Notre étude a été réalisée spécifiquement pour répondre à cette problématique. On démontre que certains éléments paysagers convergent en terme d'effets sur les flux de gènes, notamment concernant l'effet barrière des routes observé chez l'Alyte accoucheur et la Féronie noire. Cependant, de manière générale, nos résultats indiquent que chaque espèce répond différemment aux éléments du paysage en terme de flux de gènes. Par conséquent, la prise en compte de cet aspect dans les schémas d'aménagement du territoire est complexe. En effet, en se focalisant sur une seule espèce, même si celle-ci est une espèce "parapluie" à tendance généraliste, les prises de décisions favorisant la connectivité de cette espèce pourraient en affecter négativement d'autres. Néanmoins, l'approche multi-spécifique permet de déterminer les éléments paysagers limitant les flux de gènes chez différentes espèces. Il est ainsi possible de créer des carte représentant la résistance des milieux pour chaque espèce. En combinant le degré de recouvrement de ces surfaces résistantes entre les espèces, la recommandation de zones candidates pour la protection du mouvement à travers des corridors peut être proposée (Baguette et al., 2013). Dans la dernière partie de cette discussion, nous nous intéresserons aux mesures de gestion envisageables dans le paysage étudié pour améliorer la connectivité du plus grand nombre d'espèces.

La réplication spatiale

Une composante qui n'a pas pu être explorée durant cette thèse est la réplicabilité spatiale. En effet, il est très difficile de tirer des conclusions générales lorsque un seul paysage est étudiée (Richardson et al., 2016). Étudier un seul paysage revient à avoir une taille d'échantillon de N = 1, ce qui représente peu de valeur statistique (Holderegger and Wagner, 2008), d'où l'importance de répliquer les zones d'études lors des suivis génétiques. Il est vrai que les gestionnaires et les organismes liés à la conservation sont probablement plus intéressés par les études locales qui se concentrent sur les processus écologiques propres à leurs aires de gestion. Toutefois, les informations apportées par des études répliquées permettent de dresser de solides conclusions et des lignes de conduites pour la gestion applicables à d'autres paysages (Keller et al., 2015).

En réalité, très peu d'études ont réalisé de la réplication spatiale pour étudier les effets des éléments paysagers sur les flux de gènes (Richardson et al., 2016). Les rares études étudiant cette dynamique multi-sites trouvent des résultats très différents d'un paysage à l'autre. Par exemple, Short Bull et al. (2011) ont étudié les flux de gènes de l'Ours noir dans 12 paysages. Ils trouvent que les éléments affectant les flux de gènes varient d'un paysage à l'autre. De la même manière, Balbi et al. (2018) ont estimé les effets des éléments paysagers dans 12 sites sur les flux de gènes de l'escargot Petit-gris. Ils démontrent une difficulté pour généraliser les effets des éléments paysagers. Cependant, certaines études répliquées ont permis de faire ressortir des résultats cohérents entres les paysages. C'est le cas de Villemey et al. (2016) qui ont étudié la connectivité génétique du Myrtil à travers trois grands ensembles en France. Cette analyse répliquée met en évidence l'effet conductant des milieux ouverts alors que les terres arables et les milieux forestiers limitent les flux de gènes.

Dans notre étude, il était inenvisageable de traiter cette réplicabilité spatiale tout en gardant notre approche multi-spécifique ; l'investissement aurait été trop conséquent. D'autres études en génétique du paysage ont réalisé la démarche inverse (e.g. Villemey, 2015) mais les études s'intéressant à ces deux processus simultanément restent marginales (e.g. Prunier et al., 2018). Pour les futures études en génétique du paysage, nous suggérons que traiter l'aspect multi-spécifique dans plusieurs sites répliqués (au minimum trois) permet de dresser des conclusions robustes en terme de mesure de gestion.

Dimension temporelle dans les études génétiques

Lors de l'utilisation des outils génétiques, il existe nécessairement un délai entre la modification de l'habitat et la signature génétique détectable (Epps and Keyghobadi, 2015). Le nombre de générations nécessaires pour détecter un effet génétique après la création d'une barrière varie entre une et 200 générations (Landguth et al., 2010). Cette vitesse de détection est dépendante des métriques génétiques utilisées (Fst ou Mantel r par exemple) ainsi que des capacités de dispersion de l'espèce considérée. Pour une espèce avec des capacités de dispersion supérieures à 10 km, la détection de l'effet barrière peut être très rapide, de l'ordre de 1 à 3 générations (Landguth et al., 2010). La vitesse de détection de l'effet dépendra donc étroitement des abondances et du kernel de dispersion de l'espèce considérée. Le contexte paysager et les infrastructures étudiées sont également susceptibles d'influencer la vitesse de détection des effets. Dans une récente étude, Prunier et al. (2014) montrent qu'une autoroute âgée de 41 ans est implantée depuis suffisamment longtemps pour permettre la détection d'un effet sur les flux de gènes du Triton alpestre. A l'inverse, les simulations utilisées dans cette même étude identifient une voie ferrée âgée de 29 ans comme trop récente pour permettre la détection d'un effet sur les flux de gènes de ce triton. Certaines études ont cependant détecté des effets d'infrastructures récentes sur la variabilité génétique. A titre d'exemple, Yu et al. (2017) sont en mesure de détecter les effets barrières d'une voie ferrée grillagée de moins de 10 ans sur un grand herbivore. Cette étude concerne une espèce avec une grande capacité de dispersion, qui d'après notre remarque précédente, est susceptible d'engendrer une vitesse de détection de l'effet plus rapide qu'une espèce moins mobile.

Dans notre aire d'étude, hormis l'A89 qui a été mise en service récemment, les ILT étudiées ont au minimum 53 ans. Parmi elles, deux infrastructures sont particulièrement anciennes : la voie ferrée (environ 150 ans) et la D6089 (au moins 200 ans). Ces laps de temps paraissent suffisamment long pour que l'effet des ILT se soit retranscrit dans la variabilité génétique des quatre espèces étudiées.

La portion de l'autoroute A89 considérée dans notre étude est cependant récente. Elle a été mise en service en 2004 avec un début de construction en 2001, ce qui représente moins de 15 ans depuis le début de l'étude. Ce laps de temps parait relativement court au vu des travaux de Landguth et al. (2010). Nous avons néanmoins été en mesure de mesurer les effets de l'A89 sur la variabilité génétique de deux espèces (limitation des flux de gènes chez la Couleuvre helvétique et augmentation chez l'Alyte accoucheur). En revanche, l'absence d'effet détecté pour les deux espèces d'invertébrés nous invite à la prudence concernant notre capacité à mesurer les effets de l'A89 en utilisant la génétique du paysage. En effet, considérant la forte sensibilité de la Féronie noire à la fragmentation de son habitat (Charrier et al., 1997) et de l'effet barrière des routes sur sa capacité de dispersion (chapitre 1 et Keller et al., 2004), un effet barrière de l'A89 était attendu. Par ailleurs, le chapitre 2 a mis en évidence l'effet barrière conséquent de l'A89 sur les capacités de franchissement du Myrtil en utilisant des suivis CMR. L'absence d'effet détectée en utilisant les outils moléculaires chez ces deux espèces peut être due à des tailles de populations effectives importantes (Gauffre et al., 2008 ; Prunier, Dubut, Chikhi and Blanchet, 2017) ou à un délai trop récent entre notre étude et la construction de l'A89. Cette limitation montre l'importance de la réplication temporelle des études en génétique sur de grands laps de temps afin de comprendre pleinement les effets des ILT sur la connectivité et la persistance des populations (Balkenhol and Waits, 2009).

Choix des espèces

De manière générale, les lépidoptères sont des organismes difficiles à étudier par des approches moléculaires, et ce, pour plusieurs raisons. Premièrement, les lépidoptères semblent posséder une fréquence de microsatellites plus faible que d'autres organismes

(Nève and Meglécz, 2000), ce qui rend le développement de marqueurs moléculaires laborieux (Meglecz et al., 2004). Deuxièmement, ils ont un fort taux de mutation qui peut affecter les régions flanquantes des marqueurs microsatellites et générer des allèles nuls (allèles présents mais non amplifiés car les amorces ne peuvent plus se fixer) (Meglecz et al., 2004 ; Chapuis and Estoup, 2007). Ces caractéristiques se sont avérées très limitantes pour les analyses avec le Myrtil car seulement six marqueurs ont pu être exploités sur les 15 initialement utilisés. Cette difficulté semble récurrente et a déjà été identifiée dans une précédente étude sur la même espèce (Villemey, 2015). Nous ne pouvons donc que déconseiller cette espèce comme modèle d'étude pour de futures recherches en génétique du paysage. L'utilisation de nouvelles techniques de séquençage (e.g. RADseq, Davey and Blaxter, 2010) serait potentiellement en mesure de surmonter cette difficulté.

Malgré ces limites, nous avons pu expliquer une part non négligeable de la variabilité génétique chez le Myrtil avec seulement six marqueurs dans cette zone d'étude (20 %). Nous avons également identifié de l'isolation par la distance dans ce paysage et un effet notoire des milieux forestiers qui limitent les flux de gènes de ce papillon. Il est possible que pour cette espèce, l'échantillonnage population-centré, en comparaison à un échantillonnage individu-centré (Villemey et al., 2016) soit en mesure de mieux expliquer la variabilité génétique. En effet, les analyses individus-centrés engendrent une perte d'information locale car moins d'échantillons sont récoltés par site d'échantillonnage. De plus, l'augmentation du nombre de sites échantillonnés entraine un renforcement de mesures appariées qui sont susceptibles d'engendrer une augmentation du "bruit de fond" par rapport au signal génétique recherché (Prunier et al., 2013). Une comparaison approfondie entre l'approche individu et population-centré reste nécessaire.

La Couleuvre helvétique s'est avérée être un modèle d'étude compliqué. Outre la difficulté d'échantillonnage sur le terrain due à sa faible probabilité de détection, la part de variabilité génétique expliquée a été très faible (4 %) comparativement aux autres espèces étudiées dans cette thèse. Plusieurs études ont souligné précédemment cette faible variabilité génétique dans des paysages similaires en utilisant, en partie, les mêmes marqueurs microsatellites : Meister et al. (2010) ne détectent aucune structuration génétique chez cette même couleuvre dans un paysage agricole fragmenté de 90 km^2 en Suisse. Ils en concluent que cette espèce est très mobile dans ce type de milieu fragmenté et que des flux de gènes fréquents limitent la structuration génétique. A plus large échelle cependant, Meister et al. (2012b) s'intéressent à la différenciation génétique de la Couleuvre helvétique dans trois paysages différents, toujours en Suisse. Ils observent une légère différenciation génétique entre paysages qui semble être expliquée par de l'isolation par la distance plutôt que par des éléments paysagers limitant les flux de gènes (Meister et al., 2012b). Des espèces de reptiles possédant un niveau de phylopatrie plus élevé pourraient s'avérer être de meilleures candidates pour ce type de problématique. La Vipère péliade (*Vipera berus*) pourrait répondre à ce critère car elle possède des capacités de dispersion plus limitées ainsi qu'une forte structuration génétique à l'échelle locale (Ursenbacher et al., 2009 ; François et al., 2018). La Vipère péliade n'était pas présente sur notre zone d'étude mais son aire de répartition couvre une grande partie du plateau Eurasien (Arnold and Ovenden, 2010). De ce fait, cette espèce serait également idéale pour réaliser une réplication spatiale couvrant une vaste aire géographique.

Les deux autres espèces étudiées (Alyte accoucheur et Féronie noire) ont été des choix très pertinents pour cette étude. Leurs structurations génétiques étaient importantes sur la zone d'étude et la part de variabilité génétique expliquée était conséquente (entre 11 et 20 % chez l'Alyte accoucheur et entre 17 et 27 % chez la Féronie noire selon la méthode employée). De plus, les résultats issus des analyses étaient en concordance avec des études précédentes réalisées chez les mêmes espèces (Keller et al., 2004 ; Garcia-Gonzalez et al., 2012).

Il aurait été intéressant d'étudier la connectivité génétique d'autres groupes durant cette thèse comme les oiseaux et mammifères. L'étude de la connectivité de l'Ecureuil roux avait été envisagée au début du projet mais n'a finalement pas pu se réaliser faute de moyens financiers et humains.

Perspectives pour les analyses en génétiques du paysages

Dans les études génétiques s'intéressant à la relation entre paires d'individus ou de populations (link model), la non-indépendances des données se traduit régulièrement par un accroissement du risque d'erreurs de type I (rejet à tort de l'hypothèse nulle). Ce biais statistique concerne particulièrement les analyses basées sur les tests de Mantel ou partiels de Mantel (Balkenhol, Waits and Dezzani, 2009 ; Guillot and Rousset, 2013 ; Legendre et al., 2015). Ces méthodes restent pourtant, les plus utilisées encore de nos jours (Keller et al., 2015). De nouvelles méthodes permettant de contourner ce biais sont souvent citées. Parmi elles, les méthodes basées sur des analyses multivariées : dbRDA (Mcardle and Anderson, 2001) et MEM (Borcard and Legendre, 2002) ou bien des méthodes utilisant de l'inférence bayésienne (Bradburd et al., 2013). Les modèles linéaires mixtes (LMM) sont également très performants (Shirk et al., 2017) et ont été utilisés avec succès dans de nombreuses études (Richardson et al., 2016).

Cependant, comme explicité dans le chapitre 1, la colinéarité entre prédicteurs peut fortement biaiser les résultats issus des analyses génétiques en distordant les paramètres utilisés pour estimer les effets des prédicteurs (Prunier et al., 2015). La prise en compte de cette collinéarité est cruciale et pourtant largement oubliée dans les analyses en génétiques du paysage. Dans notre étude, l'analyse des commonalités a permis une estimation fine des effets des éléments paysagers sur la variabilité génétique, donnant lieu à une hiérarchisation de ces effets. On a ainsi pu montrer que la route D6089 explique plus fortement la variabilité génétique des quatre espèces (15 % de la variabilité expliquée) que la voie ferrée (2,5 %) ou l'autoroute (5 %) par exemple. Au stade actuel, seules les régressions multiples sur des matrices de distances (RDM) permettent une estimation de cette colinéarité via l'approche des commonalités (Prunier et al., 2015). On peut cependant espérer que dans le futur, des développements méthodologiques permettront de réaliser des analyses de commonalités avec des modèles mixtes.

Apport des données de CMR pour estimer la connectivité fonctionnelle

L'estimation de la connectivité fonctionnelle par des suivis génétique est très riche d'informations (Baguette et al., 2013) mais inenvisageable en pratique dans certains cas de figures (infrastructures trop récentes, échantillonnage non adapté, manque de ressources financières et de compétences, *etc.*). L'étude du mouvement direct des individus par capture-marquage-recapture est alors une alternative prometteuse qui permet d'appréhender la connectivité fonctionnelle réelle. Cependant, la mise en évidence d'événements de dispersion (qui soutiennent la connectivité fonctionnelle) peut être difficile avec ce type d'approche. En effet, la dispersion est un phénomène complexe, multi-factoriel qui sous-entend que l'individu dispersant quitte son habitat, se déplace dans le paysage et s'installe dans un nouvel habitat pendant suffisamment longtemps pour permettre la reproduction (Ronce, 2007 ; Baguette et al., 2013). L'utilisation de distributions théoriques pour générer des kernels de dispersion à l'échelle locale est une méthode permettant de prédire les mouvements de longues distances soutenant la dispersion (Baguette, 2003). Par conséquent, étudier comment les kernels de dispersion sont impactés par les infrastructures de transport est pertinent car une modification du kernel aura des répercussions directes sur la connectivité fonctionnelle de l'espèce considérée dans les paysages.

Dans le chapitre 2, nous avons développé une méthode permettant d'estimer les effets barrières des éléments linéaires dans les paysages comme les ILT. Cette méthode est basée sur les kernels de dispersion issus de suivis CMR et s'intéresse au mouvement des individus à l'échelle locale. En l'appliquant au papillon Myrtil, nous avons identifié que l'autoroute A89 est responsable d'une modification du kernel de dispersion de ce papillon et qu'elle agit comme une barrière à la dispersion. Ce résultat a de profondes implications en matière de conservation des papillons à proximité des grandes infrastructures routières (Skórka et al., 2013 ; Baxter-Gilbert, Riley, Neufeld, Litzgus and Lesbarrères, 2015 ; Skórka et al., 2015) et sera discuté dans la dernière partie.

