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Évaluer la connectivité en paysage fragmenté : de l'écologie comportementale à la biologie de la conservation

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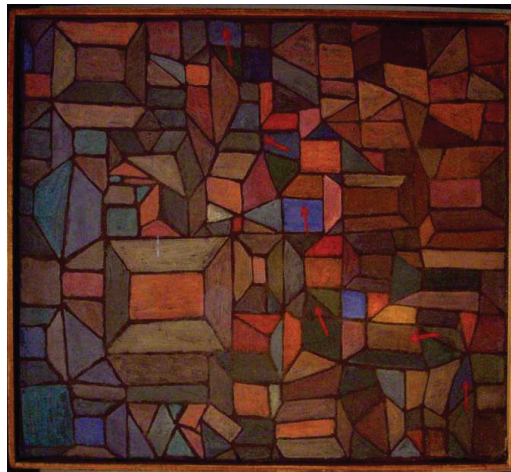
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par

JANIN Agnès



Le chemin de la citadelle, Paul Klee.

**Evaluer la connectivité en paysage fragmenté :
de l'écologie comportementale à la biologie de la conservation.**

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Ecologie des populations

Evaluer la connectivité en paysage fragmenté: de l'écologie comportementale à la biologie de la conservation.

Résumé

Dans les paysages fragmentés, les mouvements entre les patchs d'habitats jouent un rôle primordial dans la persistance des populations en assurant les flux génétiques, la possibilité de recolonisation après extinction locale, la liaison entre différents habitats pour la reproduction ou l'acquisition des ressources, Malgré l'importance de ces mouvements, la structure du paysage entre ces patchs (i.e. la matrice) est le plus souvent considérée comme un ensemble homogène de non-habitat d'intérêt mineur et les adaptations comportementales en réponse à la fragmentation du paysage sont rarement prises en compte. Pour étudier l'importance de l'influence du paysage sur les mouvements, trois approches complémentaires sont pratiquées. 1) Des études expérimentales sont menées pour évaluer les réponses comportementales et physiologiques du crapaud commun (*Bufo bufo*) juvénile et adulte soumis aux différents types d'occupation du sol. Ainsi, les types d'occupation du sol sont caractérisés par le coût que leur traversée représente pour l'espèce étudiée. 2) Ce travail s'attache également à décrypter les règles régissant les déplacements en paysage hétérogène et à mettre en évidence des différences comportementales selon le paysage d'origine (adaptations). 3) Basé sur la modélisation, le troisième volet doit permettre d'effectuer des prédictions à l'échelle des paysages.

Mots-clés

Amphibiens; Comportement; Conservation; Fragmentation; Mouvement; Paysage; Perte d'habitat.

Assessing connectivity in fragmented landscape: from behavioural ecology to biological conservation

Summary

In fragmented landscapes, movements between habitat patches play a crucial role in population persistence by allowing gene flow, rescue effect possibilities, linking different habitats for reproduction or resources acquisition, ... Although the importance of these movements, the landscape structure between patches (i.e. matrix) is too frequently considered as a homogeneous non-habitat of minor interest and behavioural adaptations in response to fragmentation are rarely taken into account. To investigate the importance of the landscape influence on movements, three complementary approaches are used: 1) experimental studies are performed to assess behavioural and physiological responses of common toads when they are confronted to different land uses. Land uses are thus characterised par the cost of their crossing for the focus species. 2) This work also aims to decipher the rules of movement in heterogeneous landscapes and to highlight behavioural differences according to the originated landscape (adaptations). 3) On the basis of modelling, the third part of this work intends to make predictions at the landscape scale.

Key-words

Amphibians; Behaviour; Conservation; Fragmentation; Habitat loss; Landscape; Movement.

Une thèse, c'est avant tout trois voire quatre ans de vie. Autant et si peu. Il est certain qu'il faut être intrépide et confiant (ou fou, la frontière étant mince) pour se lancer dans cette aventure un peu particulière.

Malgré la difficulté d'expliquer pourquoi il est important que des hommes cherchent sur Terre, mes "histoires de crapauds" ont toujours fasciné l'auditoire. Cela a été un réel plaisir de conter mes nuits aux bords des mares et le comportement de mon fabuleux sujet d'étude. Avant d'attaquer mes remerciements, laissez-moi avoir une pensée pour ces fascinants crapauds avec lesquels j'ai travaillé toutes ces années. Cela faisaient grandement partie du plaisir que de les retrouver tous les mois de mars. J'espère que leur route au côté de l'homme sera encore longue (plus pour les hommes que pour les crapauds ...).

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Introduction : objectifs et champs d'investigation

1. La fragmentation : vers une définition.

Les modifications du paysage et la fragmentation des habitats par les activités humaines sont aujourd'hui un enjeu majeur en biologie de la conservation (Meffe et Carroll 1997 ; Ewers et Didham 2006 ; Fischer et Lindenmayer 2007). Ces processus sont en effet considérés comme l'une des plus sévères menaces pesant sur la biodiversité (Sala *et al.* 2000; Foley *et al.* 2005 in Fischer et Lindenmayer 2007), leur impact se déclinant au sein de tous les groupes taxonomiques incluant les mammifères et les oiseaux (Andren 1994), les reptiles (Madsen *et al.* 1996 ; Macnally et Brown 2001), les amphibiens (Cushman 2006), les invertébrés (Didham *et al.* 1996; Wahlberg *et al.* 1995 ; Hendrickx *et al.* 2007) et les plantes (Young *et al.* 1996).. Néanmoins, pour bien appréhender les mécanismes en jeu, il est essentiel d'analyser avec précision les différents concepts en jeu. Il est tout d'abord important de définir le concept d'habitat duquel les autres concepts vont découler. L'habitat peut en premier lieu faire référence aux localisations où l'on retrouve la présence d'une espèce ou d'un ensemble d'espèces (définition corrélative). Mais cette définition ne permet pas de prédire la distribution des espèces. Il faut donc y rajouter des variables de description des lieux de vie où l'on mesure des conditions de vie similaires (définition descriptive). Cette définition de l'habitat est présente en biologie de la conservation à travers la lecture que l'homme fait du paysage. Elle a donné lieu à une nomenclature extrêmement précise, utilisée dans la codification européenne Corine (Coordination de l'Information sur l'Environnement). Néanmoins, la biologie de la conservation s'appuie la plupart du temps sur une définition plus fonctionnelle basée sur une conception évolutive impliquant de considérer la fitness. L'habitat est alors la niche écologique permettant aux organismes d'assurer survie et reproduction.

Bien que devenu un thème de recherche majeur, le concept de fragmentation demeure, quant à lui, encore défini de façon floue et variable selon les auteurs (Fahrig 2003). Dans une récente revue, Laurance (2008) décline le concept de fragmentation de l'habitat en deux processus : réduction de la quantité totale d'habitat dans le paysage et morcellement de l'habitat restant en petits fragments dont la taille et le degré d'isolation varient "*Habitat fragmentation involves two distinct but interrelated processes. First, the total amount of original habitat in the landscape is reduced. Second, the remaining habitat is chopped up into fragments of various sizes and degrees of isolation*", tandis que Ewers et Didham dans une revue de 2006 restreignent la fragmentation à l'expression du second processus. Même les revues cherchant à bien poser ce problème de définition utilise le terme de fragmentation comme mot-clé dans leur intitulé puis décline toutes les modifications du paysage que ce concept peut abriter (Fischer et Lindenmayer 2007). Il en résulte une très grande diversité de variables mesurées afin d'estimer ce paramètre (Fahrig 2003), rendant difficile l'élaboration d'un paradigme général. Néanmoins, tendre vers une uniformisation du concept ne doit pas effacer les caractéristiques liées au contexte spatial, aux espèces étudiées ainsi qu'aux objectifs visés (Haila 2002). Les travaux empiriques démontrent d'ailleurs une très large variété d'effets de la fragmentation (Ewers et Didham 2006) faisant parfois émerger des résultats contradictoires (Haila 2002) : conséquences négatives ou neutres mais aussi positives de la 'fragmentation' (de multiples exemples sur diverses espèces cités dans Fahrig 2003) au sein même d'espèces voisines (Herrman *et al.* 2010).

La notion de fragmentation de l'habitat demeure ambiguë pour trois raisons principales. Premièrement, la fragmentation *sensu lato* englobe très généralement une réduction de la surface de l'habitat couplée à un changement d'organisation spatiale de l'habitat restant modifiant la connectivité entre patches d'habitat. Or ces deux phénomènes souvent conjoints relèvent de processus écologiques différents et nécessitent donc d'être étudiés de façon

indépendante (Haila 2002, Fahrig 2003). De plus, même la fragmentation *sensu stricto* ou *per se* regroupe plusieurs changements du paysage concomitants et pouvant tous agir sur la persistance des populations : l'augmentation du nombre de fragments et la réduction de leur taille, la modification de leur forme, l'augmentation de l'isolement ainsi que l'apparition de nouveaux "effets bordure" (Fahrig 2003; Ewers et Didham 2006). Deuxièmement, différents organismes expérimentent de façon variable, parfois même contradictoire, les effets des degrés de fragmentation, selon leurs exigences écologiques et leur histoire de vie (Ewers et Didham 2006). Notamment, le degré de spécialisation influence fortement la réponse des organismes aux modifications du paysage (Devictor *et al.* 2008; Habel *et al.* 2009). Cela se décline tant à l'échelle spécifique qu'inter et intra populationnelle. Enfin, la fragmentation du paysage, classiquement, se focalise sur la structuration de l'habitat et assimile les patches restants à des îles encerclées d'une matrice considérée comme un ensemble homogène hostile (Haila 2002) (Figure 1). De plus en plus d'études ont néanmoins mis en évidence le rôle essentiel joué par l'hétérogénéité de la matrice (perçue différemment selon les organismes) sur la connectivité (Taylor 1993; Ricketts 2001; Kennedy et Marra 2010) (Figure 1). Ce rôle est d'ailleurs désormais intégré pleinement comme une composante de la fragmentation (Ewers et Didham 2006).

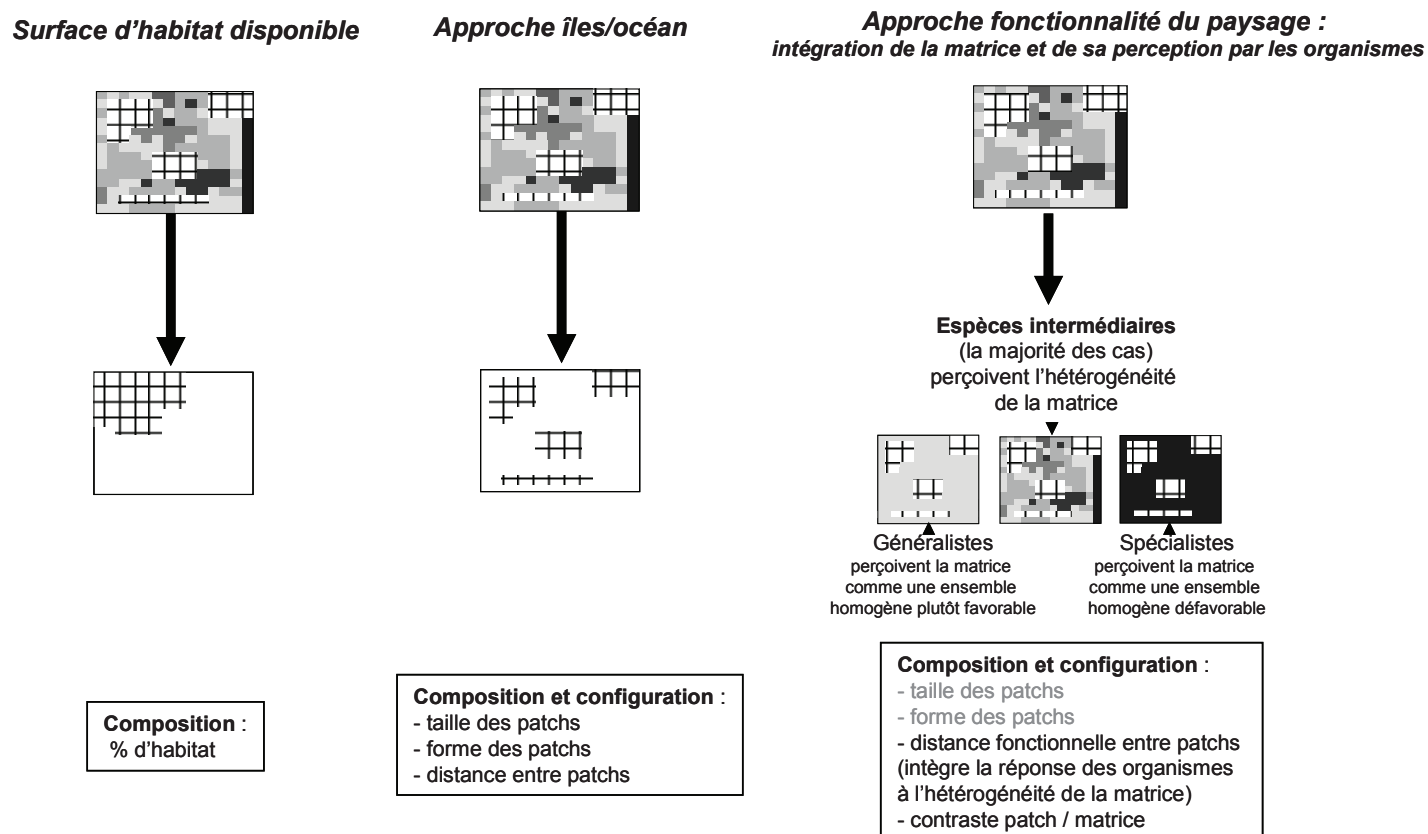


Figure 1 : Evolution du concept de fragmentation : intégrer la complexité de l'interaction organismes/paysage. Version simplifiée de Fahrig et Nuttle (2005).

De gauche à droite : historiquement, l'écologie du paysage s'est d'abord focalisée sur la disponibilité de l'habitat pour prédire la distribution des populations. La composition du paysage était alors le seul élément nécessaire (schéma de gauche). Puis, l'émergence des théories de biogéographie insulaire et du fonctionnement en réseau de populations a mis en exergue l'importance de la configuration de l'habitat. Le paysage est alors perçu de façon binaire : des patches d'habitat entourés d'une matrice de non-habitat. L'apparition conjointe des Modèles Spatialement Explicites permet de commencer à intégrer la notion d'échanges entre patches d'habitat (schéma central). Finalement, depuis le début des années 2000, l'approche fonctionnelle du paysage intégrant l'hétérogénéité de la matrice et surtout sa perception par les organismes s'est imposée en écologie du paysage (schéma de droite). Ainsi le contraste patch/matrice et la perméabilité de cette dernière ont récemment beaucoup

focalisé l'attention. Si les espèces généralistes ne subissent que très peu les effets de la matrice et perçoivent le paysage comme un ensemble plutôt homogène et favorable et si la vision binaire du paysage habitat/non-habitat reste valide pour les espèces spécialistes, une grande majorité d'espèces intermédiaires va percevoir l'hétérogénéité de la matrice et ajuster le comportement de dispersion à cette hétérogénéité.

Globalement, les changements de paysage ont un impact très fort sur les populations parce 1) une surface suffisante est nécessaire pour que les populations soient viables (dynamique des populations au sein des patches) et 2) ils affectent de façon significative et à de multiples échelles les mouvements des organismes (échanges entre patches). Or les mouvements supportent nombre de processus cruciaux en dynamique des populations à diverses échelles temporelles et spatiales (Nathan *et al.* 2008) que l'on peut résumer au travers de trois grandes catégories principalement sur la base de la fréquence (échelle temporelle) de ces mouvements: acquisition de ressources et évitement des prédateurs, migrations saisonnières, dispersion, qui s'avèrent donc soumis directement à l'influence du paysage (Figure 2).