La méthode développée s'est avérée robuste en terme de capacité à estimer les effets barrières des ILT. Pour une estimation fiable de l'effet d'une ILT, le ratio entre la largueur de l'infrastructure et la distance moyenne du kernel de l'espèce étudiée ne doit pas excéder 0,7. A titre d'exemple, cela implique qu'en étudiant l'effet barrière d'une route de 7 m de large, la capacité moyenne de déplacement de l'espèce doit être au minimum de 10 m. Un grand nombre d'organismes est susceptible d'avoir des capacités de mouvements supérieures à cette valeur. Par conséquent, cette méthode pourra être envisagée dans beaucoup de cas de figures. Ces applications sont nombreuses et pourront permettre de mesurer l'effet barrière des infrastructures mais aussi d'évaluer l'efficacité des structures de franchissements (passages à faune, éco-ponts, passages souterrains, *etc.*). En effet, il est impératif de disposer de mesures d'évaluation de ces structures de franchissement qui aillent au-delà de la simple observation d'animaux empruntant ces passages (Lesbarrères and Fahrig, 2012 ; van der Grift et al., 2013). Cette méthode peut répondre à ce type de problématique.

Limites de l'étude du mouvement

Cette méthode a été appliquée sur le Myrtil qui possède des tailles de populations très conséquentes et facilite donc l'acquisition d'un nombre important de données de capture/recapture. La puissance de détection des effets barrières de cette méthode est dépendante du nombre d'individus recapturés. Plus il sera grand, plus la méthode sera robuste pour détecter les effets. Par conséquent cette méthode ne sera pas applicable à des organismes à faibles probabilités de recapture où des suivis par télémétrie ou GPS seront plus appropriés (e.g. Dyer et al., 2002 ; Bartzke et al., 2015 ; Siers et al., 2016).

De manière similaire aux analyses génétiques, la réplication spatiale des zones d'études est en mesure d'apporter une meilleure compréhension des effets des ILT sur le mouvement. Dans notre cas, nous avons réalisé des suivis CMR sur deux sites d'études, mais chacune représentait un réplicat spatial. Bien que l'investissement de terrain soit conséquent, les futures études devront s'attarder davantage sur la réplication spatiale.

Perspectives dans l'étude du mouvement

L'étude du mouvement des organismes apporte une quantité d'information considérable. Ces informations permettent d'appréhender la connectivité fonctionnelle dans les paysages et de proposer des mesures de gestion. Pourtant, l'étude du mouvement est difficile en milieux naturels et dépend étroitement des avancées technologiques. Dans cette étude, nous avons réalisé des suivis CMR qui apportent une vision fragmentée du mouvement. En effet, entre deux événements de capture d'un individu, le mouvement observé peut différer fortement du mouvement réel. Les suivis continus des individus seraient en mesure d'apporter une estimation plus précise du mouvement, de mieux estimer les kernels de dispersion et d'étudier les comportements des papillons à l'interface avec les ILT.

Les nouveaux dispositifs de suivis, comme les balises GPS, sont de plus en plus performants et miniaturisés, permettant ainsi le suivi continu d'organismes impossible jusqu'alors. De plus, ces appareils permettent maintenant d'enregistrer simultanément des informations physiologiques de l'individu (rythme cardiaque par exemple), tout en mesurant des paramètres environnementaux (luminosité, profondeur, altitude, vitesse, *etc.*) (Kays et al., 2015). L'utilisation de ce type de dispositif chez les papillons n'est pas encore envisageable à cause du poids et de l'encombrement qu'il représente. Cependant, la miniaturisation des puces RFID (Särkkä et al., 2014), des antennes télémétriques et l'utilisation des radars harmoniques (Cant et al., 2005) permet d'entrevoir des suivis dans les milieux naturels beaucoup plus précis chez ces organismes.

Couplage des données génétiques et démographiques en écologie du paysage

Comme vu précédemment, la génétique du paysage et l'étude du mouvement par des suivis CMR sont deux approches pertinentes pour mesurer la connectivité fonctionnelle dans les paysages. Pourtant, l'emploi exclusif de l'une de ces deux approches en écologie du paysage n'apporte qu'une vision parcellaire de la connectivité. En effet, comme évoqué plus haut, les événements de dispersion sont difficiles à mettre en évidence avec les suivis CMR. Ce sont pourtant les événements de dispersion qui induisent la connectivité fonctionnelle dans les paysages. D'un autre côté, les suivis génétiques offrent une méthode idéale pour appréhender la dispersion par l'étude des flux de gènes entre les populations.

Cependant, le flux de gènes n'est pas le seul processus impliqué dans la variabilité génétique. Les adaptations locales et la dérive génétique sont d'autres facteurs qui contribuent à la variabilité génétique observée (Richardson et al., 2016). Or, ces deux autres processus sont rarement considérés malgré leur implication pour la conservation. De plus, l'étude de la variabilité génétique seule, n'apporte que peu d'information sur la viabilité des populations si elle n'est pas couplée à des suivis démographiques (Lowe and Allendorf, 2010). Dans cette partie, nous démontrons l'importance d'associer les suivis génétiques avec des suivis CMR pour (i) estimer la viabilité des populations, (ii) estimer l'importance de la dérive et (iii) nuancer l'importance des flux de gènes dans les paysages.

Estimer l'effet de la connectivité sur la viabilité des populations

La génétique du paysage offre une méthode idéale pour mettre en évidence la dispersion effective car elle s'intéresse aux flux de gènes entre les populations. Ce processus illustre la connectivité génétique qui se distingue de la connectivité démographique. Cette dernière s'intéresse aux effets de la dispersion sur les processus démographiques des populations comme leurs taux de croissance et paramètres vitaux (Lowe and Allendorf, 2010). La connectivité démographique contribue à la viabilité des populations sur le long terme et son étude est cruciale pour estimer la persistance des populations dans les paysages subissant des pressions anthropiques. Or, l'étude exclusive des flux de gènes (connectivité génétique) n'informe pas sur ces processus. Pour pouvoir estimer la connectivité démographique, il est nécessaire de mesurer les taux démographiques intrinsèques aux populations en plus d'informations sur la dispersion. Lowe and Allendorf (2010) proposent que la réalisation de suivis génétiques combinés avec des suivis directs est la seule manière de bien appréhender les effets de la dispersion sur la viabilité des populations.

Les suivis CMR, et en particulier les modèles multi-états (Brownie et al., 1993) sont idéaux pour ce type de problématique. Les modèles multi-états permettent l'estimation de paramètres démographiques au sein de chaque population tout en estimant les probabilités de transitions d'une population à l'autre (événements de dispersion). Pour cela, il est nécessaire d'établir un plan de suivi conséquent, représenté par plusieurs populations connectées entres elles par des événements de dispersion (méta-population). Une telle approche couplée avec un suivi génétique n'a encore jamais été réalisée (Lowe and

Allendorf, 2010). Elle permettrait d'évaluer le degré de corrélation entre connectivités génétique et démographique.

Dans le chapitre 3, nous avons tenté une approche de ce type avec l'Alyte accoucheur en suivant trois populations par CMR et en estimant les paramètres génétiques de neuf populations. Il était cependant impossible de mettre en place un suivi CMR multi-états avec un réseau de populations d'alytes connectées. En effet, d'une part l'investissement en temps et en moyens pour réaliser un tel suivi était inenvisageable. D'autre part, nous n'avons pas réussi à identifier un réseau de populations connectées sur la zone d'étude. Il aurait, de plus, été très intéressant d'identifier un réseau sectionné par une ou plusieurs ILT afin d'appliquer sur l'Alyte accoucheur la méthode développée dans le chapitre 2. Malgré ces limites, nous avons mis en évidence une corrélation entre paramètres démographiques et diversité génétique. Nous avons montré que les populations d'alytes accoucheurs avec des taux vitaux réduits (survies, croissance, fécondité) étaient celles qui possédaient les diversités génétiques les plus faibles. Cette viabilité limitée chez certaines populations ne semble pas être liée à une réduction des flux de gènes. Cela rejoint Richardson et al. (2016) qui stipulent que la structure génétique des populations n'est pas forcément le reflet des flux de gènes dans le paysage. Connectivités démographique et génétique ont en réalité des implications pour la conservation très différentes. A la lumière des sources multiples de structuration génétique, il est d'une importance capitale que les processus démographiques pouvant contribuer aux patrons observés soient pris en considération.

De la même manière, en considérant les sources multiples de structuration démographiques des populations, les suivis génétiques sont en mesure d'approfondir notre compréhension des processus affectant les populations. Par exemple les mesures génétiques de consanguinité (Fis) peuvent informer si des populations ont subi des phases de goulot d'étranglement “bottleneck” ou des effets fondateur “founder effect”. Des suivis démographiques seuls ne seraient pas en mesure d'identifier si une population a subi de tels processus par le passé.

Des suivis CMR permettant de mesurer les paramètres démographiques ne sont pas systématiquement envisageables. Cependant, le simple fait de marquer des individus et de les recapturer ultérieurement apporte de l'information sur les comportements et mouvements des individus. Ces informations aident à mieux comprendre les dynamiques de flux de gènes dans les paysages. Cet aspect a été exploité durant cette thèse avec le Myrtil. Pour cette espèce, les analyses des flux de gènes identifient une connectivité génétique non influencée par l'A89 en Dordogne (chapitre 1). En revanche les suivis CMR montrent que les événements de franchissement de ce papillon au dessus de l'A89 sont cinq fois réduits comparativement à une absence d'autoroute (chapitre 2). Une déconnexion similaire entre suivis génétiques et CMR a été précédemment mise en évidence sur l'Albatros hurleur (Milot et al., 2008).

Estimer l'importance de la dérive génétique

La génétique du paysage cherche à identifier des éléments paysagers influençant la variabilité génétique. Si l'on exclut les mutations, cette variabilité génétique est en réalité le sous-produit de deux processus indépendants : les flux de gènes et la dérive génétique. La dérive génétique est un processus évolutif de fluctuations aléatoires des fréquences alléliques qui est amplifié dans les petites populations (Allendorf, 1986). Par conséquent, la variation spatiale des tailles de populations peut engendrer des phénomènes de dérive et augmenter la différenciation génétique entre populations. De ce fait, les distances génétiques entre populations peuvent augmenter à cause d'une réduction de la connectivité génétique (flux de gènes) mais également à cause de la variation spatiale des tailles efficaces des populations (Richardson et al., 2016 ; Prunier, Dubut, Chikhi and Blanchet, 2017). La dérive génétique a donc des effets conséquents sur la variabilité génétique observée. Elle est pourtant rarement considérée dans les études en génétique du paysage (Richardson et al., 2016).

Une manière d'estimer la contribution de la dérive dans la variabilité génétique consiste à mesurer la taille efficace des populations et de l'intégrer dans les analyses génétiques sous la forme d'un nouveau prédicteur (Prunier, Dubut, Chikhi and Blanchet, 2017). Cependant, la taille efficace des populations est compliquée à mesurer dans la nature (Prunier, Dubut, Chikhi and Blanchet, 2017) et les estimations sont très variables selon la méthode utilisée pour la calculer (Wang, 2016). De plus, cette information reste circulaire puisque des données génétiques sont utilisées pour estimer les tailles efficaces des populations qui sont elles même comparées aux données génétiques de différenciation. L'alternative proposée par Prunier, Dubut, Chikhi and Blanchet (2017) consiste à utiliser les tailles réelles des populations. Par conséquent, les suivis démographiques par CMR permettraient de calculer ces tailles de populations afin de les intégrer dans les analyses génétiques pour évaluer la contribution de la dérive. Le couplage des suivis génétiques et CMR permettrait ainsi une estimation conjointe de la dérive génétique et des flux de gènes.

Effet de la connectivité chez les populations adaptées

Les flux de gènes entre populations sont souvent décrits comme universellement bénéfiques pour la viabilité des populations (Richardson et al., 2016). Une rupture des flux de gènes entre populations sera immédiatement perçu comme un risque conséquent pour la persistance de ces populations. On peut cependant se demander si une meilleure connectivité fonctionnelle est nécessairement le “Graal” que toute mesure de conservation devrait poursuivre. Il existe des cas de figure où une limitation des flux de gènes a des effets positifs sur les populations (e.g. Rosenblum, 2006 ; Nosil, 2009 ; Richardson and Urban, 2013). C’est notamment le cas lorsque les populations sont adaptées localement à certaines contraintes du milieu (prédateurs dans certaines mares et absents dans d’autres par exemple, Richardson and Urban, 2013). Ces populations se sont adaptées à ces perturbations locales et bénéficient d’allèles spécifiques apportant un bénéfice en terme de fitness. Cette divergence adaptative entre populations sera contrainte par l’effet d’homogénéisation porté par des flux de gènes conséquents. En effet, il existe une corrélation négative entre le degré de divergence adaptative et le niveau de flux de gènes entre populations (Nosil, 2009) (mais voir : Clobert et al., 2009 ; Edelaar and Bolnick, 2012). Un flux de gènes important pourrait s’avérer délétère car la fréquence des allèles adaptés dans les populations diminuera par l’apport continuel de gènes mal-adaptés provenant de populations extérieures (Richardson and Urban, 2013). Ce phénomène appelé “migration load” (Bolnick and Nosil, 2007) engendre une baisse de fitness des individus issus de la reproduction des parents adaptés avec des parents mal-adaptés. Par conséquent, les flux de gènes sont susceptibles de limiter la capacité d’adaptation des populations dans la nature (Rosenblum, 2006 ; Nosil, 2009). La limitation du flux de gènes aura pour effet d’augmenter la réponse à la sélection et d’améliorer la fitness des populations locales (Richardson et al., 2016). Il apparait donc évident que la mesure du flux de gènes seule ne permet pas une estimation de la viabilité des populations dans les paysages. L’estimation des taux vitaux de ces populations par des suivis CMR serait en mesure d’estimer la viabilité des populations ainsi que les conséquences des individus dispersants sur les dynamiques locales.

Application pour les gestionnaires

Dans ce travail de thèse, l’utilisation de suivis génétique et CMR a mis en évidence des effets barrières à la connectivité fonctionnelle qui concernent plusieurs espèces à travers

FIGURE H – Passages souterrains sous l'A89 et la voie ferrée (buses et passage agricole) ainsi que grillage à petite faune de l'A89 perforé

l'A89, la D6089, les routes (D6089 et réseau routier secondaire), la voie ferrée ainsi que le gazoduc. Par conséquent, certaines mesures de gestion sur cette zone d'étude peuvent être envisagées. De manière générale, nous encourageons la volonté de diversifier les structures permettant le franchissement des ILT par la biodiversité. Ces mesures peuvent concerner l'amélioration des ouvrages déjà présents sur la zone d'étude mais aussi servir de ligne directrice pour améliorer la connectivité à travers de futurs projets ou la requalification de certaines infrastructures existantes.

Les structures souterraines

Concernant les infrastructures étudiées, et en particulier l'A89, il existe actuellement plusieurs structures souterraines permettant le franchissement de la faune. C'est notamment le cas des buses ou passages agricoles (Fig. H).

Cependant, ces structures ont peu de chances d'être utilisées par les lépidoptères pour cause de perturbations thermiques et manque de lumière (Dennis, 1986). Ce phénomène a pu être observé lors des sessions de CMR sur Myrtil ; le site d'étude présentait un passage agricole sous l'A89 et les rares individus s'engageant dans ce passage ont rapidement fait demi-tour (observations personnelles). De même, les serpents sont réticents à utiliser ces structures souterraines pour des raisons thermiques principalement (Rodriguez et al., 1996 ; Baxter-Gilbert, Riley, Lesbarrères and Litzgus, 2015). Dans ce travail de thèse, nous avons mis en évidence l'effet barrière de l'A89 sur les flux de gènes de la Couleuvre helvétique malgré un nombre important de passages souterrains le long de l'A89.

Ces structures peuvent néanmoins favoriser le franchissement de certains organismes tel que les amphibiens et les mammifères (Lesbarrères and Fahrig, 2012). Pour les amphibiens, l'utilisation de ces passages nécessite qu'ils soient humides car ces organismes sont peu disposés à utiliser les passages aériens ou les tunnels asséchés (Lesbarrères et al., 2004). Dans ce travail de thèse, l'A89 semble promouvoir les flux de gènes de l'Alyte accoucheur. Ce résultat original nécessiterait cependant d'être confirmé via des suivis directs par CMR. Toutefois, il est possible que les bassins de rétentions offrent des habitats alternatifs intéressants pour cette espèce et que les passages transversaux souterrains de l'A89 soient fréquemment empruntés par cette espèce, spécialement lors des événements pluviaux qui les remplissent (Prunier et al., 2014). Concernant les espèces plus imposantes comme les grands mammifères, la dimension de ces passages souterrains doit être suffisamment grande pour qu'ils soient empruntés. Pour le passage de cerfs, Gordon and Anderson (2003) préconisent la construction de passages mesurant au minimum 6 m de large pour 2,5 m de hauteur.