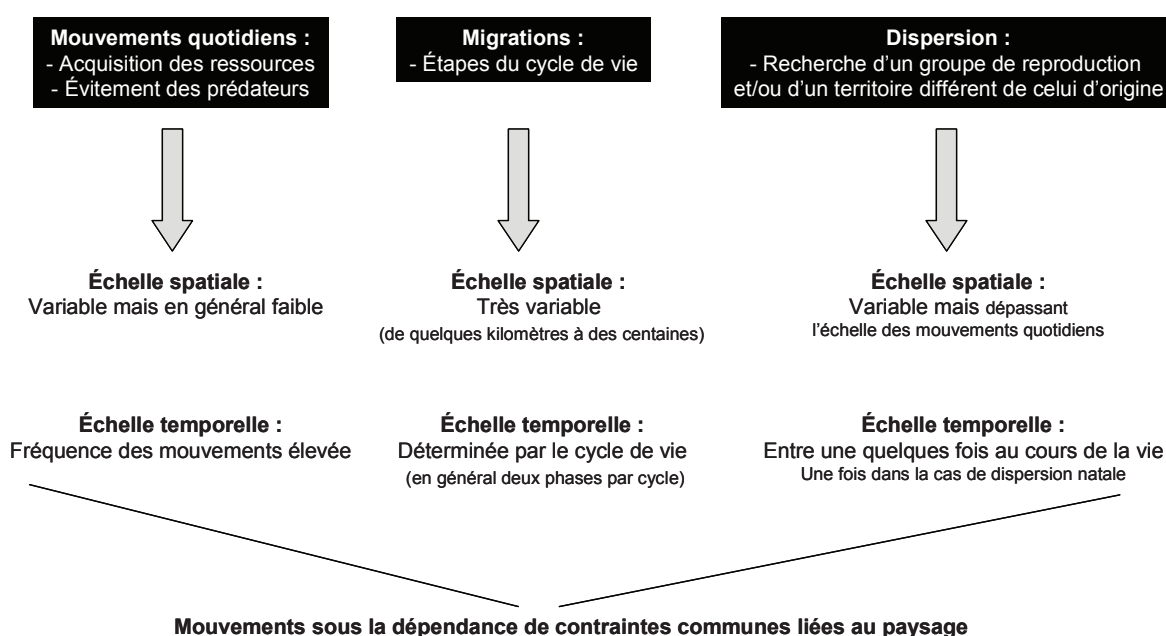


Figure 2 : Classification des différents types de mouvement.

En effet, au cours des différentes étapes liées au mouvement (émigration, transfert à travers la matrice et immigration; Baguette et Van Dyck 2007), différents éléments déterminés par la structure du paysage vont avoir une influence sur le déplacement (Figure 3). La phase d'émigration va être conditionnée par 1) la fréquence de rencontres des bordures dans le patch (dépendante à la fois de la taille et de la forme du patch d'habitat et de la propension à se déplacer) et 2) le contraste entre l'habitat et la matrice (dépendant de la nature de la matrice et du système de perception de l'espèce considérée). De façon plus indirecte, la structure du paysage via l'augmentation de la consanguinité dans les patches de petites superficies peut favoriser la motivation à disperser afin d'éviter la reproduction entre apparentés. La phase de transfert est quant à elle intrinsèquement liée à la composition et à la configuration de la matrice au travers des coûts et risques engendrés par le déplacement, des capacités locomotrices associées aux différents types d'occupation du sol traversés ainsi que de la capacité des organismes à choisir des chemins optimaux également dépendante des différents éléments du paysage rencontrés. Enfin, la phase d'installation est aussi soumise à l'influence du paysage à travers 1) l'attractivité liée à la qualité du patch, 2) la fréquence de rencontre des bordures de patches (liée au degré de fragmentation de l'habitat et à la taille, la forme des patches) et 3) la fenêtre de perception résultant d'une interaction entre capacités perceptuelles de l'organisme et structure du paysage. La fragmentation du paysage intervient donc de façon importante au cours de toutes les phases liées au mouvement, ce qui nécessite une compréhension fine de ces interactions notamment pour mettre en place des règles optimales de gestion en biologie de la conservation.

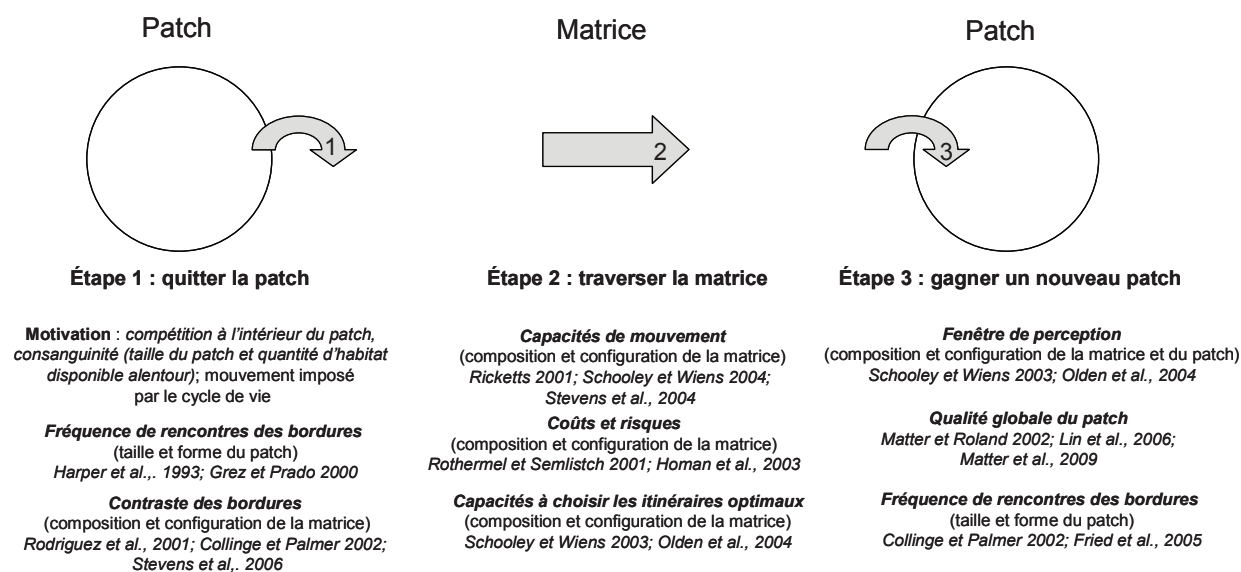


Figure 3 : Influence du paysage sur les différentes phases du mouvement. Sont détaillées les différentes composantes influençant chacune des trois étapes du mouvement. La phase d'émigration est dépendante de la motivation de l'organisme (indirectement sous la dépendance de la structure du paysage), de la fréquence de rencontre des bordures (elle-même fonction de la taille et la forme du patch) et du contraste entre patches et matrice (fonction de la nature et de la configuration de la matrice). La phase de transfert est sous l'influence des capacités de mouvement de l'organisme dans les différents types d'occupation du sol composant la matrice, des coûts et des risques engendrés par ces mouvements et des capacités à choisir un chemin optimal au sein de l'hétérogénéité de la matrice (fonction de la nature et de la configuration de la matrice). La phase d'installation est quant à elle affectée par la fenêtre de perception de l'organisme qui peut varier selon la structure du paysage, la qualité globale et de la taille et forme des patches d'immigration déterminant la fréquence de rencontre des bordures. L'influence de chacune de ces composantes sur les différentes phases du mouvement sont illustrées et soutenues par des travaux empiriques et/ou de modélisation.

1.1 Comment la fragmentation du paysage se décline-t-elle ?

Bien que la perte d'habitat soit typiquement considérée comme le facteur lié aux modifications du paysage qui impacte le plus fortement la persistance des populations (Fahrig

2003), la fragmentation *per se* des habitats restants fait l'objet d'un intérêt notoire en biologie de la conservation, notamment car ses effets plus insidieux que ceux de la perte d'habitat pure peuvent néanmoins bouleverser les dynamiques de populations (Ewers et Didham 2006). Ainsi, de plus en plus d'auteurs ont cherché à distinguer les effets de la perte d'habitat (ou composition du paysage) de ceux induits par la fragmentation *per se* (configuration du paysage) (Ritchie *et al.* 2009 ; Zurita et Bellocq 2010) comme le suggèrent les revues récentes (Haila 2002 ; Fahrig 2003). Néanmoins, trois hypothèses alternatives continuent d'alimenter la controverse sans que des avancées théoriques ou que résultats empiriques ne permettent d'aboutir à une conclusion définitive :

- l'hypothèse "composition du paysage" suggérant que la composition du paysage a un effet plus important sur les populations que le rôle négligeable joué par la configuration,
- l'hypothèse "configuration du paysage" impliquant un effet de la configuration indépendant de celui de la composition
- l'hypothèse "seuil de composition" incluant l'existence d'un seuil de perte d'habitat en dessous duquel la configuration commence à jouer un rôle important (Ritchie *et al.* 2009).

Des études théoriques suggèrent l'existence d'un tel seuil en dessous d'une proportion de 20 à 30% d'habitat dans le paysage (Fahrig 1998 ; Flather et Bevers 2002). Néanmoins bien que des résultats de revues appuient cette hypothèse (Andrén 1994), nous manquons encore de preuves empiriques aujourd'hui.

De plus, la fragmentation *per se*, lorsque son impact est identifié, intervient à travers différents processus. Premièrement, la réduction de la taille des patchs d'habitat restants induit de façon évidente une augmentation de la compétition au sein des patchs. Elle peut aussi avoir pour conséquence d'inciter les individus à s'engager dans un processus de supplémentation

d'habitat (*i.e.* se déplacer entre patchs d'habitat similaire pour acquérir une quantité de ressources suffisantes, Dunning *et al.* 1992) ce qui implique un coût supplémentaire lié aux mouvements entre patchs. Deuxièmement, la fragmentation provoque un éloignement des patchs d'habitat restants augmentant ainsi leur isolement. La contrainte de la distance se décline alors à deux échelles emboîtées : une échelle large à laquelle sont régulés les échanges entre populations locales assurant la possibilité de recolonisation et les flux géniques (Hanski et Giattoti 2004), et une échelle plus locale à laquelle peut intervenir un processus de complémentation d'habitat (*i.e.* se déplacer entre patchs d'habitats différents pour compléter un cycle de vie complexe, Dunning *et al.* 1992) qui, s'il n'est pas requis par toutes les espèces, est crucial pour la persistance des populations lorsqu'il contraint fortement le cycle de vie. La complémentation d'habitats lorsqu'elle n'est pas intégrée dans l'analyse de l'impact du paysage peut d'ailleurs obscurcir le rôle global joué par la fragmentation (Pope *et al.* 2000). Enfin, en réduisant la taille des patchs, la fragmentation induit une augmentation du linéaire de bordure, ce qui est susceptible d'affecter parfois très fortement les populations (hormis chez les espèces d'écotones) (Fletcher 2005). De façon concomitante, la dégradation de la qualité de la matrice autour des patchs va intensifier les différences entre patch et matrice et augmenter donc globalement "le contraste de bordure" pouvant limiter le taux d'émigration des individus (Collinge et Palmer 2002).

1.2 La perception de la fragmentation diffère selon les espèces.

L'impact de la fragmentation sur les organismes se révèle en effet être très dépendant de leurs traits biologiques (Henle *et al.* 2004, Ewers et Didham 2006) notamment de leurs capacités sensorielles. Les principaux traits biologiques régissant la sensibilité à la fragmentation sont la taille corporelle, la mobilité, le degré de spécialisation et le niveau trophique avec une vulnérabilité accrue pour les organismes de grande taille, spécialistes, de niveau trophique élevé et possédant des capacités médianes de mouvement (Ewers et Didham 2006). Les

synergies entre ces caractéristiques accroissent d'autant plus l'exposition aux conséquences de la fragmentation. Néanmoins, les différentes composantes de la fragmentation vont avoir plus ou moins d'impact selon les modalités des traits biologiques : par exemple, une espèce très dispersive sera plus affectée par une réduction de taille de fragment que par l'isolement (Ewers et Didham 2006). Les relations entre traits biologiques et sensibilité à la fragmentation du paysage sont cependant complexes (voir Ewers et Didham 2006 pour une synthèse des prédictions théoriques et Prugh *et al.* 2008 pour une méta-analyse), et des relations contradictoires ont été identifiées (Fahrig 2007). Ainsi, les seuls traits biologiques liés aux espèces ne suffisent pas à expliquer pleinement leur sensibilité à la fragmentation. A l'échelle inter-populationnelle, des différences sont aussi très susceptibles d'émerger selon les différents contextes de paysage auxquels les populations vont être confrontées (Fahrig 2007; Swift et Hannon 2010 ; voir paragraphe 2).

L'importance relative de la composition du paysage (perte d'habitat) vs. sa configuration (fragmentation *per se*) encore en débat aujourd'hui (Fahrig et Nuttle 2005 ; Swift et Hannon 2010) dépend également des espèces considérées. Bien que l'idée selon laquelle un effet de la fragmentation *per se* n'existe qu'à partir d'un certain seuil de perte d'habitat (Andr en 1994 ; Fahrig 1998 ; Flather et Bevers 2002, voir paragraphe 1.1), les r sultats empiriques ne permettent pas d' tablir de consensus global quant aux impacts propres de la composition et de la configuration (Fahrig 2003 ; Cerezo et al. 2010). Lorsque les types de mouvement en jeu sont la suppl mentation d'habitat et la dispersion, une interaction entre perte d'habitat et fragmentation est attendue   partir d'un certain seuil de perte d'habitat (Figures 4 a et c). En dessous de ce seuil, l'agencement des fragments restants ne rev t pas beaucoup d'importance mais il devient tr s important de consid rer ce facteur lorsque ce seuil est franchi. Il est d'ailleurs vraisemblable que le seuil de perte d'habitat   partir duquel l'agencement commence   impacter la persistance des populations est plus faible pour les types de mouvement de

routine (processus de supplémentation) du fait de la forte fréquence de ces mouvements, que pour les mouvements de dispersion, évènements plus rares (comparaison des seuils entre les figures 4 a et c). L'impact de la fragmentation peut même devenir crucial dans le cas d'espèces ayant un cycle de vie complexe nécessitant des migrations régulières entre deux types d'habitat, les populations pouvant être fortement impactées par l'isolation quelque soit le degré de perte d'habitat (Figure 4 b). Le manque de cohérence quant à l'existence d'un seuil de perte d'habitat à partir duquel la fragmentation affecte la persistance des populations émerge vraisemblablement, outre du type de mouvement en jeu, de différences de caractéristiques de mouvement entre espèces. Il est alors compréhensible que la généralisation des impacts respectifs de la perte d'habitat et de la fragmentation ainsi que de leur interaction soit difficile. Néanmoins, il est possible de formuler des hypothèses permettant de tester l'influence des caractéristiques spécifiques de mouvement sur l'impact de la perte d'habitat et de la fragmentation sur la persistance des populations. Par exemple, plus les espèces ont une forte propension à se déplacer et plus elles en ont les capacités, plus le seuil de perte d'habitat à partir duquel la fragmentation intervient est élevé et plus les mouvements vont compenser facilement la perte d'habitat (Figure 4 d).

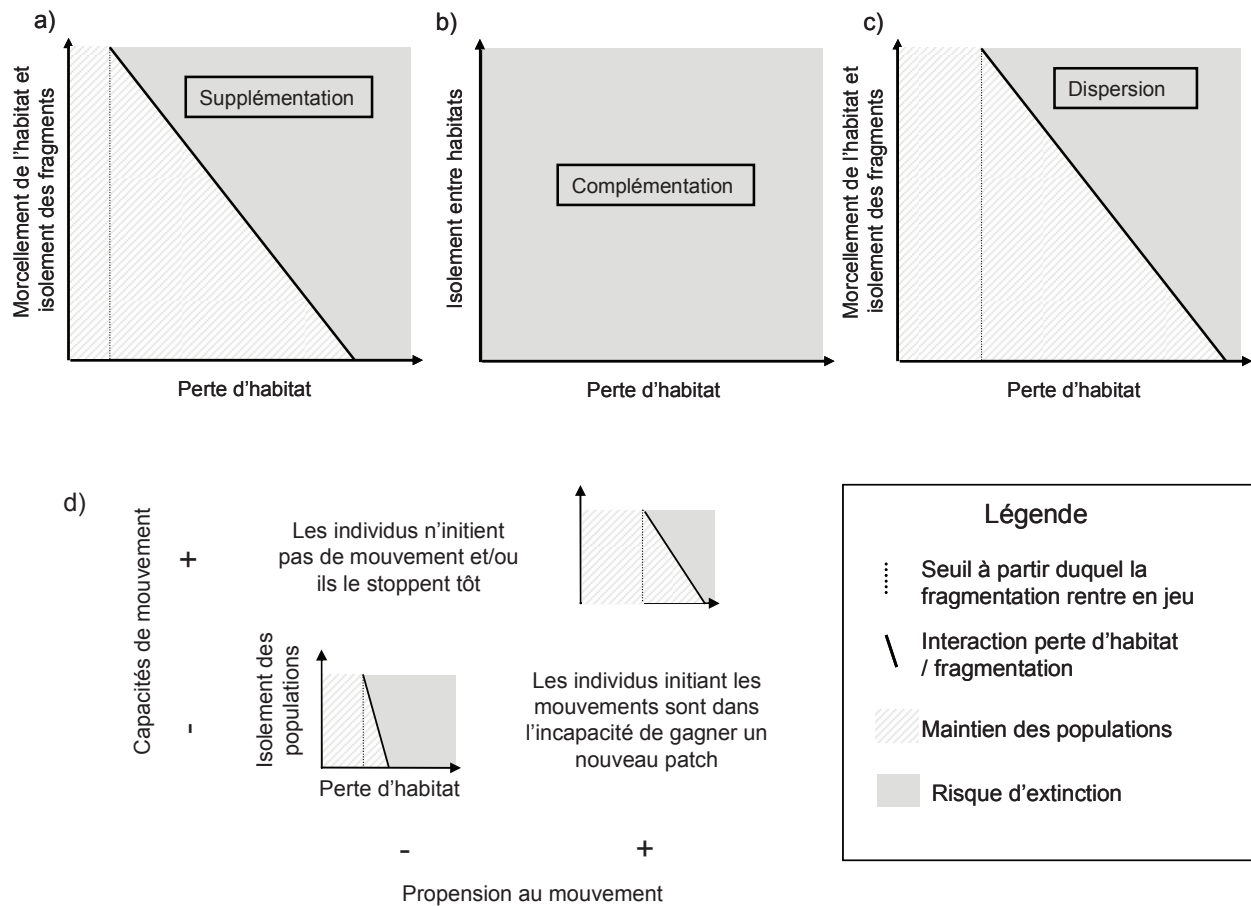


Figure 4 : Influence des différents aspects de la fragmentation en fonction des différents types de mouvement en jeu (a) supplémentation d'habitat (b) complémentation d'habitat (c) dispersion (d) influence des paramètres de mouvements.