L'aménagement des structures de franchissement des cours d'eaux est vraisemblablement la meilleure stratégie pour améliorer la connectivité (Lesbarrères and Fahrig, 2012). En effet, lorsqu'une infrastructure intersecte un cours d'eau de faible dimension, celui-ci est redirigé de l'autre côté à travers une buse ou un conduit de drainage. Le remplacement de ces buses par des structures plus imposantes permettrait le franchissement d'un plus grand nombre d'organismes. Cela se traduirait par des structures plus hautes et plus larges permettant aux cours d'eau de couler librement. Des berges importantes permettraient également aux organismes terrestres de traverser même en cas de crues et la possibilité que de la végétation puisse s'implanter.

Les structures aériennes

Les passages à faune construits au-dessus des infrastructures dédiés au franchissement de la faune offrent un complément prometteur aux passages souterrains. Sur la zone d'étude, il n'en existe qu'un seul (Fig. I). Il s'agit, cependant, d'un passage dit “mixte” ne visant pas spécifiquement la connectivité de la faune. Ce type de structure possède en plus d'un chemin, une bande enherbée permettant à certains organismes de traverser

Figure I – Unique passage à faune jusqu'à présent sur la zone d'étude

sur un substrat différent du chemin empierré. Cette seule structure est probablement insuffisante pour soutenir la connectivité de la faune présente sur la zone d'étude. Un éco-pont est cependant au cours de construction sur la commune de Limeyrat (travaux initiés en 2017). Cet ouvrage mesurera, à terme, 25 m de large et sera entièrement dédié au passage de la faune. Des mares de chaque côté ainsi qu'un muret et autres micro-habitats seront en mesure de favoriser le franchissement de l'autoroute par de nombreux organismes. Ce type de structure pourrait ainsi soutenir la dispersion de la Couleuvre helvétique qui, on l'a vu, est négativement affectée par l'A89.

Afin de limiter les effets fragmentant des ILT, un nombre plus important de ce type de structure est à envisager (Lesbarrères and Fahrig, 2012). Il existe, en France, 1685 passages pour animaux sauvages (pour l'essentiel de petits passages "mixtes") pour environ 9137 km d'autoroute concédées (chiffres 2016, ASFA, 2017). Cela représente environ un passage tous les 6 km, un nombre insuffisant pour garantir la connectivité paysagère (Carsignol, 2006).

La construction d'éco-ponts est une démarche louable, mais encore faut-il que ces structures soient empruntées par la faune. La dimension médiatique diffusée autour de ces projets masque souvent l'absence de preuves réelles de leur efficacité. Pour qu'un écopassage soit emprunté, il est nécessaire que l'infrastructure possède une structure d'exclusion comme un grillage par exemple, afin d'éviter que les organismes ne se retrouvent sur la chaussée, et de les rediriger vers la structure de franchissement (Lesbarrères and Fahrig, 2012). Ces structures d'exclusions doivent être effectives, sans quoi la construction d'éco-passages peut s'avérer inefficace (Baxter-Gilbert, Riley, Lesbarrères and Litzgus, 2015). Un entretien régulier du système d'exclusion est nécessaire pour éviter que les organismes puissent passer outre. Ce point est particulièrement important concernant la petite faune, plus difficile à rediriger lorsque le grillage possède un maillage peu dense. Une solution utilisée est d'appliquer un second grillage d'exclusion d'environ 30 cm de haut et possédant un maillage très fin (Carsignol, 2006). Cependant, ce grillage peut se détériorer avec le temps et des trous peuvent se former rapidement si un entretien régulier n'est pas réalisé (Fig. H).

Pour résumer, les passages aériens sont des structures prometteuses, mais coûteuses. Leurs efficacité est difficile à estimer (Lesbarrères and Fahrig, 2012) et elles n'empêchent pas la mortalité routière pour les espèces avec un cycle de vie aérien (insectes, oiseaux, chauve-souris) pour lesquelles les structures d'exclusions classiques sont inefficaces (Baxter-Gilbert, Riley, Neufeld, Litzgus and Lesbarrères, 2015). Elles sont, de plus, encore aujourd'hui pensées essentiellement pour le franchissement du grand gibier (Carsignol, 2006).

Elles jouent néanmoins un rôle pour la dispersion des organismes et on ne peut qu'encourager le développement de ces structures. On peut espérer que les futurs éco-ponts seront également pensés pour permettre le franchissement des espèces strictement forestières comme les carabes forestiers, très réticents à s'engager dans des milieux ouverts (Charrier et al., 1997). Des avancées techniques seront sûrement à considérer pour que ce type de structure puisse supporter le développement racinaire d'arbres matures sans engendrer de dommages à la structure du pont.

Modération du trafic et changements des comportements

L'atténuation du trafic routier par l'adoption d'une vitesse limitée est une mesure envisageable afin de luter contre la mortalité routière (Garcia-Gonzalez et al., 2012). Cette mesure s'applique également aux trains qui par l'adoption d'une vitesse réduite, notamment lors des périodes de reproductions ou de migrations de la faune, limite la mortalité (Borda-de Agua et al., 2017).

Une autre mesure permettant de limiter la mortalité routière consiste à éduquer les conducteurs de véhicules (Kioko et al., 2015). En effet, il apparait que la grande majorité des usagers de la route sont indifférents aux problèmes de mortalités routières de la faune (Kioko et al., 2015). Une sensibilisation de ces personnes parait être une mesure efficace pour favoriser des comportements responsables face à ces problématiques. Une telle mesure ne serait pas transposable pour la mortalité liée aux voies ferrées due à l'impossibilité de dévier la trajectoire d'un train. En revanche, certaines méthodes très récentes utilisant des signaux avertisseurs seraient susceptibles de diminuer drastiquement la mortalité sur les voies ferrées (Backs et al., 2017).

Les lignes électriques et gazoducs

Pour assurer le bon fonctionnement des lignes électriques ou des gazoducs, la végétation sur leurs emprises est maintenue basse avec un entretien régulier via des coupes à ras ou l'épandage de pesticides (Bramble and Byrnes, 1983). Cet entretien permet la dispersion de plantes pionnières et favorise la connectivité de certaines espèces comme les abeilles (Russell et al., 2005) ou des mammifères spécifiques (Paquet and Callagan, 1996). Cependant il peut favoriser la dispersion d'espèces invasives (Lampinen et al., 2015) et limiter la connectivité génétique de certaines espèces forestières comme la Féronie noire, très sensible à la fragmentation des forêts (chapitre 1). Par conséquent, une méthode proposée pour limiter la fragmentation des forêts à cause de ce type d'ILT consisterait à réaliser un entretien permettant le développement d'un couvert végétal plus mature. On peut encourager les projets tels que LIFE Elia-RTE qui vise justement à transformer les emprises des tracés de lignes à haute tension en corridors écologiques en Belgique et en France.

Conclusion

L'estimation de la connectivité fonctionnelle est nécessaire en écologie du paysage pour appréhender les effets de la fragmentation sur la viabilité des populations. Les résultats et réflexions qui découlent de cette thèse ont permis de clarifier les effets des ILT sur la biodiversité. En mettant en pratique les développements récents en génétique du paysage et en s'intéressant aux aspects démographiques des populations, nous avons pu mettre en évidence que la connectivité paysagère varie d'une espèce à l'autre. La prise en compte de l'aspect multi-spécifique dans les études paysagères améliore la compréhension du mouvement des espèces dans les paysages selon leur traits d'histoires de vies. En multipliant les espèces étudiées et en extrapolant les résultats obtenus à d'autres espèces présentant des traits d'histoires de vies similaires, il serait possible de dresser une vision d'ensemble des effets des éléments paysagers sur les communautés. De plus, la prise en compte de cet aspect multi-spécifique permet de recouper les éléments paysagers et/ou zones spatiales les plus sensibles concernant la connectivité de la faune afin de proposer des mesures de gestion.

Il est à espérer que les initiatives de conservation de grande envergure (TVB ou Natura 2000 au niveau européen par exemple) tiennent compte de ces prérogatives dans les schémas d'aménagement du territoire. Au vu des lourds coûts financiers et humains que représentent ce type de suivis multi-spécifiques et multi-sites, seules des structures avec des grandes capacités de coordination et possédant des moyens financiers conséquents pourront être les moteurs de tels suivis (Richardson et al., 2016). Nos résultats démontrent que les ILT ont des impacts profonds sur la connectivité de la faune et que la multiplicité des structures de franchissements des ILT est nécessaire, spécialement dans les zone de cumul d'ILT.

Par ailleurs, nous avons vu que la génétique du paysage est un outil pertinent pour estimer la connectivité fonctionnelle et les développements méthodologiques participent grandement à l'amélioration de ce champ de recherche. L'utilisation de la génétique du paysage souffre cependant de limites conséquentes : (i) les flux de gènes soutiennent uniquement la connectivité génétique et ne renseignent que partiellement la viabilité des populations ; (ii) la variabilité génétique ne reflète pas nécessairement les flux de gènes et l'effet de la dérive doit être pris davantage en considération dans les futures études ; (iii) les flux de gènes ne sont pas nécessairement bénéfiques pour la viabilité des populations si celles-ci sont fortement adaptées à leur milieu.

Dans cette thèse, nous avons mis en évidence que l'emploi exclusif des suivis génétiques ou CMR n'apporte qu'une vision parcellaire de la connectivité fonctionnelle. En revanche, le couplage des suivis génétiques avec des suivis CMR est en mesure d'apporter une compréhension profonde des effets de la fragmentation sur la connectivité et les dynamiques des populations. Ces travaux permettent de proposer un outil d'aide à la décision pour les gestionnaires souhaitant estimer les effets d'ILT (Fig. J).

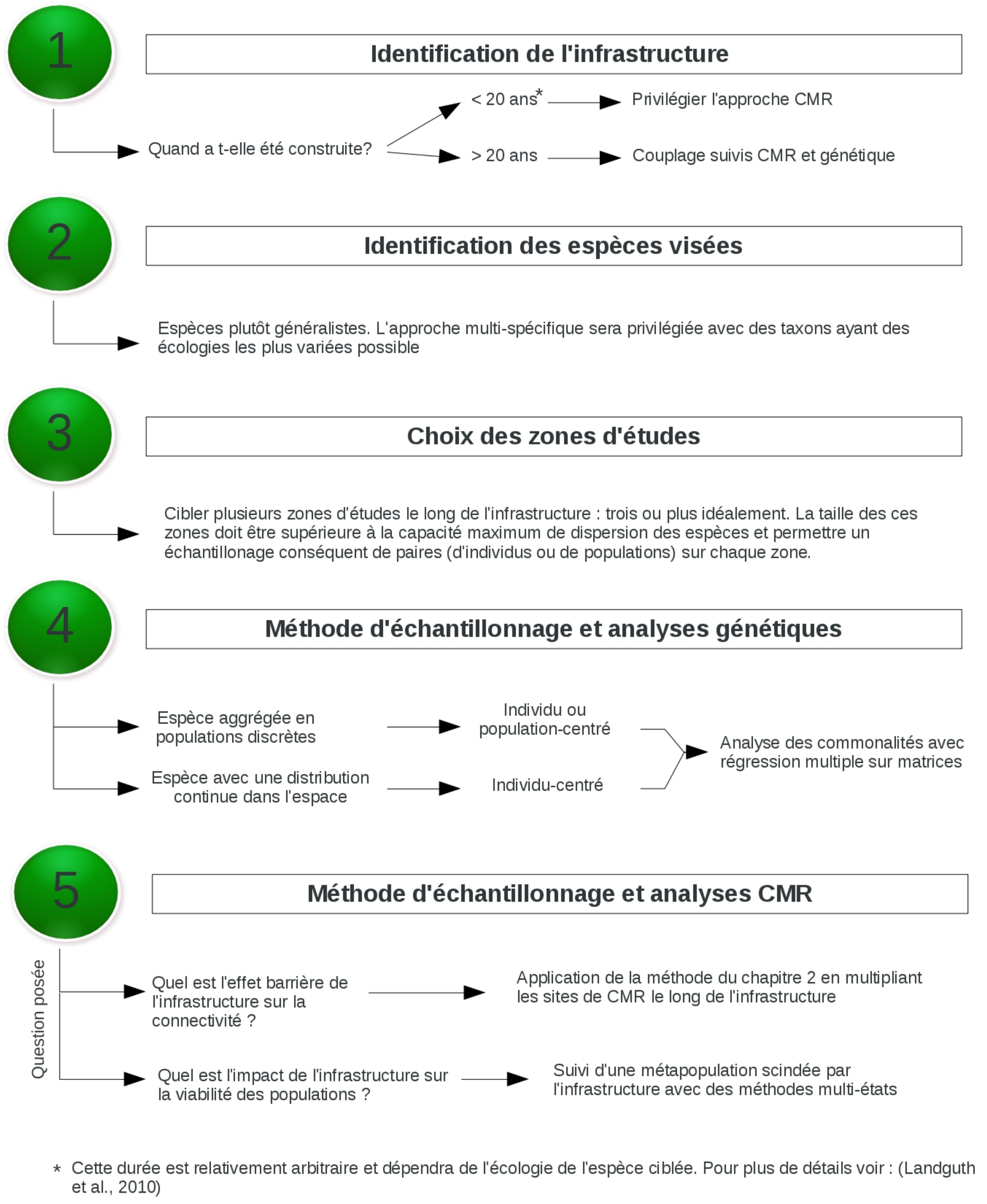


Figure J – Guide d'aide à la décision pour gestionnaires et écologues souhaitant mettre en place un suivi permettant de mesurer les effets des ILT sur la biodiversité.

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Matériel supplémentaire

Table S1 – Habitat elements defining the six main retained landscape predictors used in genetic analyses.

Water	**Crops**	**Woodlands**	**Grasslands**	**Urban**	**Roads**
stagnant water	intensive monocultures	recent logged forests	grass stripes	agricultural buildings	gravelled roads
streams	gardens	coniferous forests	forest clearings	residential buildings	paved roads
ditches	orchards	deciduous forests	openings	waste disposals	
rivers	vineyards	riparian forests	grazed pastures	electrecity pylons	
	vegetable gardens or horticultures	mixed woodlands	dry grasslands	residential habitats	
		heathland	hayed meadows	water tanks	
		hedgerows	meadows	artificial gardens	
		tree plantations	trails ans paths	domestic gardens	
		bushlands	rocky lands	cemeteries	
			abandoned arable lands	sport equipements such as football fields	
				surroundings of agricultural buildings	
				campings	
				car parks	
				greenhouses	
				open cast mines	
				stone quarry	
				industrial sites	
				urban paved areas	
				windmills	

Table S2 – Matrices of Pearson's correlation coefficients among final predictors depending on the genetic dependent variables. The genetic dependent variables are genetic distances (GD) based on the Bray-Curtis dissimilarity index (bc), Fst or hierarchical genetic distances based on first and second level of STRUCTURE outputs (HGD1 and HGD2). The variance inflation factors (VIF) are presented for each predictor.

Species	**DV**	**Pearson's correlation coefficient**					**VIF**
Alytes obstetricans 	GD(bc)	**Predictor**	IBD	Altitude	Woodlands	Roads	
		IBD					1.704
		Altitude	0.427				1.244
		Woodlands	0.304	0.141			1.164
		Roads	0.489	0.308	0.029		1.368
		D6089	0.174	0.062	-0.111	0.062	1.067
	HGD1	**Predictor**	Woodlands	Crops	Roads		
		Woodlands					1.063
		Crops	0.045				1.053
		Roads	0.119	0.224			1.071
		Railway	0.220	0.006	0.086		1.055
	HGD2	**Predictor**	Woodlands	Urban	Roads	D6089	
		Woodlands					1.132
		Urban	-0.303				1.246
		Roads	0.003	0.318			1.142
		D6089	-0.112	0.015	0.084		1.041
		Motorway	0.008	-0.023	0.042	-0.125	1.021
Natrix natrix 	GD(bc)	**Predictor**	Roads	Motorway			
		Roads					1.056
		Motorway	0.188				1.038
		Railway	0.131	-0.008			1.019
Maniola jurtina 	GD(Fst)	**Predictor**	IBD	Woodlands			
		IBD					1.049
		Woodlands	-0.197				1.111
		Power line	-0.039	-0.239			1.069
Abax parallelepipedus 	GD(Fst)	**Predictor**	Altitude				
		Altitude					1.001
		Grasslands	-0.034				1.001
	HGD1	**Predictor**	Roads				
		Roads					1.095
		D6089	0.295				1.095
	HGD2	**Predictor**	Altitude	D6089	Motorway		
		Altitude					1.010
		D6089	-0.019				1.071
		Motorway	0.046	-0.229			1.071
		Gas pipeline	0.087	0.095	0.090		1.030

Table S3 – Studies that identify the average distance travelled by the butterfly *Maniola jurtina* in different sites using Mark-Release-Recapture surveys. The averaged distance across studies is indicated in bold.

Average distance (m)	Author (year)
51	Brakefield (1982)
91	Lörtscher *et al.* (1997)
320.5	Schneider *et al.* (2003)
80	Ouin *et al.* (2008)
50	Ouin *et al.* (2008)
170	Ouin *et al.* (2008)
59	Valtonen *et al.* (2005)
120	Munguira *et al.* (1992)
39	Munguira *et al.* (1992)
79.2	Merckx and Van Dyck (2002)
95.6	Merckx and Van Dyck (2002)
70.8	Grill *et al.* (2006)
428	Ockinger and Smith (2007)
127.2	

Table S4 – Subset of candidate models with AICc weights greater than zero for the three Mark-Release-Recapture studies conducted on the toad *A. obstetricans* in south-western France. Data were analysed using the robust design method with the following parameters: survival probability (Phi), seniority probability (Y), capture (c) and recapture (p) probabilities and number of missing individuals (f0). Parameters vary either with time (t, which corresponds to primary sampling occasions), secondary sampling occasions (secondary) or time constant (.).

Model	Npar	AICc	DeltaAICc	Weight	Deviance
SITE 370					
Phi(.) Y(.) p=c(t*secondary) f0(t)	34	676.72	0.00	0.60	-515.66
Phi(t) Y(.) p=c(t*secondary) f0(t)	35	679.21	2.49	0.17	-515.81
Phi(.) Y(t) p=c(t*secondary) f0(t)	35	679.27	2.55	0.17	-515.75
Phi(t) Y(t) p=c(t*secondary) f0(t)	36	681.60	4.88	0.05	-516.06
SITE 107					
Phi(.) Y(.) p=c(t*secondary) f0(t)	34	676.40	0.00	0.41	-154.25
Phi(.) Y(t) p=c(t*secondary) f0(t)	35	677.05	0.65	0.30	-156.50
Phi(t) Y(.) p=c(t*secondary) f0(t)	35	677.71	1.31	0.21	-155.84
Phi(t) Y(t) p=c(t*secondary) f0(t)	36	679.62	3.22	0.08	-156.86
SITE 118					
Phi(.) Y(.) p=c(t*secondary) f0(t)	30	643.54	0.00	0.41	-1092.02
Phi(t) Y(t) p=c(t*secondary) f0(t)	32	643.70	0.16	0.38	-1096.61
Phi(.) Y(t) p=c(t*secondary) f0(t)	31	644.83	1.29	0.21	-1093.10

Table S5 – Score coefficients and the proportion of the total variance explained by the three first axes of the Principal Component Analyses performed on genetic metrics alone (PCA1) or on both genetic metrics and fitness parameters (PCA2). Bold scores indicated to which axis genetic metrics (observed heterozygosity (Ho), expected heterozygosity (He), inbreeding coefficient (Fis), allelic richness (AR) and number of private allele (PA)) or fitness parameters (population growth rate (r), annual survival (S), body condition (BC) and clutch size (CS)) were mostly associated.

	PCA1			**PCA2**		
	Axis 1	**Axis 2**	**Axis 3**	**Axis 1**	**Axis 2**	**Axis 3**
Ho	**0.78**	-0.62	0.03	**0.78**	-0.59	0.14
He	**0.95**	-0.19	0.18	**0.94**	-0.17	0.10
Fis	0.04	**0.96**	0.25	0.02	**0.94**	-0.17
AR	**0.90**	0.28	0.15	**0.90**	0.34	0.19
PA	**0.73**	0.51	-0.45	**0.72**	0.48	-0.25
r	-	-	-	**0.93**	0.03	0.20
S	-	-	-	**0.97**	-0.03	-0.11
BC	-	-	-	-0.03	0.32	**0.87**
CS	-	-	-	**0.59**	-0.07	-0.41
% explained	57.08	33.56	6.41	55.36	19.09	12.75

Table S6 – This table represents the p-values of the linear regression between the length of roads in a radius surrounding *A. obstetricans* populations and the first and second axis of PCA1 and PCA2. All p-values were higher than 0.05.

	PCA1		**PCA2**		
Radius (m)	**Axis 1**	**Axis 2**	**Axis 1**	**Axis 2**	**Axis 3**
250	0.69	0.79	0.73	0.66	0.29
500	0.44	0.64	0.54	0.51	0.34
1000	0.84	0.78	0.86	0.83	0.85

Appendix A

Laboratory procedures and microsatellite markers

For all species, we used a Qiagen Type-it Microsatellite kit. We extracted total DNA from invertebrate legs, scales and swabs using the DNeasy Blood and Tissue kit (Qiagen, Valencia, CA). Before enzymatic digestion, each invertebrate leg and scale was cut in 4-6 pieces to facilitate DNA extraction. Buccal swabs were used as is. For *N. helvetica* and *A. obstetricans*, we amplified 13 (Pokrant et al., 2016) and 14 (Tobler et al., 2013; Maia-Carvalho et al., 2014) polymorphic microsatellite loci, respectively. For both species, loci were amplified in 10 μl reaction volumes containing 2 μl multiplex PCR Master Mix, 1.2 to 1.6 μl of primer mix (between 0.13 and 0.25 μM of each primer), 5.4 to 5.8 μl of purified water and 1 μl of template DNA (10-20 ng μl^{-1}).

For *Maniola jurtina*, we amplified 15 polymorphic microsatellite loci (Richard et al., 2015) in three Multiplexes, in 10 μl reaction volumes containing 2 μl multiplex PCR Master Mix, 0.7 μl of primer mix (between 0.03 and 0.08 μM of each primer), 4.3 μl of purified water and 3 μl of template DNA (1-10 ng μl^{-1}). For *Abax parallelepipedus*, we amplified 14 polymorphic microsatellite loci (Marcus et al., 2013) in three Multiplexes, in 5 μl reaction volumes containing 1 μl multiplex PCR Master Mix, 0.7 μl of primer mix (between 0.04 and 0.11 μM of each primer), 2.3 μl of purified water and 1 μl of template DNA (approx. 10 ng μl^{-1}).

Polymerase Chain Reaction (PCR) conditions were set on an Applied Biosystems thermal cycler. For the two vertebrate species, conditions were set as follows: initial denaturation 10 min at 95°C; 30 cycles of 30 s at 95°C, 90 s at 51 to 60°C (depending on the multiplex) and 30 s at 72°C; final elongation of 5 min at 72°C. For the two invertebrate species, conditions were set as follows: initial denaturation 10 min at 94°C; 40 cycles of 30 s at 94°C, 90 s (for the 10 first) or 30 s (for the 30 following) at 61°C (*A. parallelepipedus*) or 56°C (*M. jurtina*) and 30 s at 72°C; final elongation of 5 min at 72°C.

All PCR products were ten times diluted and were run on an ABI 3730 DNA Analyser (Applied Biosystems) with the GeneScan-600 LIZ size standard. Genotyping was performed with GENEMAPPER 5.0 (Applied Biosystems) and all peaks were manually confirmed.

The following tables describe the specificity of the microsatellite markers tested for the four species followed in this study. Gray colours represent markers that were not used in the landscape genetic analyses either because they could not be amplified, showed sex-linkage, presence of null alleles or linkage disequilibrium (see results section).

Locus	**Primer sequences (5'- 3')**	**Repeat motif**	**Allele size range (bp)**	**Number of alleles**	**Multiplex set**	**Annealing temperature**	**Fluorescent label**	**Original reference**
Alyobs3	f – CCAACATGTTCACTTTATAGAGCAG r – GGAACCTTGAATCTCGAAAGC	(TATC)28	168-276	27	1	52	FAM	Tobler *et al.* (2013)
Alyobs4	f – TTTTCCCTTGCTAAATCCTCAG r – AAAGTGTTGATGCACATTTTCC	(CTGT)11	117-161	9	1	52	NED	Tobler *et al.* (2013)
Alyobs7	f – AAGGACGTGCTTCTATCTGC r – AGTTCGCACACATTACATTGC	(TATC)16(TG)3(TA)3(TC)(TA)4			1	52	PET	Tobler *et al.* (2013)
Alyobs28	f – CCAGTGCTGTGGTTTTCTCA r – AAATATCAAGAGCCTTAGCTAACATTT	(GT)13(GA)3(GTGA)3	96-106	4	1	52	VIC	Tobler *et al.* (2013)
Alyobs17	f – TTCTCTTCAGCTGGGCAATC r – TGGAACTGAAGAGCGAGGAC	(GT)13	146-160	8	2	56	VIC	Tobler *et al.* (2013)
Alyobs19	f – TGAATGTGCCGGTGAAGAC r – AAACACATATGAACAGGTGAAAAGAG	(GT)12	72-112	13	2	56	NED	Tobler *et al.* (2013)
Alyobs20	f – GATGCAGCACATTTCTGAGC r – GGTGCATCTGCCATAGTGTG	(GT)12	105-115	5	2	56	PET	Tobler *et al.* (2013)
Alyobs23	f – TGCAGAGCTCAGCCACTTAG r – TGACCAATCCAATCATCCAG	(GT)13	208-238	6	2	56	PET	Tobler *et al.* (2013)
Alyobs24	f – TCCTCAAAATCTTGTGATGTGC r – ATGGCCAGATGTCCCAATAC	(CA)28	75-139	23	2	56	FAM	Tobler *et al.* (2013)
Alyobs25	f – CCTTCTGTCTACCTTGTCATATTTCC r – AAAGCGACTAATACAGAACACTGC	(GT)16	141-163	9	2	56	NED	Tobler *et al.* (2013)
Aobst14	f – TGTGGGAACCTTTACATCATAA r – CCCTCCTCTAAGCCGTCA	(ACT)n	102-158	11	3	52	VIC	Maia-Carvalho *et al.* (2013)
Aobst15	f – TTGGATGGTGGGTACAATCA r – TGAGGACAAATGCCTGACAA	(AGAT)n	251-401	21	3	52	NED	Maia-Carvalho *et al.* (2013)
Aobst16	f – TCAGAATAAACAAGAGCTGCAAA r – GGAGATCCACGCTCAGGATA	(AGAT)n	445-497	8	3	52	FAM	Maia-Carvalho *et al.* (2013)
Aobst17	f – CGGTGTCCCCATCTTATCAA r – CCCAGTGCTCAAACCTCAAT	(ACC)n	244-268	6	3	52	PET	Maia-Carvalho *et al.* (2013)

Locus	**Primer sequences (5'- 3')**	**Repeat motif**	**Allele size range (bp)**	**Number of alleles**	**Multiplex set**	**Annealing temperature**	**Fluorescent label**	**Original reference**
Natnat09	f – TGTAAATAACACTGTACCATTTTGG r - TGACTGGGCAACAGAAAAGC	$(AC)_{22}$	96-132	15	1	55	FAM	Meister *et al.* (2009)
Natnat05	f – TCTGCACTGGGGATAGGAAG r - GTCCCTTTTTCAGTGCTGTTG	$(GT)_{16}$			1	55	VIC	Meister *et al.* (2009)
µNt8new	f – GTATCGTCCTTCCAGACAAG r - GCAAATCAAATAAATCTCACTGG	$(AC)_{15}$	81-123	14	1	55	NED	Meister *et al.* (2009)
Nsµ3	f – CTGACTCACTTCTGACCCTAAT r - AATATTTGCTTGGCTCAAAC	$(ATCT)_{14}$ $ATC(CA)_{20}$			1	55	PET	Prosser *et al.* (1999)
µNt3	f – GGCAGGCTATTGGAGAAATG r – GGCAAAACTCCAGGTGCTAC	$(AC)_{16}$	127-145	5	2	60	FAM	Gautschi, Widmer & Koella (2000)
µNt7	f – TTTGAAAGGAGAATGAATCGTG r – CGCGAGGAATCAGAATGAAC	$(AC)_{17}$	177-185	3	2	60	VIC	Gautschi *et al.* (2000)
Natnat11	f – GGCTGTTTTCCCAGTGAAGC r - GGTCTGGGGAAAAAGAAAGG	$(GA)_{13}$	106-118	4	3	55	FAM	Meister *et al.* (2009)
Natnat06	f – AATGGCATTCTCTCCAGCTC r - ACCCATATCCGTATCCATATCC	$(GT)_{21}$	159-187	13	3	55	VIC	Meister *et al.* (2009)
3TS	f – GGTCACTTAAATACAACGAAATTGGTTAGCT r - CGGACAGCTCTGGCTCCCTTG	$(GATA)_{19}$			3	55	PET	Garner *et al.* (2002)
30	f – CCCACTGGCTCATTTCAAGT r – CCACATTTGCATCGGAGTG	$(CA)_{14}$	250-274	13	—	60	NED	Burns & Houlden (1999)
Tbu A09	f – CATCTCAACCAAAGTCGCTTC r – GGATGTTGTGGGGTGTTTTC	$(AC)_{7}$	110-140	14	—	55	NED	Sloss *et al.* (2012)
Eobµ1	f – ATCAGTAGGAGTGAGAGCAACT r – CTGCATACTCTTCCAGAACC	$(TG)_{21}$	128-134	3	—	51	NED	Blouin-Demers & Gibbs (2003)
Eobµ13	f – TGATCTGAGTCTCTTTCTGG r - CAATTCAAATCCATTGGTTT	$(AC)_{20}$	138-162	9	—	51	PET	Blouin-Demers & Gibbs (2003)

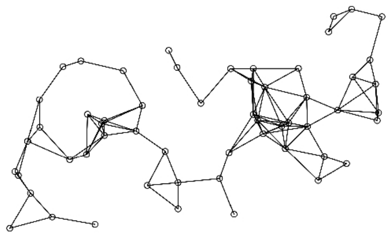
Locus	Primer sequences (5'- 3')	Repeat motif	Allele size range (bp)	Number of alleles	Multiplex set	Annealing temperature	Fluorescent label	Original reference
Mj0008	f – CGTGTCGCCTAAACCACATC r – TGGCAACCCTAAACCCTACG	(ACAT)7	91-149	5	1	56	PET	Richard *et al.* 2015
Mj5287	f – GCTAGCTCGTGGGTACTCTG r – CTCCAAGCAATAAGACCGCC	(GATA)11			1	56	FAM	Richard *et al.* 2015
Mj7132	f – ATCTGCGGATTTGCAGTTGG r – CACTATTGAGCACGTGTGTCC	(TATG)13	165-213	19	1	56	NED	Richard *et al.* 2015
Mj5647	f – GCGTTCTGATTACCACCCTG r – GCGACAGTCCCCTAAGATCG	(TATG)13			1	56	PET	Richard *et al.* 2015
Mj5563	f – CGGTTTTGCCGATAGCGTAG r – CGCAAGGCAATAGACCACTC	(ATCT)7			1	56	VIC	Richard *et al.* 2015
Mj3956	f – CAACATCGGGAGTCGAAACG r – CTCAGCCAGGATACCCACTC	(GATA)7			2	56	PET	Richard *et al.* 2015
Mj7232	f – AAGTTACAAGAGCGTTGGCG r – GCGGGAACTCTTGGGTTTTC	(CTGT)7	144-214	19	2	56	FAM	Richard *et al.* 2015
Mj5522	f – TGATCTTTGCCAGCAGGAAC r – AGTGTAAGCTGGCCCTAAAC	(GATA)8			2	56	NED	Richard *et al.* 2015
Mj0247	f – ATTCCACAAACGAGCCAACG r – ACTCCGATGGTAAGAGGTGC	(GATG)8	182-328	53	2	56	PET	Richard *et al.* 2015
Mj0272	f – GTTGCATTGGCACACTCCTC r – CAGCTGCACACTACGACAAG	(AGAT)7			2	56	VIC	Richard *et al.* 2015
Mj5331	f – TTAGACCGTGATCCCACTGC r – ATTTCGATAGGCAACGAGGC	(TATC)10	100-204	25	3	56	PET	Richard *et al.* 2015
Mj4870	f – ATGATCCATAGCTGCGTTGC r – CTCCTTAGCGCTTACACGTC	(ATGT)7	156-184	13	3	56	FAM	Richard *et al.* 2015
Mj3637	f – CTTCCGCAAAATAACGTCTGC r – AGATACTCCATTGACCCGGC	(TCTA)7			3	56	NED	Richard *et al.* 2015
Mj2410	f – TAATTAGAGTTTGCGCGGGG r – CGCACACCGCAGTATAAGTG	(TGTA)7			3	56	PET	Richard *et al.* 2015
Mj0283	f – CCCTTAGAATAAGAACTCGGCTC r – TGTTCGCACATGCTTAGTCC	(AGAT)9			3	56	VIC	Richard *et al.* 2015