(a) Dans le cas de la supplémentation (i.e. les individus se déplacent de patches en patches pour acquérir suffisamment de ressources), la perte d'habitat va interagir avec son morcellement et son isolement à partir d'un certain seuil : au-delà d'un certain pourcentage de perte, plus la perte d'habitat est élevée, plus les populations vont devenir sensibles à sa fragmentation. (b) Dans le cas d'espèces à cycle de vie complexe, quelque soit le niveau de perte d'habitat, si la connectivité est rompue entre les deux habitats (par exemple par un élément linéaire qui occupe peu de surface mais isole comme une autoroute), la persistance de la population est tout de suite en jeu. Il n'existe donc plus d'interaction entre perte d'habitat et fragmentation. (c) Dans le cas de la dispersion, on retrouve le même schéma que pour les mouvements de

type supplémentation : au-delà d'un certain pourcentage de perte, il existe une interaction entre perte et fragmentation de l'habitat faisant que plus la perte d'habitat est élevée, plus les populations vont devenir sensibles à sa fragmentation. Néanmoins, il est possible que le seuil de perte d'habitat à partir duquel la fragmentation commence à impacter la persistance des populations soit plus faible pour les types de mouvement de routine (processus de supplémentation) du fait de la forte fréquence de ces mouvements, que pour les mouvements de dispersion, événements plus rares (a) et (c). (d) Selon les caractéristiques de mouvements des espèces considérées, à la fois le seuil de perte d'habitat et l'interaction perte d'habitat / fragmentation peuvent fluctuer : plus les espèces ont une forte propension à se déplacer et plus elles en ont les capacités, plus le seuil de perte d'habitat à partir duquel la fragmentation intervient est élevé et plus les mouvements vont compenser facilement la perte d'habitat.

1.3 La matrice : une mosaïque de types d'occupation du sol

Enfin, la dichotomie simple entre patch d'habitat et matrice de non-habitat a aujourd'hui montré ses limites, de nombreuses études établissant l'impact de l'hétérogénéité et la complexité de la matrice sur la persistance des populations à travers la régulation des mouvements entre patchs (Joly *et al.* 2001 ; Dunford et Freemark 2004 ; Revilla *et al.* 2004 ; Schooley et Wiens 2004 ; Anderson *et al.* 2007). Négliger d'intégrer cette complexité dans les modèles prédisant les effets de la fragmentation peut même conduire à des conclusions erronées (Bender et Fahrig 2005). Il en découle également une perception de la matrice propre à chaque espèce en fonction de son degré de spécialisation et de ses affinités pour chaque type d'occupation du sol. Le concept de connectivité entre patchs a donc évolué de la structure (lien physique entre habitats) vers la fonctionnalité (lien fonctionnel estimé sur la base du comportement des organismes face aux différents types d'occupation du sol composant la matrice) (Tischendorf et Fahrig 2000). L'utilisation de la distance de coût notamment a connu une expansion très importante permettant d'intégrer la réponse comportementale des

organismes au paysage en utilisant des modèles spatialement explicites (Ray *et al.* 2002, Adriaensen *et al.* 2003). Le principe du modèle repose sur la construction d'une carte de friction en assignant une valeur de résistance au mouvement à chaque cellule composant la carte selon le type d'occupation du sol. Deux types de mesure peuvent alors être utilisées : le chemin de moindre coût (least-cost path) entre deux points (Adriaensen *et al.* 2003; Verbeylen *et al.* 2003) ou la surface de coûts cumulés depuis une source jusqu'à atteindre un coût cumulé seuil (Ray *et al.* 2002 ; Joly *et al.* 2003; Compton *et al.* 2007). Ainsi, toute fausse estimation de résistance est susceptible de conduire à des conclusions erronées. La fiabilité de telles mesures est donc clairement dépendante de l'estimation fiable des valeurs de résistance (Adriaensen *et al.* 2003). De très nombreux travaux alimentent aujourd'hui cette notion de connectivité fonctionnelle et cherchent à évaluer les valeurs de résistance. Deux types d'approches ont été progressivement développées pour estimer ces valeurs : la première basée sur l'étude du comportement des individus soit *in situ* via des techniques de Capture Marquage Recapture et/ou de radiopistage, soit en situation expérimentale via l'analyse des caractéristiques de déplacements ou du choix de types d'occupation du sol ; la seconde s'appuyant sur des corrélations entre patrons de populations (distribution ou patrons de diversité génétique) et différentes valeurs de résistance (Tableau 1). Les récents travaux entrepris plaident d'ailleurs en faveur de la combinaison de diverses approches pour évaluer efficacement la situation de la population face au paysage (Safner *et al.* sous presse).

Tableau 1 : Résumé des différents types d'approches développées pour estimer les valeurs de résistance des différents types d'occupation de la matrice et illustrations par des exemples que fournit la bibliographie.

Comportement		Patterns de populations		Espèce	Etude
Suivis in situ	Expérimental	Distribution	Génétique		
				Papillons	Ricketts 2001 Ovaskainen <i>et al.</i> 2008
				Hérissons	Driezen <i>et al.</i> 2007
				Caribou	O'Brien <i>et al.</i> 2006
				Amphibiens	Snafer <i>et al.</i> sous presse.
	Vitesse, linéarité des déplacements			Amphibiens	Stevens <i>et al.</i> 2004
	Choix d'éléments du paysage			Amphibiens	Stevens <i>et al.</i> 2006
			Fst entre populations	Amphibiens	Greenwald <i>et al.</i> 2009
			Hétérozygotie entre individus	Ours	Cushman <i>et al.</i> 2006
			Occurrence	Amphibiens	Janin <i>et al.</i> 2009
			Abondance	Micromammifères	Kuroe <i>et al.</i> 2010

2. Des réponses à la fragmentation qui diffèrent entre populations

2.1 Des réponses adaptatives : micro-évolution et plasticité phénotypique

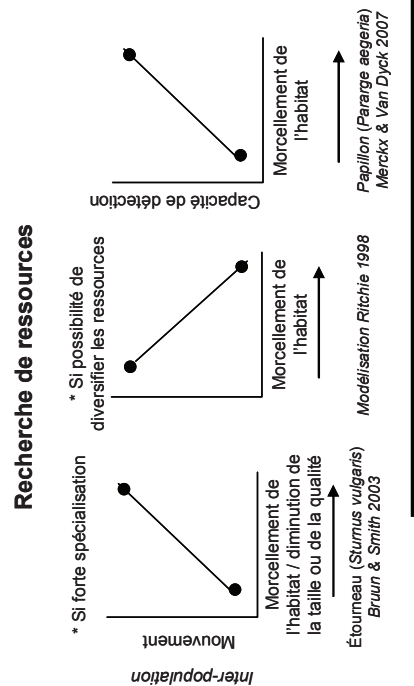
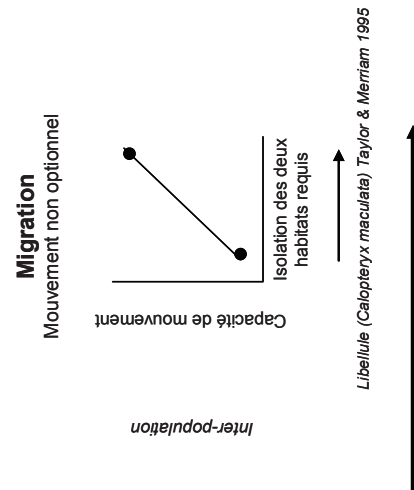
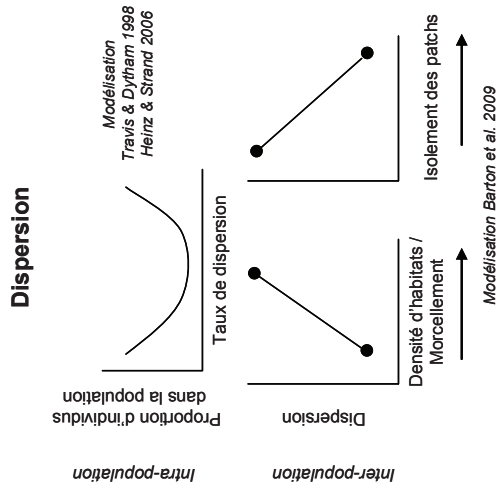
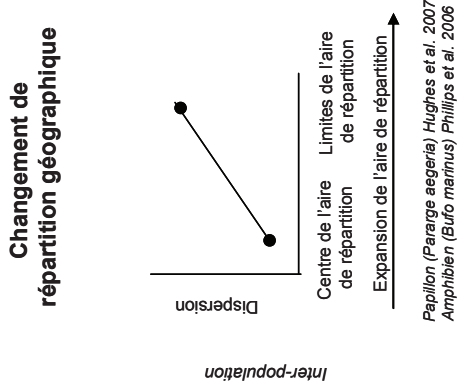
A cause de la force de la pression de sélection s'exerçant sur le mouvement, on attend que les caractéristiques de déplacement évoluent en réponse à différentes structures de paysage (Travis et Dytham 1999; Heino et Hanski 2001). Les investigations à la fois théoriques et empiriques suggèrent que l'hétérogénéité de l'habitat est un facteur dirigeant l'évolution du polymorphisme des mouvements (Roff et Fairbairn 1991). En paysages fragmentés, dans lesquels les ressources sont fractionnées, la mobilité peut être accrue ou bien limitée selon une balance coûts-bénéfices dont les contraintes sont l'échelle spatiale à laquelle la fragmentation intervient, l'étalement des ressources, les capacités d'exploration et le taux de dispersion des espèces considérées (Van Dyck et Mathysen 1999; Merckx *et al.* 2003). A l'échelle des populations, on peut ainsi soit prédire une augmentation de la mobilité en réponse à la fragmentation des ressources ou alternativement une limitation des déplacements imputable à

une réticence à franchir les bordures des patchs d'habitat (Merckx *et al.* 2003). Ces hypothèses contradictoires trouvent d'ailleurs toutes les deux des échos dans plusieurs études empiriques : Taylor et Merriam (1995) et Thomas *et al.* (1998) observent une augmentation de l'investissement dans les traits morphologiques liés au mouvement en réponse à la perte d'habitat et à l'éloignement entre patchs tandis que Dempster (1991), Schtickzelle *et al.* (2006) et Ahlroth *et al.* (2010) relatent des changements morphologiques ou des caractéristiques comportementales traduisant une contre sélection de la mobilité en réponse aux changements de paysage. Ainsi, à la fois des changements morphologiques (plutôt liés à la modification de la *capacité* à se déplacer) et des modifications du comportement (traduisant plutôt une modification de la *propension* à se déplacer) peuvent apparaître en réponse à la dégradation du paysage que ce soit au travers d'adaptations locales incluant un déterminisme génétique (Merckx *et al.* 2003) ou de façon alternative à travers l'expression d'une plasticité phénotypique (Merckx et Van Dyck 2006). Il demeure donc difficile de prévoir le sens de la pression exercée par l'altération du paysage (diminution ou augmentation de la mobilité) et de comprendre à travers quels mécanismes évolutifs les changements liés aux mouvements ont lieu (sélection en faveur d'une plasticité phénotypique ou en faveur de la fixation de traits héréditaires). En effet, la sélection peut s'exercer soit directement sur le trait ou alternativement sur le degré de plasticité du trait, un trait morphologique pouvant alors répondre de manière plastique à l'augmentation de l'exercice résultant d'une pression agissant sur un trait comportemental (Llewelyn *et al.* 2010). Bien que l'hypothèse de la fixation de traits héréditaires ne soit pas fortement supportée par la rapidité avec laquelle interviennent les changements du paysage (Fahrig 2007), il existe d'autres mécanismes de transmission trans-générationnelle d'information moins rigides tels que l'épigénétisme et les effets parentaux regroupés sous le terme d'héritabilité inclusive (Danchin et Wagner 2010). Les avantages en terme de fitness

peuvent également découler d'interactions entre caractéristiques du génome et conditions environnementales (Niitepolt *et al.* 2009).

Néanmoins, les échelles spatiales et temporelles qui président aux différents types de mouvements vont déterminer le sens des pressions de sélection (Figure 5). Pour les types de mouvements qui contraignent très fortement la fitness individuelle tels que l'acquisition des ressources et le cycle de vie, la pression de sélection ne peut tendre que vers une augmentation des capacités de mouvement et/ou des capacités d'orientation sauf si des adaptations alternatives moins coûteuses sont envisageables comme l'élargissement du spectre des ressources alimentaires ou la diminution de la fréquence des migrations saisonnières (Figure 5). Pour les mouvements se déroulant à l'échelle inter-populationnelle, différentes contraintes peuvent induire tant une contre-sélection du mouvement qu'une augmentation de la propension et ou des capacités à disperser (liée à la distance de dispersion effective) (Bonte *et al.* 2010), selon (i) le degré de spécialisation écologique (les spécialistes ayant tendance à répondre à la fragmentation par une diminution du taux de dispersion du fait des risques de mortalité très importants dans la matrice), (ii) le mode d'action de la fragmentation (le découpage en petits patches proches induisant une augmentation des mouvements tandis que l'isolement entraîne une chute du taux de dispersion), (iii) les capacités de la population à subir l'isolement, *i.e.* les potentialités génétique et démographique de la population, combinées aux caractéristiques de mouvement des individus (une population dans laquelle la plasticité phénotypique liée à des traits morphologiques de mouvement est déjà développée sera plus à même de répondre à l'isolement en augmentant l'investissement dans les organes locomoteurs par exemple) (Figure 5). En marge des aires de répartition, une favorisation de la dispersion (propension et/ou capacités dispersives) est vraisemblablement mise en place sous la contrainte des forces sélectives (Figure 5). Par ailleurs, lorsque la fragmentation réduit et isole très significativement la population, l'évitement de la dérive génétique et de la

compétition entre apparentés sont des forces sélectives qui, à l'inverse, peuvent tendre à favoriser la dispersion (Guillaume et Perrin 2006).



Échelle temporelle 1 génération

Échelle spatiale

Figure 5 : Prédications de l'évolution des capacités de mouvement en fonction des échelles spatiales et temporelles des processus en jeu et illustrations par des exemples que fournit la bibliographie.