Locus	Primer sequences (5'- 3')	Repeat motif	Allele size range (bp)	Number of alleles	Multiplex set	Annealing temperature	Fluorescent label	Original reference
apar_20	f – ACACTCCACTCAAAGTTGCG r – AAACGGTCAACTTTCCACCC	(AC)	185-189	3	1	61	PET	Marcus *et al.* 2013
apar_50	f – GCTGGACTATTACAGAAGTCTTTTGC r – ATGTGGAGGAAGCACGTGTT	(CATA)			1	61	FAM	Marcus *et al.* 2013
apar_27	f – CCTCCTTACCAAGTAACGGG r – GTTTGGAAGCGACAGTCAACGTG	(AC)	251-255	2	1	61	NED	Marcus *et al.* 2013
apar_34	f – GTTTGCCATACTAGGTGCTCTGG r – ATCTCCCGTGAAATCAACGC	(AC)	103-111	4	1	61	PET	Marcus *et al.* 2013
apar_32	f – TTTACCAACACACGCAGGC r – GTTTGGACCACAACACGTTAGCAC	(AG)	92-94	2	2	61	NED	Marcus *et al.* 2013
apar_12	f – GACCGTCGAGTGTAATGACG r – CAATCTGCTCCTCAAGTTCAAG	(AG)	123-133	4	2	61	VIC	Marcus *et al.* 2013
apar_23	f – GTGCCTATCGTTCTTTGTCAC r – GTTTGCGATATTGTCTCTTGGCGG	(AC)	156-162	4	2	61	NED	Marcus *et al.* 2013
apar_25	f – GTTTCGTAGCGAAACAGTGCCTTG r – ATACTCCGGCGCTACTTTGG	(AC)	198-204	5	2	61	NED	Marcus *et al.* 2013
apar_02	f – GCCGCACGATATTAGCGAC r – TTGGGAGTAAGTCTGTCCGG	(AC)	165-169	3	2	61	PET	Marcus *et al.* 2013
apar_46	f – CAGTTCAGTTCATCACGGGC r – GTTTGGAACCCAACGCAGAAAGTC	(AAC)			2	61	PET	Marcus *et al.* 2013
apar_05	f – CAACAACATTACCGGCGGAG r – GCCGAGTCACTTGTTACGTG	(AG)	150-156	4	3	61	FAM	Marcus *et al.* 2013
apar_44	f – GTTTCTTAATGTTCCATGCCGCG r – TCTTCTTCGGCAAGCGTTAC	(AG)			3	61	VIC	Marcus *et al.* 2013
apar_14	f – GACATCTCGACTGCACCTAC r – CCCTGTCTTTCCAACATCGC	(AG)			3	61	NED	Marcus *et al.* 2013
apar_06	f – AAACATTCTGCGGTGACACC r – CTGCTGCCCTCTTGTAAACG	(AG)	284-308	5	3	61	PET	Marcus *et al.* 2013

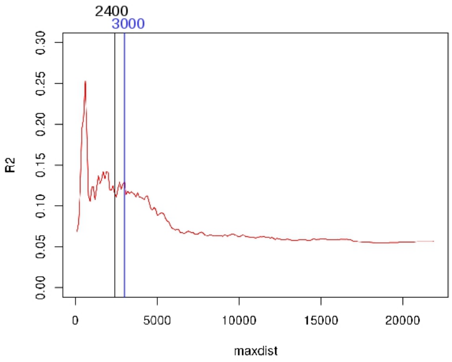
Appendix B

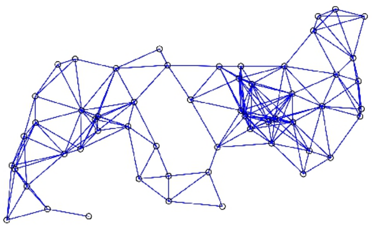
Spatial scale of analysis

Identification of the maximum neighboring distance retained among pairs of individuals or populations in subsequent analyses. Gabriel graphs are presented for the four studied species and for two types of genetic dependent variables: classic genetic distances (GD) based on the Bray-Curtis dissimilarity index (bc) or Fst and hierarchical genetic distances (HGD1 and HGD2). Left panels show the relation between the R^2 of the full model including all predictors in a classical multiple linear regression and euclidean distances among pairs of individuals or populations. Black lines correspond to the minimum distance insuring that all pairs are connected to at least one neighbor (top black Gabriel graph). Blue lines represent the retained spatial scale for subsequent analysis. Right panels represent the Gabriel graphs corresponding to the retained spatial scale.

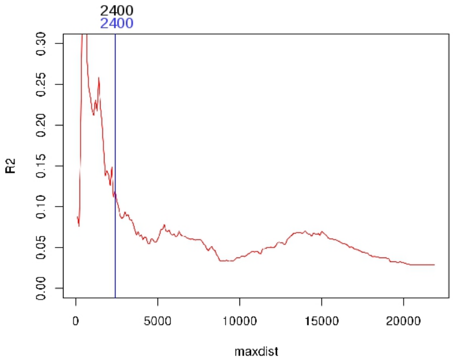


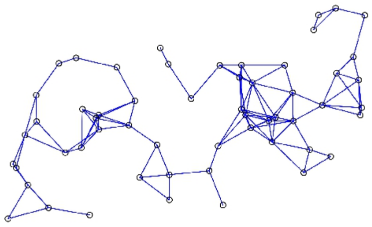
2400 m



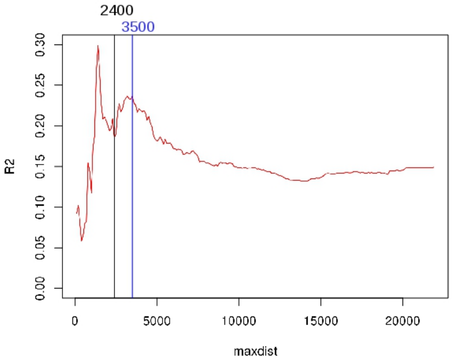


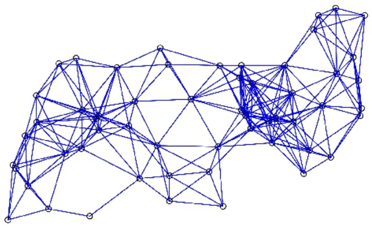
GD(bc) 3000 m



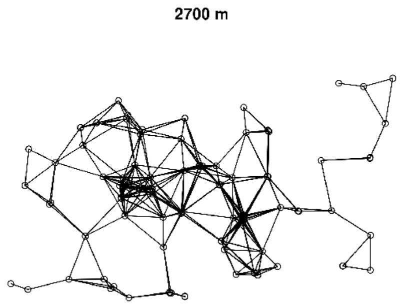


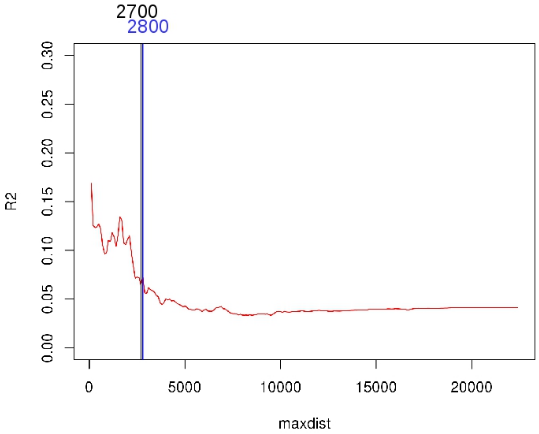
HGD1 2400 m

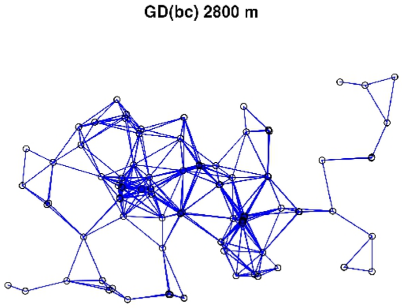


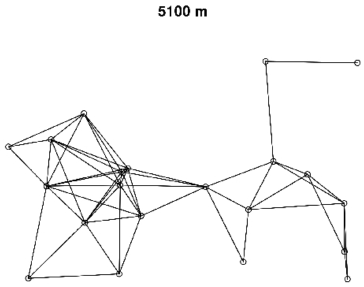


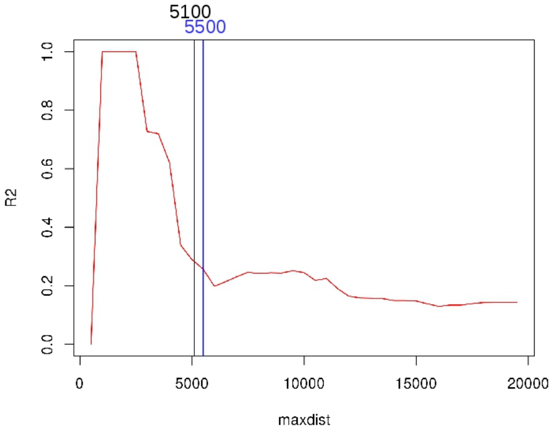
HGD2 3500 m

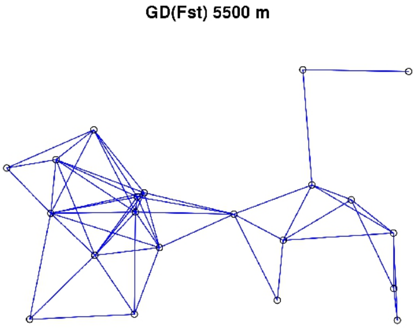

2700 m

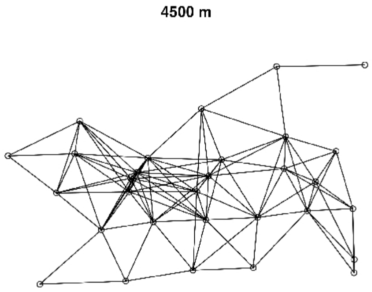

2700
2800
R2
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0
5000
10000
15000
20000
maxdist

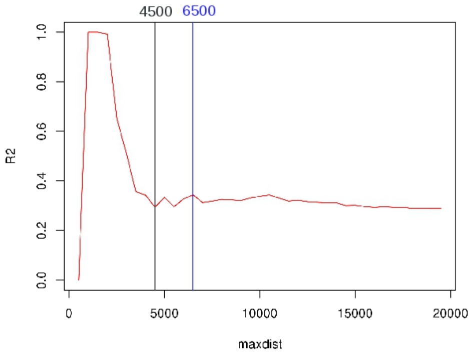

GD(bc) 2800 m

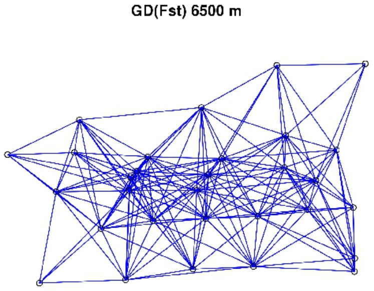

5100 m

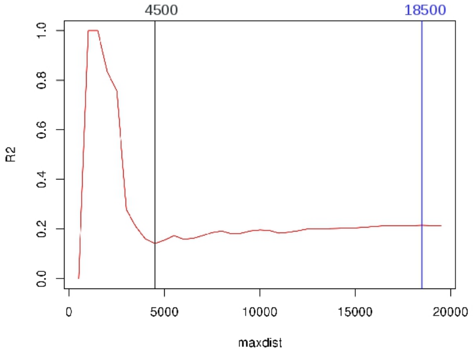

5100
5500
R2
0.0
0.2
0.4
0.6
0.8
1.0
0
5000
10000
15000
20000
maxdist


GD(Fst) 5500 m


4500 m


4500
6500
R2
1.0
0.8
0.6
0.4
0.2
0.0
0
5000
10000
15000
20000
maxdist


GD(Fst) 6500 m


4500
18500
R2
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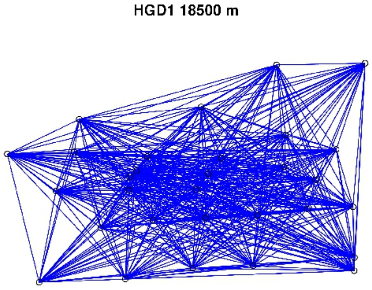
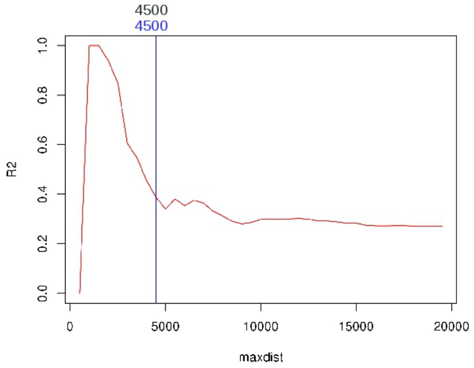

HGD1 18500 m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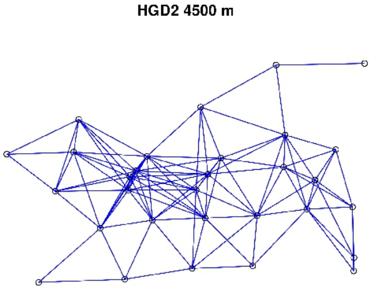

HGD2 4500 m

Appendix C

Intermediate steps of commonality analyses on vectors

Runs of identification of unnecessary predictors for each species and genetic dependent variable DV (GD: genetic distance either calculated with the Bray-Curtis (bc) dissimilarity index for individual-based method or Fst for population-based method; HGD1 and HGD2 for hierarchical genetic distance based on first and second level of STRUCTURE outputs, respectively). Distance stands for the spatial scale retained in our analysis (Appendix B). Results of the different runs of multiple linear regressions (predictors, structure coefficient rs and standardised coefficient B), in addition to parameters derived from CA: unique (U), common (C) and total (T) contributions of predictors to the variance in the genetic dependent variable. The rationale for withdrawal of predictors (Ra) is the following: CO: cross-over suppression; S: synergistic association with other predictors; PS: partial suppression (or reciprocal suppression). All predictors (IBD: isolation by distance; D6089: a large country road; Urban: urban areas; see Table S1 for additional informations on predictors) were coded as resistance. In bold: parameters allowing the identification of unnecessary predictors and suppressors. Note that situations of classical suppression were avoided by discarding any predictor with a squared zero-order correlation < 0.1.

DV	Species	Distance	Run	Pred	rs	B	U	C	T	Ra
GD(bc)	*A. obstetricans*	3000 m	1	IBD	0.809	0.172	0.012	0.068	0.080	
				Altitude	0.608	0.098	0.008	0.037	0.045	
				Woodlands	0.545	0.149	0.019	0.017	0.036	
				Water	**0.335**	**-0.078**	0.004	0.010	0.014	CO
				Roads	0.612	0.117	0.010	0.036	0.046	
				D6089	0.314	0.096	0.009	0.003	0.012	
				Railway	**0.346**	**-0.016**	0.000	0.014	0.015	CO
HGD1	*A. obstetricans*	2400 m	1	IBD	0.767	0.102	**0.004**	**0.062**	0.066	S
				Woodlands	0.451	0.081	0.005	0.018	0.023	
				Water	**0.381**	**-0.068**	0.003	0.013	0.016	CO
				Crops	0.672	0.182	0.030	0.021	0.051	
				Roads	0.660	0.135	0.011	0.038	0.049	
				Railway	0.433	0.081	0.005	0.016	0.021	
HGD2	*A. obstetricans*	3500 m	1	IBD	0.355	-0.006	**0.000**	**0.025**	0.025	S
				Woodlands	0.538	0.190	0.028	0.029	0.058	
				Urban	-0.465	-0.240	0.046	-0.003	0.043	
				Roads	0.448	0.268	0.051	-0.011	0.040	
				D6089	0.440	0.185	0.030	0.008	0.039	
				Motorway	-0.279	-0.118	0.013	0.003	0.016	
HGD2	*A. obstetricans*	3500 m	2	Woodlands	0.614	0.261	0.046	0.012	0.058	
				Urban	0.317	0.002	**0.000**	**0.015**	0.015	S
				Roads	0.512	0.187	0.035	0.006	0.040	
				D6089	0.502	0.196	0.036	0.002	0.039	
				Motorway	0.318	0.110	0.010	0.005	0.016	

DV	Species	Distance	Run	Pred	rs	B	U	C	T	Ra
GD(bc)	*N. hevetica*	2800 m	1	Roads	-0.533	-0.125	0.015	-0.003	0.012	
				Motorway	0.616	0.148	0.021	-0.005	0.016	
				Railway	-0.520	-0.088	0.008	0.004	0.011	

DV	Species	Distance	Run	Pred	rs	B	U	C	T	Ra
GD(Fst)	*M. jurtina*	5500 m	1	IBD	0.434	0.257	0.062	-0.019	0.044	
				Woodlands	0.636	0.349	0.072	0.022	0.093	
				Meadow	**-0.386**	**0.060**	0.002	0.032	0.035	CO
				D6089	-0.255	-0.178	0.030	-0.015	0.015	
				Power line	-0.553	-0.226	0.045	0.026	0.071	
GD(Fst)	*M. jurtina*	5500 m	2	IBD	0.436	0.254	0.061	-0.018	0.044	
				Woodlands	0.639	0.313	0.088	0.005	0.093	
				D6089	-0.257	-0.177	**0.030**	**-0.015**	0.015	PS
				Power line	-0.555	-0.220	0.043	0.028	0.071	

DV	Species	Distance	Run	Pred	rs	B	U	C	T	Ra
GD(Fst)	*A. parallelepipedus*	6500 m	1	Altitude	0.192	0.144	0.019	-0.008	0.011	
				Grasslands	0.918	0.683	0.213	0.031	0.244	
				Water	**0.360**	**-0.156**	0.010	0.028	0.038	CO
				Urban	**0.443**	**-0.023**	0.000	0.057	0.057	CO
				Roads	**0.445**	**-0.089**	0.001	0.056	0.057	CO
				Motorway	**0.226**	**-0.049**	0.002	0.013	0.015	CO
HGD1	*A. parallelepipedus*	18500 m	1	Grasslands	**0.283**	**-0.140**	0.010	0.006	0.016	CO
				Water	0.595	0.189	0.015	0.055	0.070	
				Crops	**0.546**	**-0.043**	0.001	0.058	0.059	CO
				Urban	**0.628**	**-0.172**	0.005	0.073	0.078	CO
				Roads	0.759	0.401	0.031	0.082	0.114	
				D6089	0.745	0.265	0.059	0.051	0.110	
HGD1	*A. parallelepipedus*	18500 m	2	Water	0.628	0.094	**0.005**	**0.065**	0.070	S
				Roads	0.801	0.194	0.018	0.096	0.114	
				D6089	0.787	0.261	0.062	0.048	0.110	
HGD2	*A. parallelepipedus*	4500 m	1	Altitude	0.440	0.212	0.044	0.009	0.053	
				Roads	0.404	0.085	**0.007**	**0.038**	0.045	S
				D6089	0.750	0.324	0.089	0.065	0.154	
				Motorway	-0.312	-0.119	0.013	0.014	0.027	
				Gas pipeline	0.511	0.226	0.050	0.022	0.072	

To explain the dependent variable based on the Bray-Curtis genetic distance in *A. obstetricans*, the predictors with a squared correlation (r^2) with the dependent variable higher than 0.1 were IBD, Altitude, Woodlands, Water, Roads, D6089 and Railway. Among these predictors, Water and Railway were cross-over suppressors and were discarded from subsequent analysis. To explain the first level of hierarchical genetic distance (HGD1) in *A. obstetricans*, the predictors with a r^2 higher than 0.1 were IBD, Woodlands, Water, Crops, Roads and Railway. IBD was a suppressor with synergistic association with other predictors. Water was a cross-over suppressor. These two predictors were discarded and the final model comprised four predictors: Woodlands, Crops, Roads and Railway. To explain the second level of hierarchical genetic distance (HGD2) in *A. obstetricans*, the predictors with a r^2 higher than 0.1 were IBD, Woodlands, Urban, Roads, D6089 and Motorway. IBD and Urban were cross-over suppressors and were discarded from subsequent analysis.

In the *N. helvetica* data set, only three predictors had a r^2 higher than 0.1: Roads, Motorway and Railway. There was no suppressors among these three predictors and all were used in the final model.

For the species *M. jurtina*, five predictors had a r^2 higher than 0.1: IBD, Woodlands, Grasslands, D6089 and Power line. Grasslands was a cross-over suppressor and the roads D6089 was a partial suppressor. These two predictors were discarded from subsequent analysis resulting in a final model with three predictors: IBD, Woodlands and Power line.

To explain the Fst genetic distances in *A. parallelepipedus*, six predictors had a r^2 higher than 0.1: Altitude, Grasslands, Water, Urban, Roads and Motorway. Water, Urban, Roads and Motorway were cross-over suppressors. All were discarded from subsequent analysis. Only two predictors remained in the final model: Altitude and Grasslands.

To explain the first level of hierarchical genetic distance (HGD1) in *A. parallelepipedus*, we retained the predictors: Grasslands, Water, Crops, Urban, Roads and D6089 ($r^2 > 0.1$). Grasslands, Crops and Urban were cross-over suppressors and Water was a suppressor with synergistic association with other predictors. Therefore, we retained only Roads and D6089 to explain the dependent variable in the final data set.

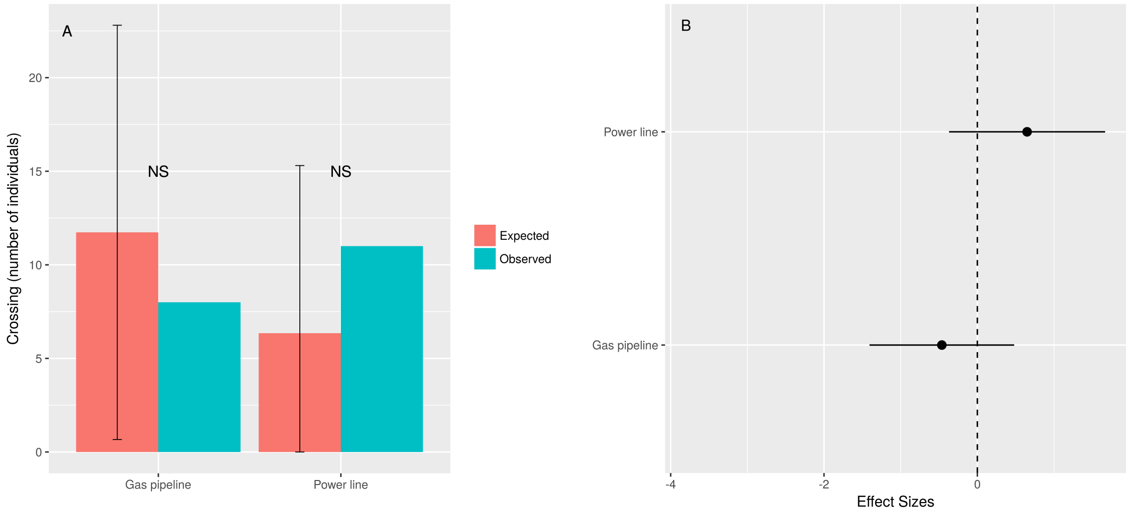
To explain the second level of hierarchical genetic distance (HGD2) in *A. parallelepipedus*, we retained the predictors: Altitude, Roads, D6089, Motorway and Gas pipeline ($r^2 > 0.1$). The predictor Roads was a suppressor with synergistic association with other predictors and was discarded from subsequent analysis.

Appendix D

Infrastructures on the control site

On the control site, two infrastructures were present: a gas pipeline and a power line. There was no real reason to suspect that these two infrastructures might influence movements of butterflies as the vegetation cover on these LTIs was strictly similar to the vegetation in surrounding meadows. Nevertheless, we tested this assumption by applying our method to test the barrier effects of these two infrastructures using a dispersal kernel based on the literature. We identified nine published studies where the average movement distances travelled by *M. jurtina* were reported or could be calculated. In these studies, the average mobility capacity ranged from 39 m to 428 m (Brakefield, 1982; Munguira and Thomas, 1992; Lörtscher et al., 1997; Merckx and Van Dyck, 2002; Schneider et al., 2003; Valtonen and Saarinen, 2005; Grill et al., 2006; Öckinger and Smith, 2007; Ouin et al., 2008) with an average of 127 m resulting in a value of α of 0.0079. We used this value to calculate the expected probability of crossing either the gas pipeline or the power line. These expected probabilities were compared to the crossing observations applying the framework developed in the manuscript.

We found that butterfly movements were not affected by the two LTIs present. Eight (7.8%) butterflies crossed the gas pipeline whereas 12 crossing events were expected. Although we observed less crossing events than expected, this result was not significant (logOddsRatio -0.46 [95% CI -1.410.48]; binomial test $p = 0.35$). On the same site, a higher number of butterflies crossed the power line than expected by our method (11 (10.7%) crossing events against 6 expected). This difference was also not significant (logOddsRatio 0.65 [95% CI -0.371.67]; binomial test $p = 0.063$).



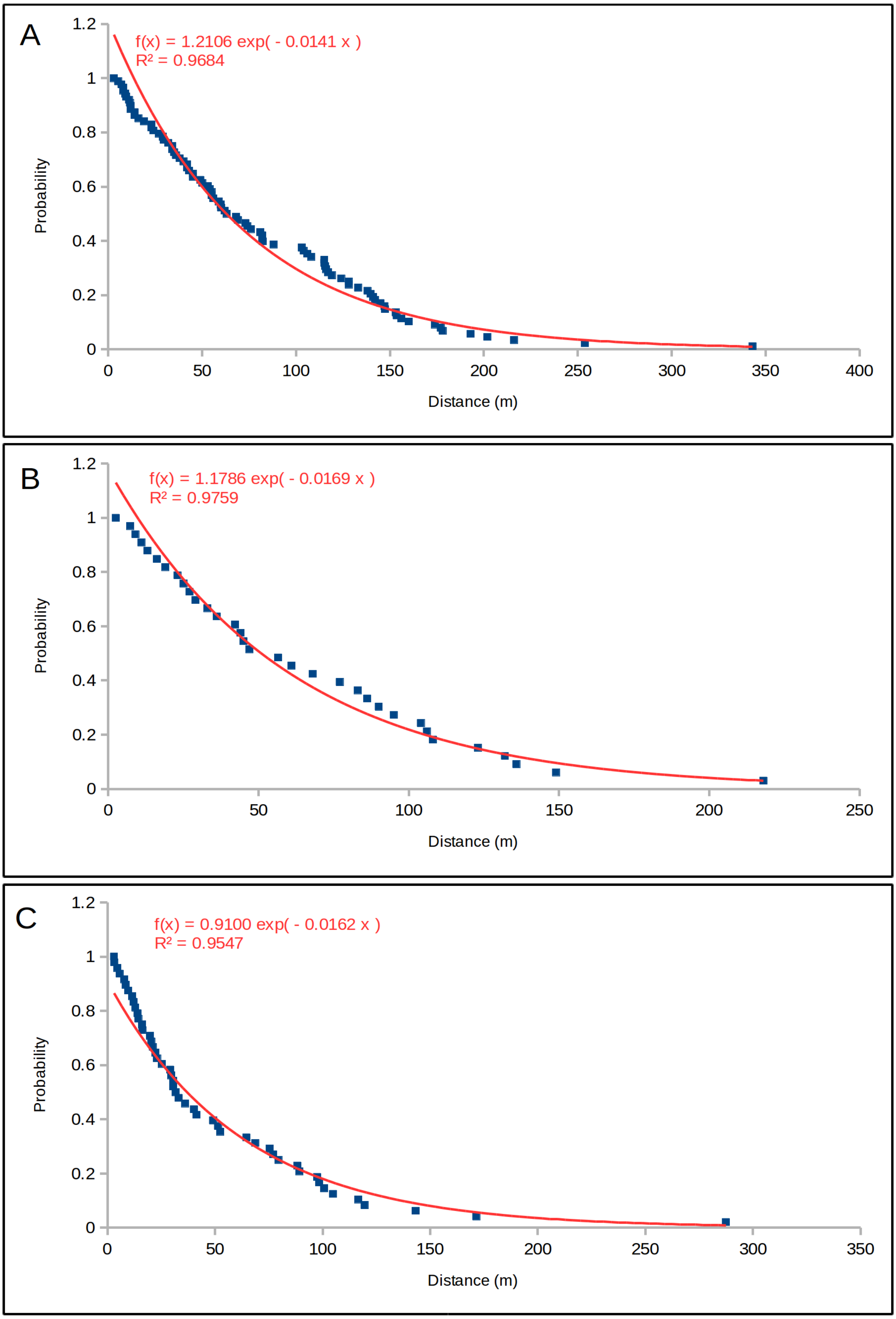
Comparison between expected and observed probability that *Maniola jurtina* individuals cross two types of LTIs on the control site. Expected probabilities were calculated from a theoretical distribution fitted to a dispersal kernel as if LTIs were completely permeable. Panel A shows the comparison between expected and observed number of crossing events. Error bars represent mean ± SD. Significance was based on binomial tests. NS : Non Significant. Panel B shows effect sizes (logOddsRatio) ± 95% confidence intervals.

Appendix E

Seasonality variation in movements

It is likely that seasonality variation in movements occurs (Schtickzelle et al., 2012). On the study site, a capture-recapture data set was available from 06 June to 16 August 2016. In the manuscript, a reduced data set was used for the study site (from 04 July to 16 August) to match the sampling period performed on the control site (from 13 July to 26 August). However, we used the entire data set to test the hypothesis of seasonal variation in movements. Accordingly, we analysed the monthly variation in the dispersal kernels on the study site (June, July and August). These three kernels are only informative about the seasonal variations as the infrastructures present (motorway and railway) are likely to modify butterfly movements. We fitted Negative Exponential Function ($P(x) = \beta e^{-\alpha x}$) to the kernels and exported the value of α to compare the dispersal kernels.

We revealed that the mobility of butterflies was higher in June ($1/\alpha = 71$ m) than in July ($1/\alpha = 59$ m) or August ($1/\alpha = 62$ m). In July and August, the dispersal kernels were relatively similar but we detected an increase in butterflies movements in June. In *Maniola jurtina*, individuals tend to become less active during the hottest months (July, August), than directly after emergence (June) (Grill et al., 2013), which might explain the observed pattern. This highlights the importance of considering the use of a proper control site (similar time frame and similar landscape characteristics to the case study site). Indeed, using the kernel distribution computed with data from July and August would have led to underestimating the expected probability of crossing events in June, with possible spurious outcomes.



Seasonal variability in the dispersal kernel calculated on the study site when only data from the month of June (A), July (B) or August (C) were used. Red lines indicate the Negative Exponential Functions fitted to the recapture data. Equations and model fit are indicated on each graph.

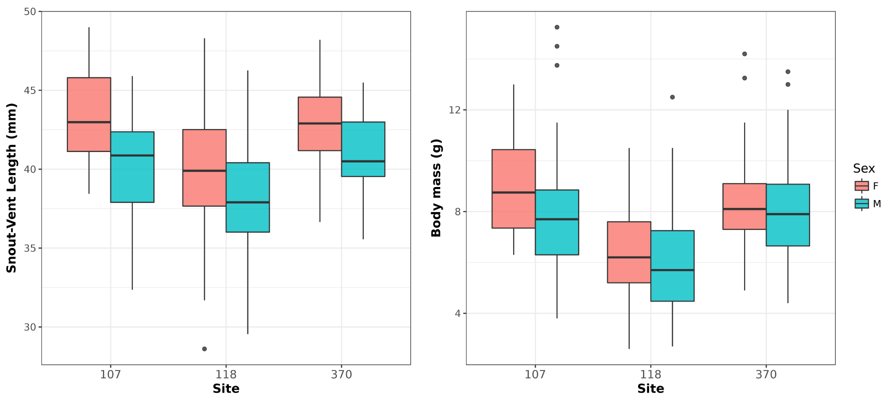
Appendix F

Additional informations provided by the MRR surveys

This Appendix provides additional results obtained from the three year surveys on the Midwife toad (*Alytes obstetricans*) that we performed on sites **107**, **118** and **370** in south-western France.