A faibles échelles temporelles et spatiales, les mouvements ont principalement pour but la recherche de ressources. La contrainte liée à ce type de mouvement est donc forte. Ainsi, on peut prédire que la fragmentation de l'habitat va plutôt induire une augmentation du mouvement (les ressources doivent de toute façon être acquises) hormis chez les espèces généralistes capables de diversifier les ressources utilisées. On attend également que les capacités de détection des patches de ressources soient améliorées en réponse à la fragmentation. A échelle temporelle faible mais échelle spatiale large, les mouvements vont correspondre à des flux migratoires saisonniers. Là encore la contrainte portant sur ces mouvements est très forte puisque ce sont eux qui permettent aux individus de compléter leur cycle de vie. On attend donc encore une fois une augmentation du mouvement en réponse à la fragmentation en particulier à l'isolement des habitats. Lorsque l'échelle temporelle des mouvements s'accroît, le type de mouvement en jeu correspond aux évènements de dispersion. La modélisation prédit alors que le taux de dispersion va augmenter dans les populations subissant les effets du morcellement de l'habitat et diminuer dans les populations subissant un isolement des patches. Au sein des populations, la modélisation prédit aussi une distribution bimodale des taux de dispersion lorsque les effets de bordure sont forts. Enfin, à larges échelles temporelle et spatiale, dans un contexte de changement d'aires de répartition géographique, on attend que soit sélectionnée une dispersion plus forte en limites de l'aire qu'en son centre.

Néanmoins, prédire la direction de la réponse évolutive à l'échelle du fonctionnement en métapopulation demeure un challenge. Dans les paysages où la dispersion est très risquée, les

nombreux échecs de tentatives de mouvement résultent dans la vacance d'un grand nombre de patches ce qui augmente paradoxalement la fitness des rares animaux ayant réussi leur dispersion, et favorise donc la sélection de l'aptitude à la dispersion (Kokko et López-Sepulcre, 2006). De plus, la fragmentation semble générer à l'intérieur des populations une distribution bimodale des taux de dispersion des individus (Travis et Dytham 1999). Il est donc également primordial d'étudier les variations inter-individuelles des caractéristiques de la dispersion. Les pressions de sélection aboutissant à la variation inter-individuelle de la dispersion ne favorisent pas de façon automatique l'émergence de règles de dispersion permettant aux populations d'optimiser l'utilisation du paysage disponible (Kokko et Lopez-Sepulcre 2006; Gibbs *et al.* 2010).

2.2 La fragmentation, un piège évolutif ?

Les modifications rapides du paysage font aussi émerger le risque d'apparition de trappes écologiques. Le phénomène de trappe écologique se met en place lorsque les organismes délaissent des habitats de bonne qualité au profit d'alternatives par lesquelles ils sont attirés mais offrant de mauvaises conditions (Gilroy et Sutherland 2007), *i.e.* il existe un décalage entre les indices sur lesquels s'appuient les organismes pour évaluer les habitats et leur qualité réelle (Kokko et Sutherland 2001). A la différence d'un puits, une trappe écologique ne constitue donc pas un choix par défaut mais un "leurre" pour les individus. Ce phénomène a reçu une attention grandissante en biologie de la conservation ces dernières années, notamment car les altérations du paysage induites par l'homme se déroulent à un rythme rapide provoquant des changements brutaux qui peuvent créer une divergence entre les indices utilisés par les organismes pour effectuer leur choix et la réelle qualité des habitats sélectionnés durant au moins le laps de temps nécessaire à l'adaptation au nouvel environnement. De plus, même lorsqu'une petite part seulement du paysage est affectée, les conséquences sur la survie de la population restent très fortes (Schlaepfer *et al.* 2002). Le

phénomène de trappe écologique est donc symptomatique des mécanismes de perte d'habitat et de fragmentation du paysage qui ont tendance à dissocier les informations perçues par les organismes de la qualité des habitats et donc à amener les individus à préférer des habitats suboptimaux. Par exemple, Weldon et Haddad (2005) ont montré que les bordures de patches pouvaient jouer le rôle de trappe écologique chez une espèce d'oiseaux qui sélectionne préférentiellement des patches avec un fort ratio bordure/surface mais y subit un fort taux de prédation de ses oeufs. Ce phénomène a par ailleurs plus d'impact sur les petites populations, retrouvées très fréquemment en milieu fragmenté, dans lesquelles la compétition est faible laissant libre choix aux organismes de s'installer dans leur habitat préféré qui est aussi celui de moins bonne qualité (Schlaepfer *et al.* 2002). Une trappe écologique peut avoir une existence très transitoire et peu dommageable pour la population si les règles de sélection d'habitat sont flexibles ou avoir au contraire un impact très fort si les règles de choix sont très rigides. Selon l'histoire de vie des espèces, l'efficacité de la sélection à éviter le phénomène de trappe écologique va varier. Les espèces avec un faible temps de génération et pour lesquels la diversité génétique est forte réaliseront de meilleures performances si leurs préférences sont fixées génétiquement et inversement, les espèces à temps de génération long et à faible variabilité génétique réussiront mieux si leurs règles de choix d'habitat sont plastiques (Kokko et Sutherland 2001) (Figure 6).

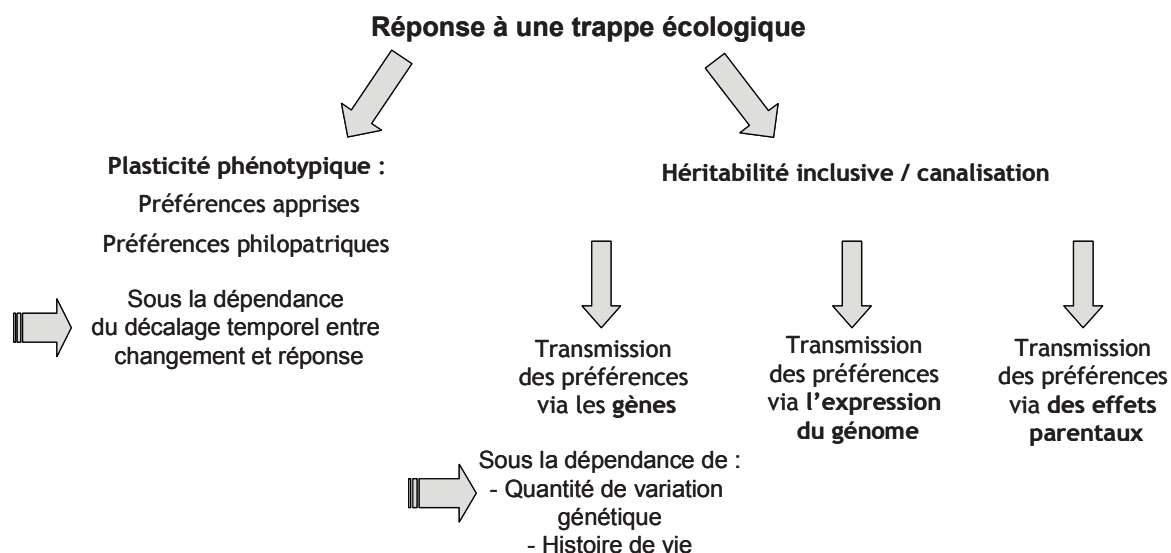


Figure 6: Réponses adaptatives possibles à l'émergence d'un piège écologique.

Selon que la préférence des organismes soit l'expression de la plasticité phénotypique ou bien de l'héritabilité, la réponse des organismes à l'apparition d'une trappe écologique va être sous la dépendance de différents mécanismes. Si la préférence est l'expression de la plasticité phénotypique, la possibilité de faire face à une trappe écologique sera dépendante du décalage temporel entre le changement créant la trappe et l'expression du phénotype permettant d'y échapper. Si la préférence est l'expression de l'héritabilité de traits fixés génétiquement, la possibilité de réponse sera soumise à la fois au pool de diversité génétique dans la population et à l'histoire de vie des organismes (les espèces avec un faible temps de génération et pour lesquels la diversité génétique est forte réaliseront de meilleures performances si leurs préférences leur permettent de faire face). La préférence peut aussi être modulée par des effets parentaux permettant de modifier les critères de sélection d'habitat dès la génération suivante si les parents pâtissent directement du caractère délétère de l'habitat choisi (par exemple au travers d'une mauvaise condition physique) et non pas au travers de leur succès reproducteur. Enfin la transmission d'une préférence via l'héritabilité de l'expression du génome (épigénétisme) peut aussi permettre aux populations de répondre plus rapidement aux changements du paysage que via la seule transmission de gènes.

De façon plus large, les changements abrupts et cadencés de l'environnement sont susceptibles de pousser à l'extrême les modifications comportementales, physiologiques ou morphologiques. Toute pression intense notamment en faveur d'une augmentation de la dispersion peut induire, au-delà du piège écologique que constitue un découplage entre la qualité d'habitat et les niveaux de préférence des organismes, des modifications dues à l'exagération de traits particuliers servant au mouvement qui peuvent engendrer des coûts importants et rendre ces modifications non-adaptatives sur le long terme (Brown *et al.* 2007).

3. La fragmentation : une question étroitement liée aux traits d'histoire de vie

3.1 Quelle écologie du paysage pour des animaux à cycle de vie complexe ?

Les populations d'organismes à cycle de vie complexe dans lesquelles adultes et juvéniles occupent différents habitats (complémentation d'habitat), comme de très nombreuses espèces d'amphibiens mais aussi d'autres groupes taxonomiques tels que les libellules (Taylor et Merriam 1995), sont potentiellement régulées au cours des multiples stades d'histoire de vie se succédant (Harper et Semlistch 2007). Ainsi, ces populations sont d'autant plus exposées au risque d'extinction causé par la perte d'habitat et la fragmentation (Semlistch 2003). Notamment, la connectivité entre les multiples habitats nécessaires au cycle de vie est dans ce cas-là complètement obligatoire (Becker *et al.* 2007). Les organismes sont donc contraints de se déplacer entre ces types d'habitats et de traverser une matrice parfois hostile. La dégradation du paysage constitue dans ce cas une pression de sélection n'agissant que dans un sens, celui de la favorisation des capacités de mouvement et de la propension à engager le déplacement. Néanmoins, pour que les populations se maintiennent, il est vraisemblable que la pression de sélection que constitue le paysage dégradé va aussi agir sur l'efficacité des déplacements afin que ceux-ci soient le moins coûteux possible. Ainsi, à la fois les capacités locomotrices, la résistance au stress et les capacités d'orientation dans le paysage peuvent être

optimisées. Pour comprendre l'impact de la fragmentation sur les espèces à cycle de vie complexe, il est aussi indispensable de tenir compte des différents niveaux de régulation des populations (Figure 7). A l'échelle intra-habitat, chacun des deux habitats complémentaires peuvent être limitants. La fragmentation au sein de chaque habitat est donc à prendre en compte car elle peut conditionner la nécessité de déplacements entre différents fragments d'habitats au sein d'une même période du cycle afin d'acquérir une quantité de ressources suffisantes (initiation du processus de supplémentation). A l'échelle inter-habitats, la connectivité entre habitats complémentaires dépendant de l'altération du paysage est bien évidemment à considérer. Enfin, à l'échelle inter-populations, doit être intégré l'impact de la fragmentation sur les échanges d'individus pour comprendre les phénomènes de persistance des populations à moyen/long terme. Ne pas prendre en considération un de ces trois niveaux d'impact de la fragmentation peut aboutir non seulement à une vision tronquée du rôle joué par le paysage mais aussi masquer l'influence d'un des autres niveaux (voir par exemple Pope *et al.* 2000).

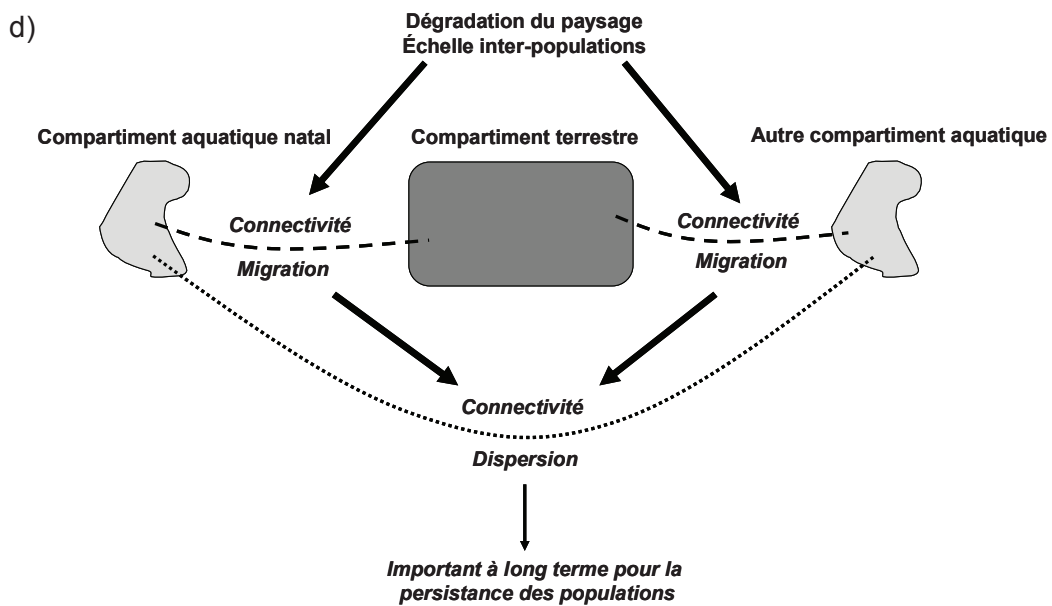
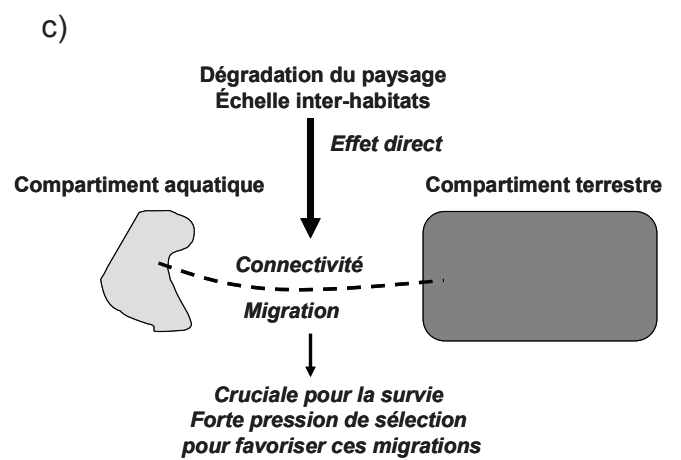
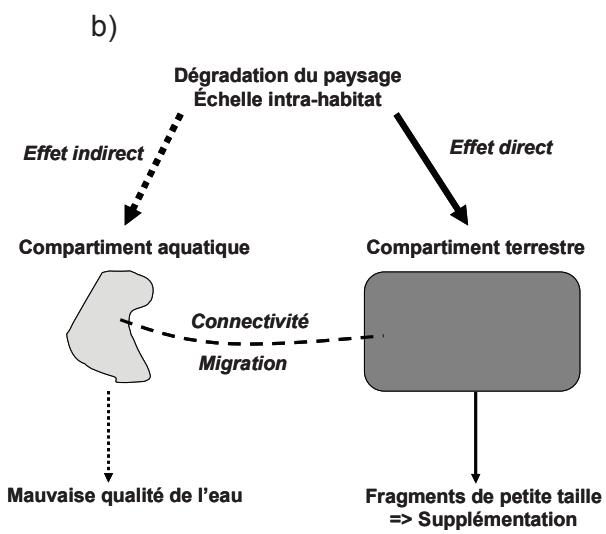
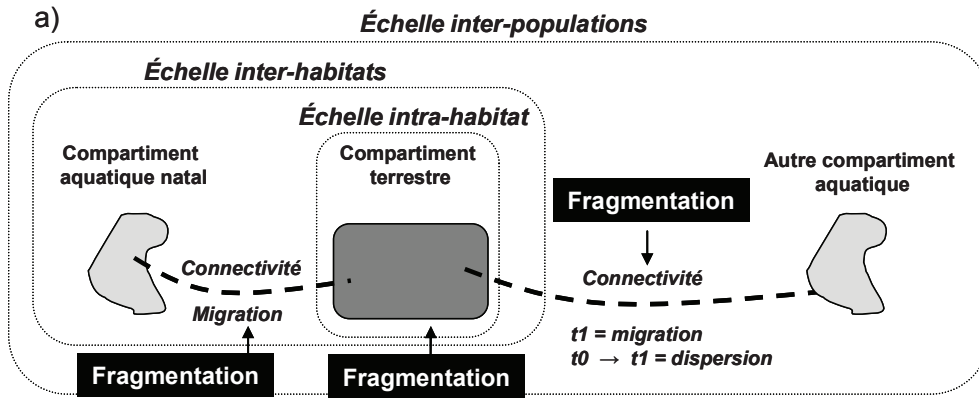


Figure 7: Déclinaisons des influences de la fragmentation aux différentes échelles de mouvement des espèces à cycle de vie complexe (exemple d'un cycle de vie habitat aquatique durant la phase larvaire / habitat terrestre durant la phase adulte. a) Les différentes échelles de mouvement à considérer : l'échelle intra-habitat à laquelle interviennent les mouvements de types supplémentation (mouvements de fragments d'habitat en fragment d'habitat pour acquérir suffisamment de ressources) ; l'échelle inter-habitats à laquelle se déroule les mouvements de type complémentation (migrations saisonnières entre deux habitats complémentaires nécessaire au cycle de vie) ; l'échelle inter-populations à laquelle interviennent les évènements de dispersion. b) Influence de la fragmentation à l'échelle intra-habitat : de façon indirecte, la qualité de l'habitat aquatique peut être altérée par la fragmentation si elle implique la présence de sols cultivés à proximité, le point pouvant se charger en divers pesticides ; de façon directe, la fragmentation peut morceler l'habitat terrestre induisant le recourt à des mouvements de supplémentation. Selon, le degré de fragmentation de l'habitat terrestre, ce dernier peut devenir un très limitant pour la population si les ressources trop fragmentées deviennent trop coûteuses à acquérir. c) Influence de la fragmentation à l'échelle inter-habitats : la fragmentation et la dégradation du paysage vont altérer la connectivité entre les deux habitats, or ces mouvements sont cruciaux pour la persistance des populations et doivent pouvoir être maintenus. d) Influence de la fragmentation à l'échelle inter-populations : la fragmentation et la dégradation du paysage vont altérer la connectivité entre les populations rendant plus coûteux les évènements de dispersion, pourtant importants pour la persistance à long terme des populations.

3.2 Les amphibiens comme modèle pour appréhender les effets de la fragmentation

Les amphibiens sont de très bons candidats pour étudier l'interaction entre mouvement et paysage. Premièrement, les amphibiens sont utilisés depuis longtemps comme modèle dans des études de choix d'habitat, d'orientation et de capacités locomotrices. Leur fort évitement

des zones d'agriculture intensive (Joly et al. 2001; Rothermel et Semlistch 2001; , notamment, permet d'évaluer l'impact de ce changement d'occupation du sol. Deuxièmement, à cause de leur faible capacité de mouvement, de leur déplacement au sol et de la perméabilité de leur peau, on attend un fort impact de la nature de l'occupation des sols sur le coût lié des mouvements. Enfin, la connectivité du paysage intervient pour eux à deux échelles imbriquées. Au cours de la réalisation de leur cycle de vie, les amphibiens ont à effectuer des migrations saisonnières obligatoires entre habitat aquatique et habitat terrestre. La connectivité entre ces deux habitats intervient comme un élément crucial dans le maintien des populations. De plus, ils présentent typiquement des distributions apparentées aux *patchy populations* (Marsh et Trenham 2001). La taille des populations locales étant souvent petite (quelques dizaines de couples), la persistance dépend du fonctionnement de réseaux de populations pour lesquels la dispersion constitue donc un facteur de persistance crucial pour ces organismes (Semlistch 2003). Cette forte pression du paysage implique donc que les traits liés au mouvement subissent une sélection relativement intense.

4. Déclinaisons des impacts de la fragmentation sur les amphibiens : altération des populations, coûts des mouvements et modifications du comportement en paysage fragmenté.

4.1 Le crapaud commun comme espèce focale

Parmi les amphibiens, le crapaud commun *Bufo bufo* se révèle un excellent modèle pour appréhender les différents impacts du paysage. Espèce plutôt ubiquiste et largement répandue en Europe, il se reproduit dans une grande diversité de points d'eau (Loman et Lardner 2006). Comme de nombreux amphibiens, il est principalement terrestre et requiert la présence de point d'eau uniquement pour sa reproduction. Néanmoins, à la fois sa présence et son abondance sont mieux prédites par l'environnement terrestre que par la qualité du point d'eau

(Scribner *et al.* 2001). En effet, la tolérance de ces têtards vis-à-vis de la qualité du point d'eau repose 1) sur la flexibilité du régime alimentaire (Diaz-Paniagua 1989), 2) sur une relative tolérance vis-à-vis des fertilisants (Xu et Oldham 1997) et des pesticides (Mandrillon et Saglio 2007) et 3) sur leur caractère impalatable vis-à-vis des poissons natifs (Glandt 1984) et allochtones (Reshetnikov 2003). Ainsi, la présence de l'espèce semble beaucoup plus contrôlée par le paysage autour des points d'eau que par la qualité du point d'eau lui-même. De plus, de multiples études renseignent à la fois les distances de mouvement (Scribner *et al.* 2001, Smith et Green 2005), le degré de préférence de l'habitat terrestre qui va à la forêt (Denton et Beebee 1994) ainsi que leurs facultés d'orientation (Sinsch 2006). Enfin, cette espèce est relativement facile à maintenir et peut être élevée en captivité quand nécessaire, ce qui permet de mener à bien des études expérimentales sur leur comportement de déplacement et ce sans préjudice du point de vue de la conservation étant donné leur relative abondance.

4.2 Objectifs du travail de recherche

Ce travail focalise sur les interactions entre le mouvement et le paysage. La source principale de fragmentation sur laquelle nous avons travaillé et qui est présentée dans ce manuscrit est l'intensification agricole qui tout autant que l'urbanisation et les infrastructures routières contribue à la dégradation du paysage et au morcellement du territoire (Joly *et al.* 2001, Quinn 2004, Tschardtke *et al.* 2005). Si le mécanisme d'action de ce type de "fragmenteur" peut différer de celui des linéaires de transports (notamment de par leur géométrie) et des extensions d'urbanisation (plus ici par les caractéristiques écologiques liées à chaque type d'occupation du sol), il en reste néanmoins proche et nous renseigne sur la façon dont agit la plus grande cause de changement d'occupation du sol à l'heure actuelle. Deux grandes questions ont particulièrement attirées notre attention : (1) Quelle est l'influence directe du paysage sur les populations ? A travers a) l'étude de leur distribution b) l'étude de leur "état de santé" et c) l'analyse des coûts liés au mouvement dans différents types d'occupation du sol et

(2) la fragmentation du paysage constitue-t-elle une pression de sélection modifiant les caractéristiques de mouvements de l'espèce étudiée ?

Dans la première partie, nous présenterons les résultats d'une étude corrélative liant la perméabilité de la matrice du paysage à l'échelle des migrations saisonnières à l'occurrence du crapaud commun. Dans les parties suivantes (parties 2 et 3), nous nous attacherons à comprendre les mécanismes impliqués dans l'action du paysage sur la persistance des populations en focalisant sur des indicateurs de "santé" mesurés à l'échelle individuelle. Enfin, les parties 4 et 5 développent deux illustrations de modifications, potentiellement adaptatives, du comportement inné d'émergence des juvéniles en réponse à différents processus de fragmentation.

L'influence de la fragmentation du paysage sur les populations se révèle être, en raison des différents mécanismes précédemment développés, un processus complexe se déclinant à de multiples niveaux. Son étude intégrative constitue un challenge majeur pour mettre en place des politiques de conservation pertinentes dans les années à venir. Cette thèse a pour objectif de contribuer à une compréhension plus globale des effets de ces changements du paysage en abordant à la fois les conséquences en terme démographique et de fitness et les réponses adaptatives possibles des populations. Comprendre l'impact de la fragmentation du paysage sur la persistance des populations est une thématique qui commence à être étayée par de nombreuses études qui donnent une vision de plus en plus précise des mécanismes impliqués. Néanmoins, la prise en compte de l'hétérogénéité de la matrice dans ces travaux est longtemps restée lettre morte et seules quelques études récentes ont permis de précieuses avancées dans ce domaine. Notamment, l'enjeu primordial a longtemps été d'être en mesure de calibrer la résistance des différents types d'occupation du sol composant la matrice (voir paragraphe 1.3). Aujourd'hui différentes méthodologies ont démontré leur intérêt pour répondre à cette question (voir Tableau 1), notamment des études à l'échelle du paysage comme celles

constituant la première partie de cette thèse. En effet, par une approche corrélative entre des aires de migrations prédites sur la base de valeurs de résistance de la matrice calibrées, cette première partie permet d'étudier l'hétérogénéité de la matrice et son impact sur l'occurrence des populations de crapauds communs.

Les études s'attellant au décryptage des mécanismes sous-jacents aux extinctions locales restent cependant très restreintes limitant notre compréhension du rôle précis joué par la dégradation du paysage (mais voir Cardozo et Chiavariglio 2008; Hinam et St Clair 2008). Dans l'objectif d'alimenter cette question, nous nous sommes attachés dans les seconde et troisième parties à analyser les potentielles pertes de fitness associées 1) à la perte d'habitat terrestre et au degré de fragmentation de ce dernier à travers l'analyse de paramètres physiologiques individuels (condition corporelle et stress) dans des populations sauvages puis 2) à la traversée de différents types d'occupation du sol pendant les phases migratoires (au cours d'expérience en conditions semi-naturelles). Ces deux approches conjuguées permettent d'interroger deux niveaux d'action de la fragmentation : premièrement, celui des mouvements quotidiens pour l'acquisition des ressources qui peut aboutir à la nécessité de supplémenter un patch d'habitat par d'autres et deuxièmement, celui des déplacements migratoires.

Pour mieux comprendre la variation des réponses des populations à la fragmentation, nous nous sommes focalisés dans les parties 4 et 5 sur les possibles adaptations comportementales de populations de crapaud commun confrontées à deux types de fragmentation différents. Dans la partie 4, nous explorons la possibilité de mise en place de préférence d'orientation basée sur la détection du champ magnétique chez des populations de crapaud commun se reproduisant dans des sites présentant depuis plus de trente générations une configuration dichotomique de l'habitat terrestre qui n'est présent que sur un des côtés du point d'eau contraignant fortement les possibilités de survie à l'émergence. Enfin, dans la partie 5, nous cherchons à savoir si le degré de morcellement de l'habitat terrestre autour des sites de

reproduction peut induire chez les juvéniles issus des populations utilisant ces points d'eau des modifications comportementales innées leur permettant de faire face à la nécessité d'une plus grande mobilité notamment lors de la phase cruciale d'émergence.

Partie 1 : Estimer la connectivité fonctionnelle en paysage fragmenté

Avant-propos

La dégradation du paysage constituant aujourd'hui une des causes majeures d'érosion de la biodiversité, les scientifiques et les gestionnaires de territoire ont besoin d'outils pertinents permettant d'évaluer, de prédire et de simuler des scénarios de gestion pour répondre à la crise actuelle. De grands progrès en modélisation fondés sur les modèles spatialement explicites (MSE) ont permis le développement de multiples outils dont certains émergent par leur simplicité d'évaluation. Néanmoins, certains obstacles liés à leur paramétrage persistent selon le niveau de connaissances sur les déplacements des espèces focales.

L'outil d'analyse coût-distance a progressivement fait ses preuves pour alimenter cette demande et constitue aujourd'hui un outil particulièrement prometteur car facilement transférable tant que les valeurs de résistance, paramètres essentiels de ces modèles, puissent être clairement estimés par des études préalables basées soit sur de calibration soit sur l'expérimentation.

Cette première partie constitue une illustration de l'application du modèle coût-distance à l'évaluation de la connectivité à l'échelle migratoire chez le crapaud commun. Ce travail a permis à la fois de calibrer les valeurs de résistance du paysage pour cette espèce et de valider ces valeurs et la méthodologie utilisée sur différents jeux de données. De plus, en testant séparément l'influence de la composition du paysage versus celle de la configuration, cette première partie pose des bases importantes pour comprendre à travers quels mécanismes la dégradation du paysage affecte les populations.

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ASSESSING LANDSCAPE CONNECTIVITY WITH CALIBRATED COST-DISTANCE MODELLING: PREDICTING COMMON TOAD DISTRIBUTION IN A CONTEXT OF SPREADING AGRICULTURE

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Summary

1. Capturing the relative influence of landscape composition and configuration in real landscapes remains a challenge. Cost-distance modelling provides an interesting approach to the assessment of landscape complexity in a functional way. However, resistances allotted to landscape elements in cost-distance modelling frequently remain defined on the basis of expert advice. To overcome this weakness, we computed resistance coefficients without *a priori* knowledge through a calibration/validation method enabling us to test the impact of the matrix heterogeneity on the occurrence of the common toad *Bufo bufo*, the cycles of which imply migrations between complementary habitats.
2. We used cost-distance modelling to elaborate an integrative parameter of landscape composition and configuration in the form of a potential movement zone. We first applied a calibration procedure that systematically tested different resistance values for each landscape element with a large dataset. The robustness of the calibrated resistances was then evaluated using two supplementary validation datasets from contrasted landscapes. Finally, in order to isolate the relative influence of landscape configuration, we compared the predictive power of the calibrated potential movement zone with that of landscape composition only.
3. The landscape matrix strongly influences common toad occurrence: selected resistances were low for forests and meadows and intermediate to high for crops. Within the two validation datasets, the potential movement zone was positively and significantly related to toad occurrence and had a better predictive power than landscape composition.
4. *Synthesis and applications.* This study provides a tool to manage landscape structure in accordance with the ecological requirements of amphibians' populations, especially habitat complementation. This method has minimal biological information requirements and therefore could be widely used to investigate the crucial influence of landscape composition and configuration on a broad range of species, and to design functional ecological networks.

Key-words: calibration, composition, configuration, fragmentation, landscape complementation, potential movement zone, resistance.

Introduction

As human activities increase, more natural areas are converted into agricultural and urbanised landscapes. Such landscape conversions lead to habitat fragmentation which is recognised to be one of the major threats to the long-term persistence of biodiversity (Fahrig 2003). In a fragmented landscape, population viability is expected to depend on functional connectivity (i.e. the permeability of the landscape matrix to movement) which influences the success of migration and dispersal (Baguette 2004). Connectivity is determined first by the resistance which the diverse land uses composing the matrix present to animal movement, and secondly by the configuration of those land uses (Wiens 2001). Despite their crucial importance for wildlife management, only a few studies have segregated the effects of landscape composition from those of configuration (Fahrig 2003). Recent advances in Geographical Information Systems allow the integration of landscape features into spatially explicit models (Verbeylen *et al.* 2003), making it possible to link spatial landscape organisation to animal movements (Driezen *et al.* 2007; Epps *et al.* 2007). More particularly, cost-distance modelling (e.g. Ray, Lehmann and Joly 2002) presents the advantages of requiring a restricted set of biological hypotheses and of being easily transferable to landscape managers.

The principle is to build a friction map by assigning a resistance to each cell of landscape according to local land use and its assumed effects on animal movements. Two types of measures are usually derived from this approach: the measure of the least-cost path from one point to another (Adriaensen *et al.* 2003; Verbeylen *et al.* 2003), or the measure of the accumulated cost surface from a source to its surroundings under a threshold value (Ray, Lehmann and Joly 2002; Joly, Morand and Cohas 2003; Compton *et al.* 2007). Whatever the approach adopted, any false assumption about resistance may result in misleading conclusions. Nonetheless, the accuracy of cost-distance modelling remains largely untested despite its wide use, as resistance is often arbitrarily established on the basis of expert opinion

(Driezen *et al.* 2007; Epps *et al.* 2007). The reliability of the resistance values thus clearly constitutes the Achilles' heel of the cost-distance approach (Adriaensen *et al.* 2003). Resistance could be estimated from behavioural experiments conducted at a fine scale, concerning for example habitat selection (Stevens *et al.* 2006; Mazerolle and Desrochers 2005) or locomotion performances (Stevens *et al.* 2004; Jonsen and Taylor 2000). However, such experiments poorly capture at the landscape scale the complex movement processes underlying local population persistence on which cost-distance modelling focuses (Adriaensen *et al.* 2003). An alternative approach would be to calibrate these resistance values with large and contrasted datasets.

Amphibians are susceptible to the impacts of land use changes because their ground-dwelling habits and the permeability of their skin result in close exchange with their immediate environment (Rothermel and Semlitsch 2002). Moreover, their life cycles involve seasonal migrations between terrestrial and aquatic habitats (i.e. landscape complementation, Pope, Fahrig and Merriam 2000), which could compel them to regularly traverse an inhospitable landscape matrix. Areas of intensive agriculture are predicted to expose amphibians to desiccation and chemical agents (Joly *et al.* 2001; Rothermel and Semlitsch 2002). The presence of amphibians in ponds depends both on the connectivity between populations and on the costs generated by seasonal migration. Amphibians present an opportunity to analyse the effects of landscape structure on population persistence, and more particularly that of landscape configuration, which is increased by their need to move between complementary habitats (Fahrig and Nuttle 2005).