Size and body mass

On the three sites where we performed MRR surveys (**107**, **118** and **370**), we estimated the size and body mass of individuals. These two parameters were compared among sites using one-way ANOVA. Normality of residuals was tested using Shapiro tests and the dependent variable "body mass" was log transformed to satisfy normality criterion. Males carrying clutches were excluded from analysis as their weight included the eggs.



Snout-vent length (SVL) and body mass of male and female midwife toads among three studied populations (**107**, **118** and **370**) in south-western France

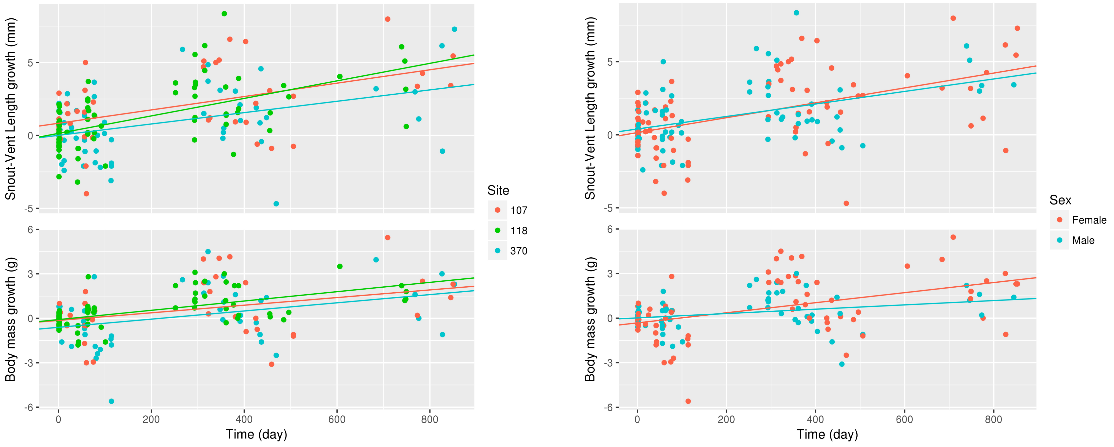
We found that females were always bigger than males ($F_{1,299} = 29.6$, $p < 0.0001$). Averaged SVL of males and females across sites were 39.5 ± 0.3 mm and 41.6 ± 0.3 mm, respectively. SVL varied among sites ($F_{2,299} = 24.6$, $p < 0.0001$) with individuals from site **118** being smaller than the two other ($p < 0.0001$). This pattern was consistent with the sex of individuals. Females from site **118** were smaller than females from the two other sites (all $p < 0.001$), as were males (all $p < 0.05$). However, individuals from sites **107** and **370** were similar in sizes ($p = 0.66$).

In a similar way, we found that females were always heavier than males ($F_{1,327} = 6.0$, $p = 0.015$). Averaged body mass of males and females across sites were 7.1 ± 0.2 g and 7.5 ± 0.2 g, respectively. Body mass varied among sites ($F_{2,327} = 50.1$, $p < 0.0001$) with individuals from site **118** having reduced body mass compared to the two other populations ($p < 0.0001$). This pattern was consistent with the sex of individuals. Females from site **118** had lower body masses than females from the two other sites (all $p < 0.0001$), as had males (all $p < 0.0001$). However individuals from sites **107** and **370** had similar body masses ($p = 0.97$).

Two main explanations can be proposed to explained why individuals are smaller on site **118**. First, their might be smaller because of reduced adult survival compared to the two other sites. Site **118** is located in the middle of a village of 450 inhabitants with potentiality more susceptibility to be killed because of human disturbances including road mortality. In fact, this site had the highest density of surrounding secondary roads (Table 3.1). However, the MRR analyses suggested that adult survival on this site was not different from the two other sites (Fig. 3.4), indicating that this hypothesis is unlikely. A second explanation might be a strong recruitment of young and small individuals in this population. A strong recruitment would lead to an over-representation of young age classes compare to old age classes. High recruitment could be the result of great hatching success and high tadpole survival stemming from optimal water bodies. In this study we could not test this hypothesis, but the high number of juveniles found on this site gave additional support to this hypothesis. Future studies should aim to assess demographic parameters of larvae in addition to adult metrics. We found that size and body mass were higher in females than in males, a typical pattern in amphibians and previously described in this species Márquez et al. (1997).

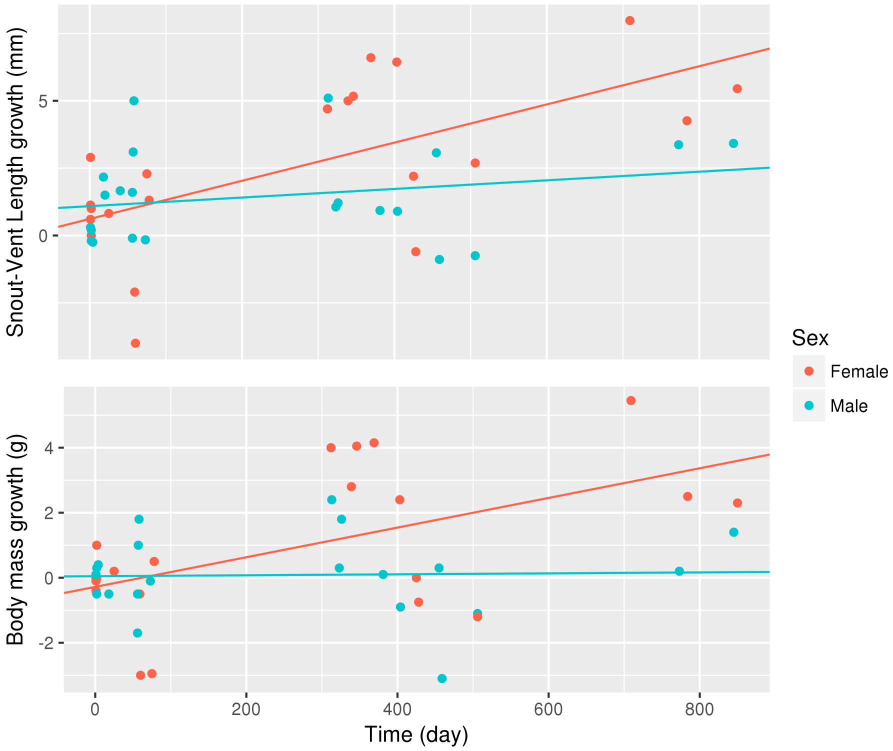
Individual growth rate

We explored how the individual growth (size and body mass) varied between sex and among sites using ANCOVA, using the time interval (time) between two measures as covariable.



SVL and body mass growth of midwife toads among three studied populations (**107**, **118** and **370**) or between sex in south-western France

We found that midwife toad grew similarly among sites either considering SVL ($F_{2,147}$ = 2.0, $p = 0.14$) or body mass ($F_{2,136} = 0.9$, $p = 0.42$). There was a difference between sex: females grew more rapidly than males when considering the body mass ($F_{1,136} = 3.9$, $p = 0.049$). However, males and females grew similarly when considering the SVL ($F_{1,147}$ = 0.5, p = 0.49). We found that this pattern was site-specific. On the sites **118** and **370**, males and females had similar growth rates either considering SVL or body mass (all $p > 0.05$). On site **107**, females grew faster than males (SVL: $F_{1,39} = 5.1$, $p = 0.029$; body mass: ($F_{1,37} = 4.8$, $p = 0.034$).



SVL and body mass growth of midwife toads on the site 107 in south-western France

Fecundity

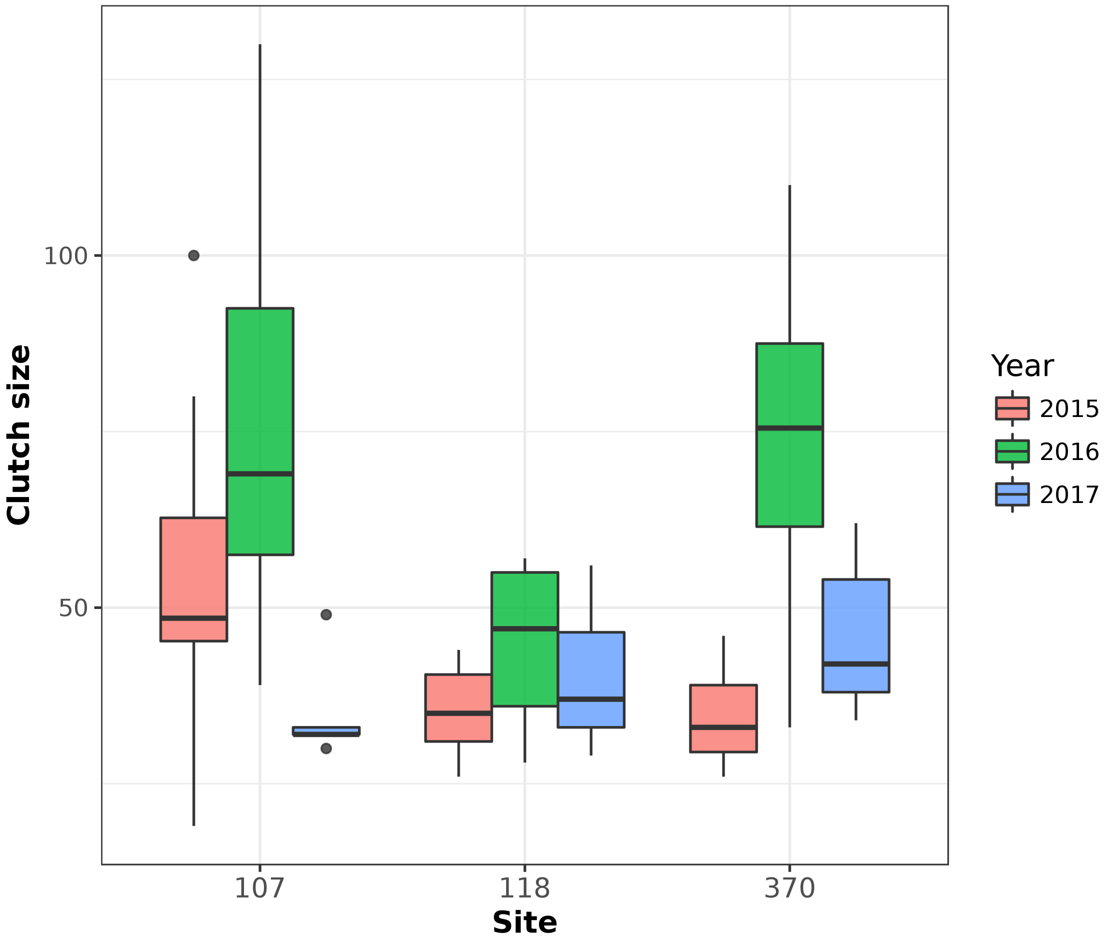
The proxy for population fecundity on the three sites was assessed using clutch sizes (number of eggs per clutch) carried by all encountered males. We compared clutch sizes among years and among sites using two-way ANOVA. We log transformed the dependent variable (clutch size) to met normality of residuals assumption and used TukeyHSD for post-hoc tests.

In total, we captured 67 males carrying clutches; 21 on site **107**, 24 on site **118** and 22 on site **370**. The averaged clutch size carried by males across sites and years was 51.4 ± 2.8 eggs and varied between 19 and 130 eggs.

Some fecundity parameters of the clutches carried by midwife toads in three populations in 2015, 2016 and 2017 in south-western France. We recorded the number of males carrying a clutch (NC), the total number of eggs (Eggs) which is the clutch size (CS) multiplied by the number of clutches (NC). Clutch sizes are indicated with standard errors (CS-se).

	NC			**Eggs**			**CS**			**CS-se**			**Total**			
Pop	**2015**	**2016**	**2017**	**2015**	**2016**	**2017**	**2015**	**2016**	**2017**	**2015**	**2016**	**2017**	**NC**	**Eggs**	**CS**	**CS-se**
107	8	8	5	442	601	176	55.2	75.1	35.2	8.7	10.8	3.5	21	1219	58	6.2
118	6	15	3	212	675	122	35.3	45	40.7	2.9	2.8	8	24	1009	42	2.2
370	7	10	5	242	744	230	34.6	74.4	46	2.7	7.8	5.2	22	1216	55.3	5.4

The clutch size differed among sites ($F_{2,58} = 3.8$, $p = 0.027$) with males from site **118** carrying smaller clutches than males from site **107** ($p = 0.038$). However, clutch size was not different between the sites **370** and **107** ($p = 0.94$) and between the sites **370** and **118** ($p = 0.08$). There was a strong evidence of clutch size variability across years ($F_{2,58}$ $= 13.2$, $p < 0.0001$). In 2015 and 2017, clutches carried by males were similar in sizes ($p = 0.98$). But clutches contained about 50% more eggs in 2016 than in the two other years (all $p < 0.001$).



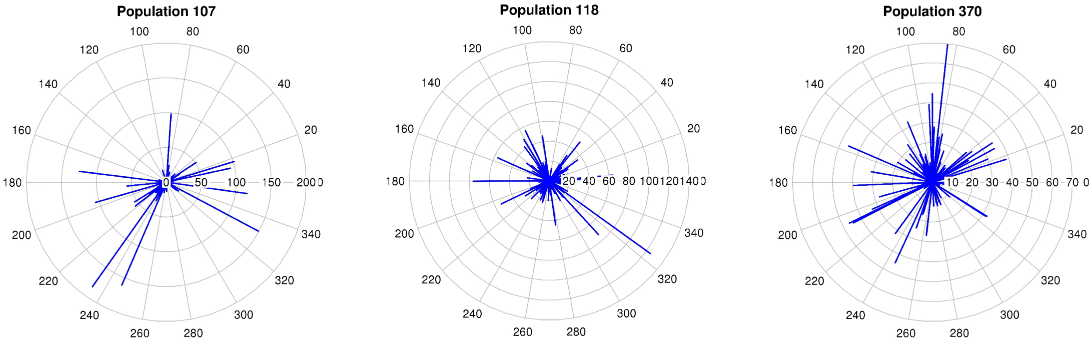
Clutch sizes carried by male midwife toads among three sites in 2015, 2016 and 2017 in south-western France

The average clutch size carried by males was 51 eggs. *A. obstetricans* can carry from one to three clutch batches at the same time (Reading and Clarke, 1988). This value was comparable to the number of eggs of a single batch (Reading and Clarke, 1988), but greater than the mean number of eggs delivered by females in a clutch (42 eggs) (Márquez, 1993). This suggest that most of the encountered males with eggs, were carrying a single batch clutch.