In this study, cost-distance modelling is used to test the impact of modifications to landscape connectivity on the occurrence of common toads *Bufo bufo* Linnaeus. Although our focus species is a generalist, landscape variables determine, as for many amphibians, the distribution and the size of populations (Scribner *et al.* 2001). We used an integrative

parameter based on accumulated cost surface: the area within which common toads can move from the focal pond to their growth and maintenance habitats (i.e. the potential movement zone). The matrix resistance constrains the potential movement zone, which thus constitutes a reliable indicator of local landscape suitability (Ray, Lehmann and Joly 2002). Consequently, we expect a positive relationship between the potential movement zone and species occurrence. We adapted a cross-validation method to avoid the arbitrary assignation of resistance values. First we applied a calibration procedure to estimate resistances, without using *a priori* knowledge of the influence of land use on toad movements. Then, we tested the validity of the calibrated resistances by predicting toad presence using two different pond datasets, one in the region used for calibration, and the second in another region differing in landscape structure. Finally, we tested the relative effect of landscape configuration by separating the predictive power of landscape composition from that of the potential movement zone, which integrates both composition and configuration.

Materials and methods

FOCUS SPECIES AND SAMPLING METHOD

As demonstrated by Scribner *et al.* (2001), both presence and population size of the common toad were better predicted by the terrestrial environment than by pond quality. The common toad is a ubiquitous and widespread species in Europe that breeds in a great diversity of wetlands including farmland ponds (Loman and Lardner 2006). The tolerance of tadpoles to the quality of their breeding pond relies on feeding flexibility (Diaz-Paniagua 1989), relative tolerance to fertilizers (Xu and Oldham 1997) and to pesticides (Mandrillon and Saglio 2007), and unpalatability to both native (Glandt 1984) and invasive fishes (Reshetnikov 2003). Therefore, the absence of toads at a pond is more likely to result from the characteristics of the surrounding landscape rather than those of the pond. Moreover, movement and habitat preferences have been well studied in this species. Using genetic

markers, Scribner *et al.* (2001) reported genetic structuring between populations breeding in ponds at least 2 km distant from one another. This dispersal distance is also consistent with the range of migration distances reported in Sinsch (1988) (i.e. 50 - 1600 m), while recent reviews have reported non-anecdotic long distances of migration reaching 3 km or more (Smith and Green 2005). We therefore assumed an optimum maximal migration distance of 2 km, but we also examined the sensitivity of our analyses to this hypothesis using both a lower and a higher distance (respectively 1 and 3 km). Forests were identified as the usual terrestrial habitat (Denton and Beebee 1994), although we deliberately did not settle an *a priori* hypothesis about forest resistance to movements in our simulations. Additionally, the placidity and explosive breeding of common toads make them easily detectable when present at ponds. These features reduce the risk of false absence even when the local abundance is low. We monitored the presence/absence of the common toad by visiting ponds at night during the breeding period. Toads were detected and sexed visually using floodlights. Since a preliminary survey did not reveal any change in toad occurrence within 14 ponds sampled twice during the breeding period, we decided to sample each pond only once each year.

STUDY REGIONS AND SAMPLED PONDS

To explore a wide range of landscape structures, we took advantage of different degrees of agriculture intensity in the lowland landscapes of the Rhone-Alps region (south-eastern France). We selected six areas characterized by the dominance of croplands (two areas), meadows (two areas) and forests (two areas), thus reflecting different landscape compositions (Fig. S1 in Supporting Information) and configurations (Table S1 Supporting Information).

We sampled 212 ponds distributed among these six areas in 2006 (between 21 and 49 ponds per area). These ponds were selected according to the following criteria: surface exceeding 40 m², distance from any large urbanised area and mountains, absence of acute

pollution, presence of vegetation and of gently sloping banks. Furthermore, ponds were also selected to be separated from one another by at least 2 km to avoid spatial autocorrelation of toad occurrence. These ponds were divided in two sets: 129 ponds constituting the calibration dataset and 83 ponds providing the first validation dataset. The 129 ponds of the calibration dataset were monitored during two consecutive years (2004, 2005) preceding the present study. As none of the empty ponds in 2006 were previously occupied (during 2004 and/or 2005), the risk of false absence in the 2006 dataset is very limited.

A second validation dataset located outside the Rhone-Alps region was analysed to avoid pseudoreplication problems (Hulbert 1984). We obtained access to a dataset previously used by Ray, Lehmann and Joly (2002) that provides male common toad occurrence in ponds of the Canton of Geneva (246 km², Switzerland) during 1998-1999. We did not use ponds located within the city core but focused on 77 ponds (exceeding 10 m²) located in the countryside. The landscape of this region differs markedly from that of the Rhone-Alps region, and falls into one of the two following types: intensively-managed agricultural areas including small fragments of meadows and woodlands, contrasting with large forested areas (Fig. S1).

LAND USE MAPS

Five land use types were considered: standing waters (marshes and water bodies), forested areas, meadows (pastures and open areas), croplands, and urban areas (towns and suburbs, industrial areas). Four linear landscape elements were also integrated: roads, rivers, highways and large rivers.

Forests, croplands, meadows and standing waters were identified by automatic classification from satellite images (ASTER/TERRA images with 15 m resolution) using the ENVI software (ITT, Boulder, CO, USA). Linear elements and urban areas were extracted from national maps (BD Carto® from National Geographic Institute, France). Gaps (cells

without attribution: < 2% in our map) were filled with the spatially closest land use using the NIBBLE function (SPATIAL TOOLS extension in ArcView 3.2, ESRI). Breaks in linear structures (Rothley 2005) were avoided by the reinforcement of the size of the linear elements, particularly those acting as barriers. For the Geneva dataset, we used the map built by Ray, Lehmann and Joly (2002). We used ArcView 3.2 (ESRI, Redlands, CA, USA) and its extension SPATIAL ANALYST to store, manage and rasterise all the landscape elements.

ESTIMATION OF THE POTENTIAL MOVEMENT ZONE

The methodology of cost-distance modelling, originating from graph theory, is briefly described below (for detailed information see Adriaensen *et al.* 2003). The resistance of a landscape element expresses the degree by which a potential movement is impeded when compared to the most permeable element (i.e. having a resistance of 1). Given the resistance for each landscape element, a friction map is created where a cost value is assigned to each cell of the grid corresponding to the product of the cell resolution by the resistance of its landscape attribute. A negative growth algorithm was used to calculate the accumulated cost surface and the resulting potential movement zone. The maximal migration distance was set as the starting value of the algorithm. This starting value equals the distance that could be covered in the landscape element offering the lowest resistance. For each cell crossed, the potential for movement was decreased according to the cost value assigned to that cell. Therefore, the potential movement zone takes into account both the composition and the configuration of landscapes. Indeed, two circular landscape areas, each composed of fifty per cent of each of two elements (suitable and unsuitable), but with contrasted configurations (one semicircle of each element *versus* a circle of unsuitable element surrounded by a ring of suitable element) would possess different potential movement zones (an extended one for the semicircle configuration *versus* a restricted one for the pond embedded in the unsuitable element). The exhaustion of the starting value defines the edges of the potential movement

zone that hence represents the terrestrial area which can be reached by toads breeding in/emerging from a focal pond under the hypothesis of a matrix effect (see Supporting Information, Fig. S2).

STATISTICAL ANALYSIS

The uneven sex ratio prevents us from properly interpreting the interaction between sex and landscape although we expect that the sensitivity of common toads to landscape greatly varies between sexes due to sex-biased reproductive investment and nomadic behaviour (Frétey *et al.* 2004). We thus chose to analyze occurrence data separately for each sex, which approach is more robust to sex differences in landscape sensitivity, although less powerful than an analysis on the whole dataset.

Calibration

The calibration procedure is based on a “brute force” method to examine all possible combinations of resistances for several landscape elements. Since their impact is clearly defined, the resistance was kept at the lower bound (i.e. 1) for standing waters whereas highways and large rivers were set as barriers (i.e. resistance > 200). We excluded roads and small rivers from the calibration procedure because their resistance is obviously intermediate but hard to define without risk functions. Indeed, both roads and rivers are linear elements which are not very costly to cross, but the risk associated to crossing them can be very high. Therefore, since they are risky rather than costly to cross, their effects cannot be properly calibrated through cost-distance modelling (see Discussion). Moreover, the number of ponds separated by small rivers is restricted (less than 8% of ponds). We thus attributed an arbitrary resistance value of 12 to small rivers and a value of 7 to roads (Ray, Lehmann and Joly 2002). We focused the calibration on the four following landscape elements: forests (constituting both the target terrestrial habitat of common toads and a matrix element through which they

move), meadows, crops and urban areas (three matrix elements). We used a sequential procedure to determine the optimal resistance value as well as its 95% confidence interval (95% CI) within the parameter space for each focus landscape element. This process was performed in five steps of increasing resolution.

At the coarsest resolution, five resistance values (1, 25, 50, 75, and 100) were used as cut points (bounds) to parse the parameter space. This resulted in 5^4 models (i.e. combinations of resistance values for the four landscape elements) for each tested maximal migration distance (i.e. 1, 2 and 3 km). Each model was then evaluated using a logistic regression of the potential movement zone on toad occurrence (male and female). Some models among those tested are likely to be the mirror of others (i.e. inverting resistance values for two negatively correlated landscape elements inevitably leads to different models with the same correlation strength but with opposite slope signs). In this case, only models resulting in a positive slope, which transcribes the effect of an increasing potential movement zone (i.e. of a more permeable landscape) on toad occurrence, are appropriate to elaborate a spatial predictive tool. We selected the optimal resistance of each landscape element among the first five tested values from the model exhibiting the lowest residual deviance. A 95% confidence interval was also constructed around optimal resistance using the approximate Chi-square distribution of the residual deviance (5% threshold = 3.84): for each element, we excluded from the 95% CI the resistance values among those tested for which the residual deviance of the resulting model exceeded 3.84 compared to that of the optimal model, thus defining the bounds of the selected parameter space.

Then, the coarse scale was refined using a finer scale of the parameter space to investigate more precise values included in the previously selected space (optimal resistances and their 95% CI). After completion of the multiple refining sequences, a last sequence was performed to obtain the final resistance value and its 95% CI. We used the 95% CI to

segregate the effects of landscape elements according to their resistance since the absence of overlap between confidence intervals can be considered as a formal test of divergence between landscape elements. The sensitivity of the calibration method to the maximal migration distance was also examined using evidence ratios (EV) computed on all explored models.

Validation

Validation analyses were performed with potential movement zones constructed with the resistances calibrated for the optimum maximal migration distance (2 km). To assess the validity of the selected resistances, we performed logistic regressions of the “calibrated” potential movement zone on toad occurrence for each dataset. We first checked the model adequacy using the Hosmer-Lemeshow goodness-of-fit test (GOF) for continuous explanatory variables (Hosmer and Lemeshow 1989), and we estimated thereafter the predictive power of the potential movement zone using a Chi-square test on deviance (McCullagh and Nelder 1989). Finally, we also checked whether the slope estimated by the logistic regression performed on each validation dataset was included in the 95% CI of the slope estimated on the calibration dataset. This verification was performed only for the male dataset since female occurrence was not available for Geneva.

Moreover, in the Geneva dataset, because sampling was exhaustive, the mean distance (670 m) from one pond to another was shorter than in the Rhone-Alps dataset. As a consequence, we expected spatial autocorrelation in species distribution. To assess the effect of such autocorrelation, we tested the predictive power of the potential movement zone on occurrence within sets of spatially independent ponds (resampling method: Holland, Bert and Fahrig 2004). Since our results remained stable after resampling, we finally decided to work with the entire Geneva dataset to avoid reduction in analysis power.

Relative influence of composition and configuration

In order to evaluate the respective influences of landscape configuration, we compared the predictive power of landscape composition alone with that of the potential movement zone, which integrates both composition and configuration. For this purpose, we described landscape composition by the amounts of each main landscape elements (forests, meadows, crops, urban areas, barriers, roads and rivers) in a circular area of 2 km radius centred on the focal pond. The regression model was therefore structured in a conservative way, as follows:

$$\ln [p_i / (1 - p_i)] = \text{forests} + \text{meadows} + \text{crops} + \text{urban areas} + \text{barriers} + \text{roads} + \text{rivers} + \text{potential movement zone} \quad \text{eqn 1}$$

We examined effect of the landscape composition by evaluating the significance of the explanatory terms using an order-dependant test. Introduced in this way, the effects of landscape composition should be inflated relative to the one actually shared by the potential movement zone. Such a method is therefore conservative since it downplays the predictive power of configuration relative to that of composition. This analysis was performed on each validation dataset. The landscape element "rivers" not being available on the map of the Geneva region, we could not incorporate it into the analysis for the Swiss dataset. All statistical analyses were performed with R 2.5.0 (R Development Core Team, 2007).

Results

TOAD OCCURRENCE

In the Rhone-Alps, female toads were detected in 70% of the calibration ponds and in 62% of the validation ponds. Across the same datasets, the occurrence of males was of 90% and 81%, respectively. In Switzerland, male toads were present in 52% of ponds.

CALIBRATION

For both males and females, the coarse calibration step segregated resistances for forests, crops and meadows but not for urban areas (Fig. 1). The lowest resistance value was selected for both forests and meadows. Conversely, the lowest resistance was rejected for crops. For urban areas, no resistance stood out against others across the entire range of explored values (Fig. 1). The resistance of this landscape element was therefore kept fixed at the upper bound (i.e. 100) for the subsequent analyses because of its negative impact on common toad populations reported in previous studies (e.g. Hitchings and Beebee 1998). After the final calibration sequence, the optimal resistance was found at the lower bound for both forests and meadows, whereas it was found at a higher value for crops whatever the sex considered (Table 1). The 95% CI for forests and meadows did not overlap that obtained for crops when calibrated on the females' dataset. Using the males' dataset, the 95% CI for forest and crops were well segregated but the 95% CI for meadows overlapped that obtained for both forests and crops (Table 1). These results were consistent across the three tested maximal migration distances, although one can notice a slight upward shifting of the 95% CI for crops when the maximal migration distance increased.

For females, the lowest maximal migration distance (1 km) was clearly less supported than the other two distances according to their respective evidence ratio ($EV_{(2km/1km)} = 10.9$; $EV_{(2km/3km)} = 0.44$). For males, the weak relative support of the lowest maximal migration distance is less apparent ($EV_{(2km/1km)} = 3.6$; $EV_{(2km/3km)} = 0.59$).

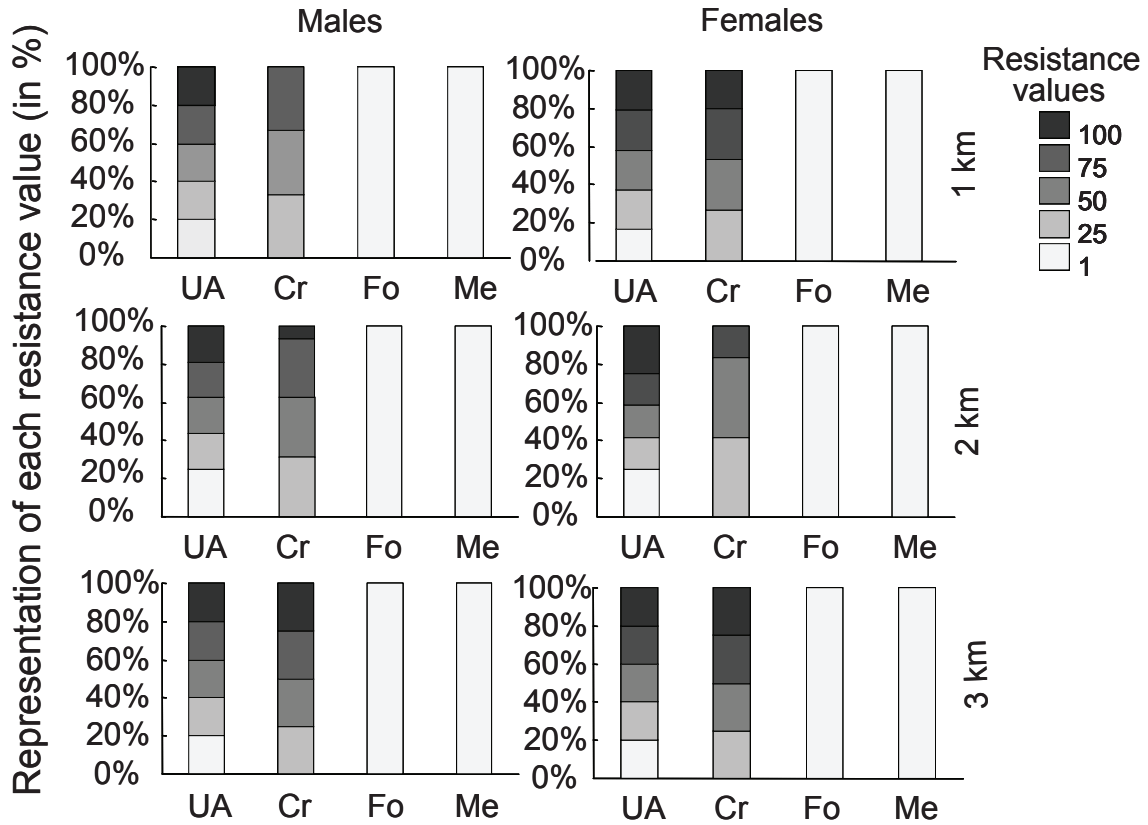


Fig. 1. Resistance values for each landscape element selected by the coarse step calibration (described by the % of representation of each value within the set of selected models). UA = Urban Areas; Fo = Forests; Cr = Crops; Me = Meadows.

Table 1. Results of the last calibration sequence: final optimal resistance and its 95 % CI for each land-use type. Fo = Forests; Cr = Crops; Me = Meadows; M = males; F = females.

		1 km			2 km			3 km		
		Fo	Me	Cr	Fo	Me	Cr	Fo	Me	Cr
F	Optimal value	1	1	7	1	1	15	1	1	28
	95 % CI	[1-2[[1-2[]2-50]	[1-2[[1-2[]3-90]	[1-2[[1-2[]4-100]
M	Optimal value	1	1	10	1	1	7	1	1	6
	95 % CI	[1-2[[1-13[]1-50]	[1-2[[1-7[]1-90]	[1-2[[1-7[]1-100]

VALIDATION

For both validation datasets, the potential movement zone was simulated using optimal resistances obtained by calibration (see Table 1 for details) for the maximal migration distance of 2 km which was supported by literature and EV. For each validation dataset, the GOF test of the logistic regression of the potential movement zone on toad occurrence was non-significant whereas the predictive power of the potential movement zone was always significant as indicated by the deviance analysis (for the Rhone-Alps females' dataset, GOF: $P = 0.64$; explained deviance = 18%; analysis of deviance: $P < 0.001$; for the Rhone-Alps males' dataset, GOF: $P = 0.70$; explained deviance = 11%; analysis of deviance: $P = 0.003$; for the Geneva males' dataset, GOF: $P = 0.24$; explained deviance = 11%; analysis of deviance: $P = 0.001$).

For each dataset, the occurrence significantly increased with the extent of the potential movement zone (Fig. 2, see Supporting Information, Fig. S2). Furthermore, logistic regressions of the potential movement zone on toad occurrence showed that the slope estimated for each validation dataset (for Rhone-Alps: 0.42, 95% CI = 0.11 - 0.73; for Geneva: 0.48, 95% CI = 0.17 - 0.79) was encompassed within the 95% CI of the calibrated slope estimate (0.55, 95% CI = 0.20 - 0.90).

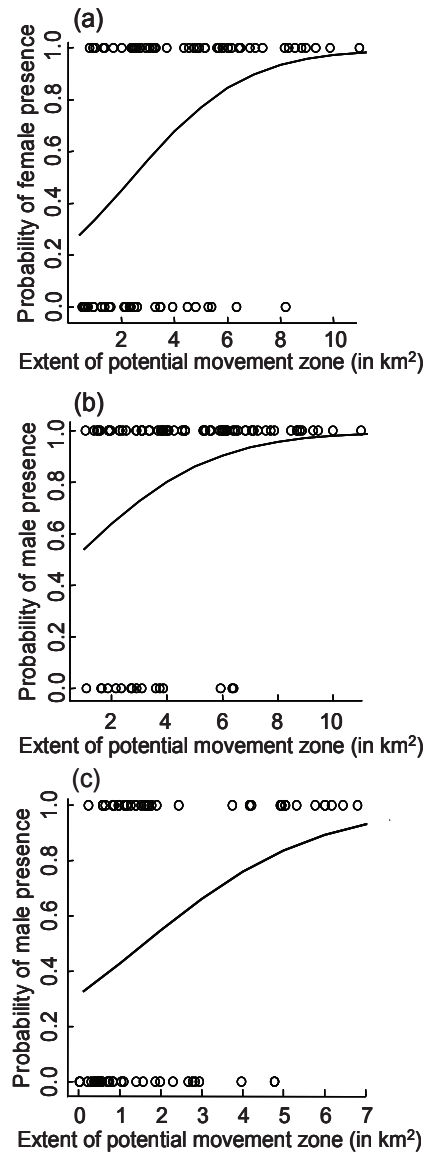


Fig. 2. Logistic regressions linking the potential movement zone (in km²) computed with the optimal resistance values for maximal migration distance = 2 km (for details on resistance values see Table 1) to: (a) female presence probability in the Rhone-Alps validation dataset; (b) male presence probability in the Rhone-Alps validation dataset; (c) male presence probability in the Geneva dataset.

RELATIVE INFLUENCE OF CONFIGURATION AND COMPOSITION

The analysis of the relative influences of composition and configuration showed that potential movement zone explained a significant part of the residual deviance after adjustment

for the effects of composition variables (Table 2). This analysis therefore showed that potential movement zone had a significantly better predictive power than all the other composition variables. Globally, amounts of forest, crops and linear elements (barriers, roads and rivers when available) only had a gentle predictive power on toad presence, whereas the amounts of meadows and urban areas had a very restrictive one (Table 2), even if these predictive powers fluctuate slightly between the two validation datasets (Table 2).

Table 2. Analysis of deviance of the sequential regression for the Rhone-Alps and Geneva validation datasets. The amount of each land use type was computed within a 2-km radius around each pond. d.f.: degree of freedom; asterisks represent the significance level for each term of the model: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Explanatory variables	Rhone-Alps (Females)			Geneva (Males)		
	d.f.	Residual deviance	P	d.f.	Residual deviance	P
Null model		109.7			106.4	
Area of forests	1	107.7	0.164	1	101.3	0.024*
Area of crops	1	104.1	0.058	1	96.2	0.023*
Area of meadows	1	102.8	0.241	1	96.1	0.786
Urban areas	1	102.7	0.743	1	96.0	0.905
Area of barriers	1	98.4	0.039*	1	94.2	0.166
Area of rivers	1	93.6	0.029*	1	-	-
Area of roads	1	90.1	0.061	1	91.6	0.107
Potential movement zone	1	73.2	0.000039***	1	80.9	0.001**
Residuals	74			69		

Discussion

Our results clearly show, first, the influence of functional connectivity on the occurrence of common toads, and secondly, the alteration of connectivity by agriculture. Confidence in our analysis derived from the concordance among the results obtained (on male and female occurrences, with different maximal migration distances and in different regions).

BIOLOGICAL RELEVANCE

Potential movement zone and matrix heterogeneity

The first objective of this study was to evaluate the influence of the landscape matrix heterogeneity on amphibians in the context of habitat complementation. Common toads have to cross the matrix when migrating between breeding pond and forested habitat. Although not *set a priori*, forest resistance was always found at the lowest bound (i.e. 1) as is expected of a target habitat. Above all, the significantly lower resistance of meadows compared to crops clearly reveals the role of the matrix heterogeneity. High resistance values for crops is corroborated by their avoidance by the common toad (Sinsch 1989), and the negative association between this species and arable land (Piha, Luoto and Merilä 2007). Croplands, which offer detrimental conditions for amphibians such as exposure to predation, the presence of pesticides, an arid microclimate, and ground ruggedness, have a negative impact on connectivity. The low resistance for meadows is consistent with the beneficial conditions (relative ground smoothness and moisture, absence of pesticides) characterising this landscape element. This result is also supported by correlative studies. For example, Scribner *et al.* (2001) established a positive relationship between common toad occurrence and the area of meadow in the landscape. However, the calibration surprisingly confused the resistance of forests, characterized by a dual function (habitat and matrix), and of meadows which are a simple matrix element. This lack of discrimination power is likely to originate from our

calibration method (see *Improvement of the method* below). Overall, these results are corroborated by the resistances attributed to landscape elements on the basis of expert opinions (Ray, Lehmann and Joly 2002; Joly, Morand and Cohas 2003). Nevertheless, although urban areas are reported to negatively impact toad populations (e.g. Hitching and Beebee 1998), setting their resistance to the lower bound rather than the upper bound did not modify the outcome of the projection. Such a flawed calibration is not surprising given the weak representation of this landscape element in our datasets, and the fact that it encompasses a wide variety of land uses (industrial areas, suburbs and villages with gardens).

Stability of the results

Globally, our results were similar for both sexes, although the contrasting effects of crops and meadows are non-significant for males. This reveals a lower influence of the matrix heterogeneity on male occurrence than on that of females. This is consistent with the different breeding behaviour of males and females. Indeed, because of their high food requirements and their high reproductive investment, females are expected to be more sensitive to habitat quality (e.g. for *Bufo viridis*, Sinsch *et al.* 2007). Furthermore, the operational sex-ratio being highly biased in favour of males, they could choose to avoid competition at densely occupied sites. Notably, they have been shown to exhibit higher nomadic behaviour than females (Frétey *et al.* 2004), which implies the exploration of ponds associated with different landscape qualities. They thus have a higher probability of being observed in unsuitable landscapes resulting in a lower apparent selectivity.

With respect to migration distance, our results remained consistent across the range of maximal migration distances investigated in this study, even at the distance of 1 km that had low supporting evidence ratios. Finally, despite differences in sampling methods between the two datasets (more restricted studied area, higher autocorrelation and smaller ponds included in the Swiss dataset), the results remain congruent. Indeed, the impact of the potential

movement zone computed with calibrated resistance values was well supported when applied to the different validation datasets, which highlights the wide applicability of this method.

Deciphering the meaning of the potential movement zone

The potential movement zone is an “omnibus” method to evaluate the impact of landscape elements on local populations for several reasons. First, resistance reflects a wide range of ecological processes underlying the connectivity between target habitats (Adriaensen *et al.* 2003) such as locomotor (Stevens *et al.* 2004) and physiological constraints (Rothermel and Semlistch 2002), predation (Russel, Swihart and Feng 2003) or the risk of being run-over (Joly, Morand and Cohas 2003). Secondly, given that forest constitutes the terrestrial habitat, its resistance reflects availability (and perhaps quality) rather than permeability to movement (see *Improvement of the method* below).

Finally, cost-distance modelling approaches integrate both landscape composition and landscape configuration. The risk of confounding effects between landscape configuration and water quality in ponds was limited by the avoidance of eutrophic ponds. Besides, most ponds were presumably free of pesticides since they were devoted to fish farming. The potential movement zone can thus be used to track the effect of connectivity. Indeed, a configuration effect was detected despite large landscape compositional effects (in the Geneva dataset): the arrangement of the different landscape elements has a largely predominant effect (see Table 2). Landscape configuration is theoretically predicted to have an important influence on populations, yet few large-scale empirical studies confirm this prediction (Harrison and Bruna 1999) since the effects of habitat loss are often not separated from those of configuration (Fahrig 2003). In contrast, our study supports this theory in demonstrating the determining influence of configuration on population distribution.

This conclusion highlights the importance of connectivity between complementary habitats (Pope, Fahrig and Merriam 2000; Becker *et al.* 2007) which must be considered at a

similar decisive level as connectivity at a regional scale (patchy populations) in landscape management (Fahrig and Nutton 2005).

METHODOLOGICAL IMPLICATIONS

The consistency of our results provides support for the validity of the calibration method as a way of overcoming the caveat of subjective resistance estimation. Even if this method requires both large datasets and intensive computer use, it allows conclusions to be drawn at the landscape scale, unlike empirical estimations of resistance values.

Improvement of the method

First, occurrence data only roughly reflect the suitability of habitats, since local populations can persist for several years in degraded landscapes before going extinct (Piha, Luoto and Merilä 2007). Combining less time-lagged parameters of population dynamics, such as abundance, reproduction cues and phenotypic indicators reflecting sub-lethal effects of fragmentation, such as body condition or stress level, could result in a substantial gain in accuracy. Additionally, pond characteristics might also alter population persistence independently of the potential movement zone. However, such an effect should result in inflated background noise whatever the potential movement zone, while our results indicate that predictive power seems higher for large potential movement zones than for small ones (Fig. 2).

Although the use of an integrative measure is a considerable advantage, the decomposition of the actual contribution of each landscape element would constitute an interesting development. This is particularly true for forests which constitute both a matrix element (used only for movement) and the target habitat of our focus species. Thus, their resistance has a particular meaning since it reflects both permeability to movement and carrying capacity. It would be interesting to determine which part of the variation is due to the

sole influence of forest configuration and which is explained by the structure of each other matrix element separating the forest from the breeding habitat.

Moreover, because resistance in cost-distance modelling is expressed in rasterised cells, tested values need to be integers (cells cannot be split). Therefore, the minimal inflation of resistance value is from 1 to 2, which corresponds to a 50% reduction of the achievable distance since it is defined by the ratio between the potential of movement and the resistance value. Nonetheless, it is likely that some landscape elements, particularly meadows, have a resistance close to 1 but slightly higher than this minimal value. Consequently, to refine calibration, resistance values between 1 and 2 should be investigated.

Finally, because roads are risky rather than costly to cross, cost-distance modelling is not the best way to calibrate their effective impact. Although an *a posteriori* analysis indicated that results were robust to variation of resistance in a range from 2 to 50 for roads, such a landscape element is likely to have a real impact on amphibian populations (Eigenbrod *et al.* 2008). Models able to simulate risks of being run-over at the individual scale such as cellular automaton or more complex individual-based models could greatly improve the evaluation of the impact of roads.

CONSERVATION IMPLICATIONS

Our study demonstrates the efficiency of the potential movement zone to capture both composition and configuration effects of landscape on species distribution. The potential movement zone enables landscape management in accordance with the ecological requirements of local populations, especially habitat complementation. It constitutes an integrative tool to design ecological networks and identify blocking points for connectivity restoration. This study illustrates how a confident parameterisation can be implemented in cost-distance modelling to avoid the subjective assignation of resistances. Because it requires little biological information, this method could be widely used to track landscape effects,

whatever the focus species, to examine landscape connectivity and to investigate the crucial influence of matrix composition and configuration on biodiversity.

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Supporting Information

The following supporting information material is available online from Blackwell-Synergy.com:

Figure S1. PCA on landscape elements composition.

Figure S2. Illustration of the potential movement zones.

Table S1. Fragmentation indexes of landscape.

Figure S1. Principal Component Analysis PCA (centred, non-normed) on proportions of land use types within a disk of 2-km radius around each pond. Principal components (PC) express variance between data points (a) PC1-PC2 factorial map according to the sectors (each sector being defined by ellipses of inertia which represent approximately 70% of point dispersion centred on the barycenter) and correlation graphic for the calibration dataset: sectors L and C, and in a minor way sector I, were dominated by woodlands whereas sectors E and V were dominated by croplands and sector B was characterized by a great proportion of meadows (b) PC1-PC2 factorial map, correlation graphic and Kernel delimitations (5, 25, 45, 65 and 85%) for the Rhone-Alps validation dataset: as for the calibration dataset, there are strong opposition between forested and cultivated grounds on the first component, and a structuring by meadows on the second component. (c) PC1-PC2 factorial map, correlation graphic and Kernel delimitations (5, 25, 45, 65 and 85%) for the Geneva validation dataset revealing a different organisation of landscape: intensive agriculture was also opposed to forested areas on the first component but the second component segregated these two land use types from urbanised areas whereas meadows do not contribute to landscape contrasts; moreover, two kernels were identified for the 45% and 65% delimitations, which revealed a deficit of sites surrounded by medium proportions of forest and thus illustrating that the Geneva landscape is structured according to a strong compositional effect (i.e. sites were either surrounded by a large proportion of forests or by a very low proportion of forests but there was no intermediary patterns). As expected, the landscape patterns greatly vary among studied sites and studied regions and calibration ponds were adequately sampled throughout areas that exhibit sufficient contrasts between landscape structures. d is the grid unit; each letter (B, C, E, I, L, V) represents a landscape sector of the calibration dataset; Fo=Forests; Cr=Croplands; Me=Meadows; UA=Urban Areas.