Intra-population movements

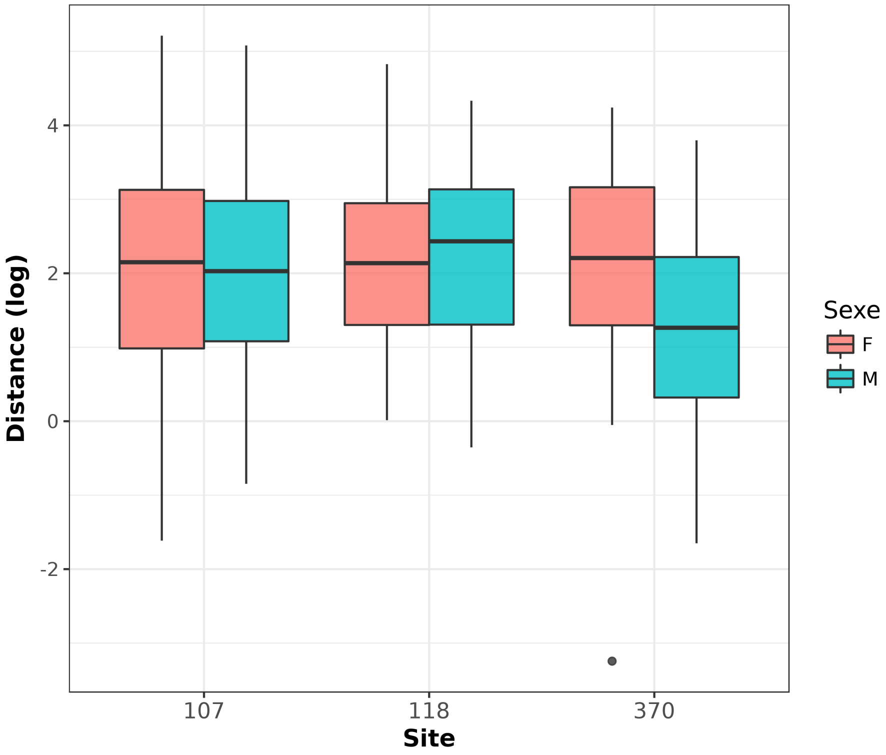
Travelled distances of *A. obstetricans* were estimated by calculating an euclidean distance between each pair of capture events with QGIS (V. 2.8). To determine whether the average direction of observed trajectories were random or showed a direction trend, we performed Rayleigh tests at the site level (pooling all recapture events from a given site). In addition, we investigated how traveled distances varied between sex and among sites using Linear Mixed Models (LMMs). The time interval (time) between pairs of capture events was always kept as an interacting factor. We first built the most complete model: sex:site:time+sex:time+site:time; then we constructed reduced models by removing predictors one by one until the last model contained only the intercept. All models were ranked based on their Akaike Information Criterion (AICc)(Burnham and Anderson, 2002) and the most parsimonious model was selected based on delta AICc. In all models, we used individual (ID) as a random variable.

A total number of 409 individual movements were recorded on the three sites (102 on site **107**, 174 on site **118** and 133 on site **370**). We recorded many small movements (median = 7.6 m) but some long distances were detected (max = 183.5 m for an individual on site **370**). Midwife toads were not moving toward specific directions in any of the three sites (Rayleigh test, all $p > 0.05$).



Polar plots of the three studied sites. Each blue line corresponds to a capture-recapture event with the distance and the direction represented.

The length of travelled distances was moderately correlated with time (r = 0.11, $p = 0.025$) indicating that the longer the time interval between two capture events, the greater distance they were able to travelled. Based on the AICc criterion, the most parsimonious model was time+sex:time (AICc = 1400.8) but this model was not different from a simpler model including only time as predictor (ΔAICc = 0.2, $\Pr(> \chi^2) = 0.12$). This suggest that distance travelled by midwife toads was similar between sex and among sites.



Distance travelled (log) by male and female midwife toad among three sites in south-western France.

Appendix G

Pradel robust design model selection

Candidate models for the three capture-mark-recapture sites

Site	Model	AICc	Delta AICc	AICc Weights	Model Likelihood	Num. Par	Deviance
107	{Phi(.)Y(.)p=c(t*session)F0(t)}	676.40	0.00	0.41	1.00	34	-154.25
107	{Phi(.)Y(t)p=c(t*session)F0(t)}	677.05	0.65	0.30	0.72	35	-156.50
107	{Phi(t)Y(.)p=c(t*session)F0(t)}	677.71	1.31	0.21	0.52	35	-155.84
107	{Phi(t)Y(t)p=c(t*session)F0(t)}	679.62	3.22	0.08	0.20	36	-156.86
107	{Phi(t)Y(t)p=c(session)F0(t)}	701.05	24.65	0.00	0.00	10	-68.88
107	{Phi(t)Y(t)p=c(.)F0(t)}	705.56	29.16	0.00	0.00	8	-59.98
118	{Phi(.)Y(.)p=c(t*session)F0(t)}	643.54	0.00	0.41	1.00	30	-1092.02
118	{Phi(t)Y(t)p=c(t*session)F0(t)}	643.70	0.16	0.38	0.92	32	-1096.61
118	{Phi(.)Y(t)p=c(t*session)F0(t)}	644.83	1.29	0.21	0.52	31	-1093.10
118	{Phi(t)Y(t)p=c(.)F0(t)}	791.07	147.53	0.00	0.00	8	-895.61
118	{Phi(t)Y(t)p=c(session)F0(t)}	791.97	148.43	0.00	0.00	10	-898.91
370	{Phi(.)Y(.)p=c(t*session)F0(t)}	676.72	0.00	0.60	1.00	34	-515.66
370	{Phi(t)Y(.)p=c(t*session)F0(t)}	679.21	2.49	0.17	0.29	35	-515.81
370	{Phi(.)Y(t)p=c(t*session)F0(t)}	679.27	2.55	0.17	0.28	35	-515.75
370	{Phi(t)Y(t)p=c(t*session)F0(t)}	681.60	4.88	0.05	0.09	36	-516.06
370	{Phi(t)Y(t)p=c(session)F0(t)}	695.57	18.84	0.00	0.00	10	-439.70
370	{Phi(t)Y(t)p=c(.)F0(t)}	698.48	21.75	0.00	0.00	8	-432.49

All sites were studied with the Pradel seniority robust design with the parameter of: survival probability (Phi), seniority parameter (Y), capture probability (p), recapture probability (c) and number of missed individuals (F0), tested for the effect of primary sampling occasions (t), secondary sampling occasions (session) or constant across sessions (.). Models were compared using the Akaikes Information Criterion adjusted for sample size AICc.

Annexe H

Article de vulgarisation “vivre à Brouchaud”

PROJET CIRFE

La fragmentation des habitats

Avez-vous entendu parler de la sixième extinction de la biodiversité? C'est ainsi qu'est nommée la crise écologique actuelle. Cette crise se traduit par une extinction massive d'espèces. La dernière extinction de biodiversité remonte à 65 millions d'années, lorsque les dinosaures ont disparu. Autant dire qu'il est urgent de comprendre les mécanismes participant à cette grande extinction.

Parmi les causes de cette perte de biodiversité, la fragmentation des habitats représente une grande menace. Un habitat fragmenté est un milieu naturel morcelé, séparé en plusieurs 'morceaux' isolés les uns des autres. Par exemple, une grande étendue de forêt peut être coupée en deux par une route, grignotée par l'urbanisation ou la création de parcelles agricoles, traversée par une ligne électrique… Au final, cette forêt sera organisée en différents fragments entourés par des milieux ouverts ou artificialisés.

L'isolement des 'morceaux' d'habitats naturels empêche certains animaux de se rendre d'un fragment à un autre. Par exemple, un chevreuil aura du mal à rejoindre un morceau de forêt située de l'autre côté d'une grosse infrastructure. Or, les animaux ont besoin de se déplacer pour trouver des sources de nourriture et des partenaires pour se reproduire. Si les animaux vivant dans ces 'morceaux' d'habitats sont trop isolés les uns des autres avec l'impossibilité de réaliser des échanges entre habitats, il y a un véritable risque de voir décliner rapidement l'espèce (c'est ce qui est arrivé au panda géant en Chine).

Dans nos sociétés développées, les infrastructures de transport (routes, autoroutes, voies ferrées...) participent grandement à la fragmentation des milieux naturels. Mais toutes n'ont pas les mêmes effets selon qu'on s'intéresse à un papillon ou un crapaud. Pour comprendre quelles sont les structures paysagères qui empêchent les animaux de se déplacer, des suivis approfondis sont nécessaires. L'enjeu est de mieux comprendre la biologie des espèces et de cibler les menaces pour pouvoir préserver au mieux la nature qui nous entoure.

Un projet de recherche pour mieux comprendre les effets des infrastructures sur la faune

Afin d'étudier le lien entre déplacement des animaux et fragmentation, le bureau d'étude TerrOïko, (situé à Sorèze dans le Tarn), le CNRS de Moulis (Ariège) et la société d'ingénierie Setec se sont associés pour mettre en œuvre un projet de recherche. Il consiste à évaluer les effets barrières de plusieurs types d'infrastructures de transport sur la capacité de mouvement des animaux. Ce projet intitulé CIRFE (Cumul d'Infrastructures linéaires de transport terrestre et Relations Fonctionnelles Écologiques) se déroule sur 3 ans (2015-2017) et est fiancé par le CIL&B (le Club des opérateurs d'Infrastructures Linéaires et Biodiversité).

Une zone de quelques centaines de kilomètres carrés entre Périgueux et Brive-la-Gaillarde offre le territoire idéal pour réaliser ce suivi de la faune. En effet, il existe peu d'endroits en France qui présentent un maillage d'infrastructures de transport aussi diversifié sur une zone aussi restreinte. Sur l'aire d'étude, l'A89, une voie ferrée, l'ancienne N89, un gazoduc et une ligne moyenne tension s'entremêlent.

Sur cette zone d'étude, nous avons étudié comment le mouvement des organismes présents est perturbé par ces infrastructures en nous focalisant sur 4 espèces communes : la Couleuvre à collier, l'Alyte accoucheur, le papillon Myrtil et un coléoptère forestier (Féronie noire). Une méthode très utile pour étudier le mouvement de la faune consiste à prélever un peu d'ADN de plusieurs individus d'une espèce et de regarder s'ils sont plus ou moins proche génétiquement entre eux. On réalise un arbre généalogique en quelque sorte. Les individus sont recherchés de part et d'autre des différentes infrastructures afin de vérifier si certaines empêchent les individus de traverser.

Couleuvre à collier 	***Papillon Myrtil*** 
Féronie noire 	***Alyte accoucheur*** 

Les premiers résultats montrent que les routes, et tout particulièrement l'ancienne N89, sont d'importantes barrières pour le déplacement de l'Alyte accoucheur et de la Féronie noire. Aucun effet des autres infrastructures n'a été détecté pour les quatre espèces étudiées. Il semblerait cependant que l'A89 soit trop récente pour pouvoir observer un effet barrière dans la signature génétique des individus (il faut attendre un certain nombre de générations pour que la divergence génétique puisse être détectée de chaque côté de l'autoroute).

Notre travail sur la commune de Brouchaud

Brouchaud est l'une des 15 communes sur lesquelles nous avons réalisé le suivi. Elle est située au nord de la zone étudiée. Deux des quatre espèces ont été suivies au niveau de La Rousselie : le Myrtil et la Féronie noire. D'autres espèces remarquables présentes sur la commune ont également pu être observées. Il s'agit en particulier de trois espèces de serpents: la Couleuvre à collier, la Couleuvre verte et jaune (appelé localement 'cingle') et la Couleuvre vipérine. Cette dernière est d'un intérêt particulier car très peu présente sur l'ensemble du territoire prospecté. A Brouchaud, ce sont plusieurs individus adultes et juvéniles de Couleuvre vipérine qui ont été observés le long du Blâme (principalement près de l'ancien lavoir entre les lieus dits 'La Rue' et 'La Rousselie'). C'est une espèce de serpent totalement inoffensive, plutôt petite (< 1 m), qui subit la crainte des Hommes à cause de sa ressemblance avec la vipère. La Vipère aspic, bien que présente en faible densité sur des communes alentours (détectée à Saint-Orse, Saint-Antoine d'Auberoche ou encore Milhac d'Auberoche), n'a d'ailleurs pas été observée sur la commune de Brouchaud (ce qui ne veut pas dire qu'elle n'est pas présente!). Avec un œil averti, ces deux espèces sont facilement distinguables. La Couleuvre vipérine est fine, possède des pupilles rondes, de grandes écailles sur la tête et ne s'éloigne que très peu des cours d'eaux (on peut fréquemment l'observer en train de nager et de chasser des poissons). La Vipère aspic, elle, est trapue, possède des pupilles verticales avec de petites écailles sur la tête, un nez retroussé et elle n'apprécie guère l'eau.

Couleuvre vipérine à Brouchaud
Notez la forme ronde de pupilles

Vipère aspic à St Antoine d'Auberoche

En ce qui concerne les amphibiens, Brouchaud regorge de Grenouilles rieuses (notamment au niveau de l'ancienne pisciculture et le long du Blâme). Elles sont très actives au printemps, période à laquelle il est possible d'entendre leurs chants caractéristiques qui donnent l'impression qu'elles rient (d'où leur nom). Ce n'est pas la seule espèce présente à Brouchaud : des Grenouilles agiles, des Salamandres tachetés, des Rainettes méridionales et des Crapauds communs se baladent également entre les milieux humides et forestiers de la commune.

Grenouille rieuse

Grenouille agile

Une dizaine d'espèces de papillons a également été identifiée (comme le grand mars changeant, le grand nègre des bois ou encore le tabac d'Espagne), et de nombreux oiseaux, libellules et mammifères observés. Le Blâme apporte à la commune de Brouchaud une fraîcheur et une biodiversité remarquable. Ce cours d'eau emblématique nécessite une attention particulière car il abrite un foisonnement de vie (libellules, amphibiens, poissons, couleuvres, oiseaux...) que ce soit dans l'eau ou sur ses berges.

Grand mars changeant	***Grand nègre des bois***	***Tabac d'Espagne***
		

Nous tenons à remercier la Mairie de Brouchaud de nous avoir permis de mener à bien ce suivi ainsi que les propriétaires pour l'accès à leurs terrains privés.

Grâce à cette étude, les effets des infrastructures sur la faune dans ce territoire seront mieux compris. En attendant que nos routes ne bénéficient de structures de franchissements pour petite faune (crapauduc par exemple) nous vous invitons à lever le pied sur les routes de campagne. On ne se sait jamais, un amphibien, serpent ou mammifère pourrait être tenté de traverser.

Article rédigé par Jonathan Remon, TerrOïko.

Pour en savoir plus sur CIRFE et TerrOïko : http://www.terroiko.fr/CIRFE.php
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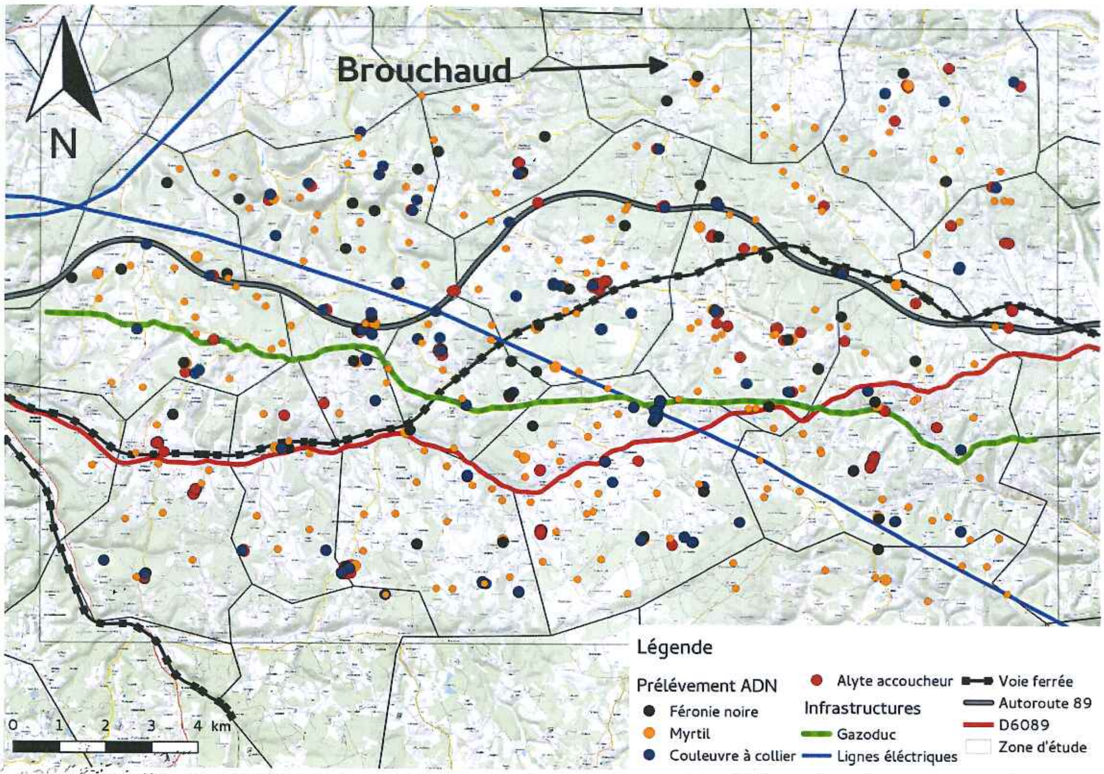

Zone étudiée en Dordogne. Les polygones noirs représentent la délimitation des communes.