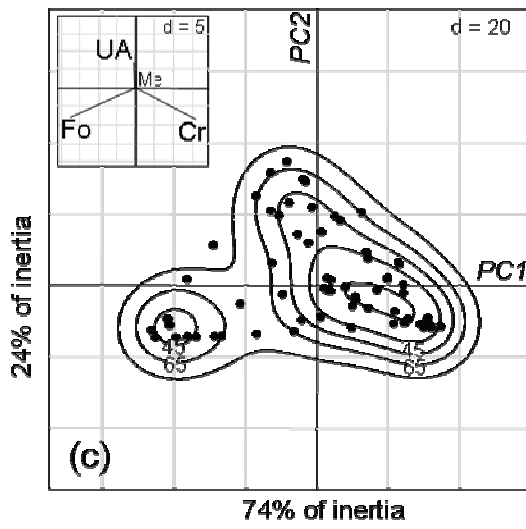
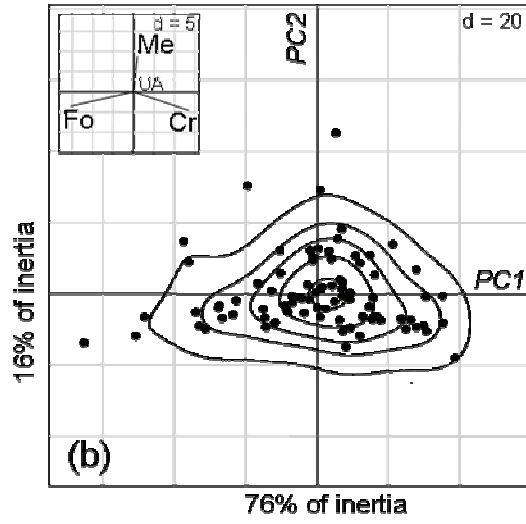
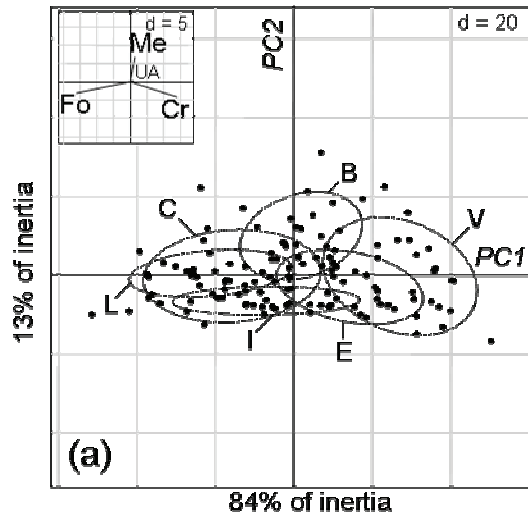


Figure S2. Potential movement zones (PMZ) applied to ponds of the two validation datasets. (a) for the Rhone-Alps dataset (each focal pond is separated from others ponds by at least 2 km); (b) for the Geneva dataset (all ponds of the Geneva area are studied).

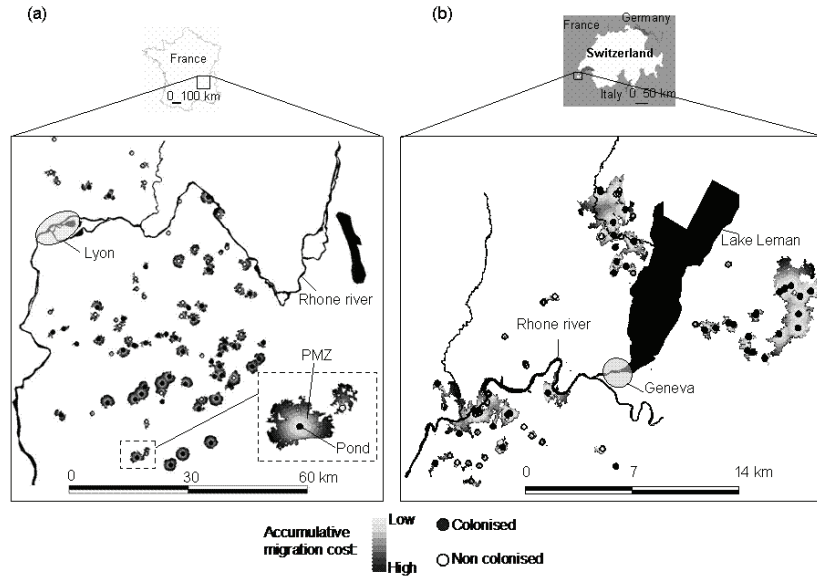


Table S1. Fragmentation index of the two principal landscape elements (forest and crops) within a disk of 2-km radius around each pond according to sectors. The fragmentation index corresponds to the mean fragment size (in m^2) for a given total surface (1 ha) for each focus landscape element. The lower is the mean fragment size, the higher is the fragmentation of the landscape element. Each letter (B, C, E, I, L, V) represents a landscape sector of the calibration dataset; Fo = Forests; Cr = Croplands.

Land use type		B	C	E	I	L	V
for 1 ha of Fo	mean fragment size (m^2)	0.65	1.21	0.94	0.85	1.06	1.45
	standard error	+/- 0.10	+/- 0.11	+/- 0.07	+/- 0.08	+/- 0.09	+/- 0.18
for 1 ha of Cr	mean fragment size (m^2)	0.47	0.77	0.75	0.67	0.61	1.01
	standard error	+/- 0.02	+/- 0.03	+/- 0.03	+/- 0.03	+/- 0.03	+/- 0.05

Partie 2 : Evaluer les coûts de la dégradation du paysage à l'aide d'indicateurs physiologiques

Avant-propos

Dans la première partie, le lien fort existant entre l'occurrence du crapaud commun et l'extension de l'aire de migration potentielle simulée sur la base des résistances du paysage a pu être mis en évidence. De même, ce premier travail a permis de révéler le rôle fondamental joué par la configuration du paysage. Néanmoins, les mécanismes sous-jacents par lesquels la dégradation du paysage affecte la distribution des populations doivent nécessairement être identifiés pour comprendre comment s'articulent les syndromes d'extinction de population avec à la perte d'habitat et à sa fragmentation. Si la première partie a mis en évidence le caractère primordial joué, dans la distribution des populations par la connectivité entre habitat aquatique et terrestre i.e., à l'échelle inter-habitats qui met en jeu des mouvements de type complémentarité, l'impact de la dégradation du paysage joue vraisemblablement un rôle fort à d'autres échelles de mouvement (Figure 7). De plus, afin d'appréhender par quels mécanismes les populations s'effondrent sous la pression de la dégradation du paysage, l'utilisation d'autres indicateurs que l'occurrence donnant accès à des changements fins et permettant donc d'intervenir avant l'extinction apparaît comme primordiale pour approfondir notre compréhension des réponses populationnelles à la dégradation du paysage et particulièrement à la fragmentation.

Dans cette seconde partie, nous interrogeons deux indicateurs d'état physiologique, la condition corporelle et la sécrétion d'hormones de stress, afin d'évaluer les impacts physiologiques liés à la dégradation du paysage. Nous travaillons à l'échelle populationnelle afin d'évaluer les différences de condition corporelle et de stress entre populations habitant des paysages subissant différents degrés de perte d'habitat et de fragmentation. Cette approche nous permet alors d'identifier les populations "à risque" avant qu'un seuil critique ne soit

atteint et constitue donc un outil très prometteur pour la gestion des populations. Enfin, ce travail permet de démontrer l'utilité de l'usage d'indicateurs plus précis et moins soumis à l'inertie que l'occurrence, pour mettre en évidence le rôle de certains aspects de la dégradation du paysage, notamment celui de la fragmentation et pour permettre de réaliser des expertises à échelle locale.

Cet article est en cours de révision dans *Biological conservation*.

**BEYOND OCCURRENCE: BODY CONDITION AND STRESS HORMONE AS
INTEGRATIVE INDICATORS OF HABITAT AVAILABILITY AND
FRAGMENTATION IN THE COMMON TOAD**

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Running title: indicators of landscape impacts

Abstract

Availability and fragmentation of habitat are today identified as key determinants of population maintenance. Disentangling their relative effects is of crucial importance to adequately manage landscapes. However, the classical use of occurrence or abundance data as proxy to assess the responses of populations could be insufficient to detect less drastic impacts than short-term population extinction. We evaluated the effects of both habitat availability and fragmentation at three spatial scales on both the occurrence and two "physiological state" indicators (body condition and level of stress hormone) reflecting the physical state of common toads (*Bufo bufo*). Our study showed that occurrence was negatively affected only by habitat availability at the largest spatial scale whereas the both physiological state indicators studied at the population scale are significantly altered by both habitat availability and fragmentation at the finest spatial scale. Results obtained with occurrence and physiological state approaches substantially diverge, which highlights the need to also investigate proximal processes to deeply understand how populations are threatened by landscape modifications. The use of physiological state indicators, particularly of body condition, powerful and easy to obtain, could be particularly relevant to detect early warnings of population decline allowing a management before extinction. Only a complete investigation of the potential impacts of landscape on the different population processes could offer a realistic picture of the requirements of populations to avoid drastic alterations.

Keywords

Amphibians; Body condition; Corticosterone; Fragmentation; Habitat availability; Physiological state.

1. Introduction

Both habitat loss and fragmentation resulting from land-use intensification are identified as main drivers of population extinction (Brook et al., 2008). Although disentangling their relative influence on populations is still a current challenge (Fahrig, 2003), both habitat loss and fragmentation are identified as processes that directly weaken population persistence through reduction of both population size and dispersal rates (Fahrig and Nuttle, 2005). Beyond these primary processes, fragmentation can also alter population functioning through other more species-specific processes. First, fragmentation inevitably results in increasing the edge/area ratio, thus implying that the area of core habitat decreases exponentially with habitat destruction. Secondly, for animals with complex life cycle fragmentation induces an increase of migration distances between patches of complementary habitats (i.e. landscape complementation, Dunning et al., 1992). Finally, by cutting up habitat in small pieces, fragmentation elicits the use of several patches to gather enough resources thus implying additional migration between patches of similar type (i.e. landscape supplementation, Dunning et al., 1992).

Most studies that addressed the effects of landscape alteration mainly used occurrence or count data as proxy to measure responses at population level (Fahrig, 2003; Cushman, 2006; Hinam and St Clair, 2008). However, this approach encompasses two important drawbacks. First, accuracy of occurrence or count data depends heavily on detection probabilities and thus involves, to be valuable, the use of complex and time-consuming sampling schemes and/or sophisticated statistical analyses. Secondly, these measures constitute time-lagged responses that may obscure the actual population status (Ewers and Didham, 2006; Piha et al., 2007). This may be particularly problematic for long-lived species with a long delay to population extinction.

Consequently, there is a great need to focus on individual processes which reflect proximal ecological mechanisms underlying population responses to landscape alterations (Ewers and Didham, 2006; Gardner et al., 2007). Individual physical state, estimated by several physiological indicators such as body condition and hormonal status, might be particularly relevant for this purpose since it provides integrative information on sub-lethal impacts of habitat degradation on individuals (Homan et al., 2003). Body condition of animals is expected to be lowered in degraded landscape due principally to nutritional deficiency (Cardozo and Chiavariglio, 2008; Hinam and St. Clair, 2008). Additionally, environmental stress due to landscape alterations (Romero, 2004) is expected to increase the level of glucocorticoids (Homan et al., 2003; Suorsa et al., 2003).

Surprisingly, although physiological state indicators offer advantages in investigating the potential impact of landscape conversion on organisms, this approach remains still poorly developed. Indeed, the studies that separated the effects of habitat loss from the ones due to fragmentation are few (but see Hinam and St. Clair, 2008) and few have documented the relevance of the impact at the population level. As outlined by Laaksonen et al., (2004), such studies cannot therefore conclusively distinguish whether the documented correlation between individual physiological state and landscape pattern results from an effect of landscape on fitness components at the population scale rather than from the variation of the competitiveness among animals to access suitable habitat within populations i.e. good-quality animals access to the best territories.

We elected to investigate the impacts of landscape fragmentation on populations of common toads (*Bufo bufo*). The choice of this focal species is appropriate given the high sensitivity of amphibians to habitat loss and fragmentation (Cushman, 2006). As the common toad is an explosive breeder, a representative part of local populations can be sampled at the breeding ponds during breeding. Moreover, both presence and population sizes of the

common toad are better predicted by landscape structure surrounding the breeding pond than by pond quality (Scribner et al., 2001). In addition, both composition and configuration of landscape elements have substantial impacts on population processes, indicating that whole landscape organisation is a key factor of population persistence (Janin et al., 2009). Given the sensitivity of amphibian populations to landscape structure, it is likely that animal physiological state will also depend on the composition and configuration of habitat at the landscape level.

We propose and investigate three scenarios to assess habitat availability and fragmentation: survey of large sample of ponds which is time consuming but not financial consuming, survey of "well-selected" sub-sample of populations where physiological state is investigated using only biometry (body condition) which is less time consuming and not financial consuming (one team of technicians required), survey of a very reduced sample of populations where physiological state is investigated using physiological descriptor (hormonal status) which is also less time consuming but financial consuming. The objectives of the present study were thus, (1) to estimate effect of habitat availability and fragmentation on toad occurrence and physiological state (2) to examine whether these approaches lead to a different interpretation of landscape effects.

2. Material and Methods

2.1. Study sites and toad occurrence

Our study was conducted in lowlands of the Rhone-Alps region (south-eastern France). Landscape greatly varies within this region as revealed by the varying proportion of croplands, meadows and forests (see Janin et al., 2009 for a detailed description of the studied area). 212 ponds were sampled in 2006 within 4000 km² study area according to the following criteria: surface exceeding 40 m², disjointed from any large urbanised area and mountains by

at least 10 km, absence of visible acute pollution, presence of aquatic vegetation and of gently sloping banks (at least $\frac{1}{2}$ of banks with slope inferior to 40%). To avoid spatial autocorrelation, we selected ponds distant from one to another by at least 2 km since genetic structuring becomes significant between breeding populations separated by this distance (Scribner et al., 2001).

We investigated landscape structures with special emphasis to the landscape structure of forested areas that constitute the usual habitat of toads apart from the reproductive period (Denton and Beebee, 1994). We thus focused on forest availability and forest fragmentation using respectively the area of forested grounds around ponds and the number of forest fragments (each pixel or group of pixels completely surrounded by another landscape elements) as landscape descriptors. Among the multiple fragmentation metrics (Calabrese and Fagan, 2004), we chose one, the number of forest fragments, that reflects the primary effect of fragmentation, i.e., the cutting up of habitat in small pieces. It also transcribes the need of supplementation for toads within their summer habitat. The influence of those descriptors was examined at three spatial scales (500 m, 1 km and 2 km from the focal pond) given the range of migration distances reported for the common toad: from 50 m to 3 km (Sinsch, 1988, Smith and Green, 2005) with a migration distance including 95% of the population averaging 700 m for frogs and toads (Semlitsch, 2008).

We surveyed the occurrence of toads in 2006 by visiting the 212 selected ponds at night during the breeding period (see Janin et al., 2009 for details on sampling) (scenario 1). Toads were detected and sexed visually using floodlights. We focused our analysis on ponds where females were detected because of the greater demand of this gender on landscape quality (Janin et al., 2009). To monitor toad physiological state in 2007, we selected a subsample of 17 ponds that spreading along the both gradients defined by forest availability and fragmentation (Table 1) and for which at least five females were detected in 2006. We

excluded from our selection ponds with a size less than 100 m² to avoid elevated stress levels emerging from strong male-male competition and female harassment at high male density.

In this sub-sample of 17 ponds, two physiological state indicators were appraised on toads: we first measured body condition of 315 males in the selected 17 populations (scenario 2), and we determined urinary corticosterone concentration of 81 males and 74 females from a reduced sample of 8 populations (scenario 3) (see Table 2 for details). The close surroundings of the ponds were visited at night in February and March 2007 during the migration peak. The toads were detected using floodlights and carefully captured by hand. Body condition was assessed through the classical measurement of both snout-urostyle length and body mass. We thus aim to explore three different methods to evaluate landscape effects and their potentiality to be used by conservationists in applied situations.

Table 1. Ranges, medians and standard deviations of forest availability (area of forest in ha) and fragmentation (number of forest fragments) for the ponds from the occurrence dataset in which at least five female toads were present in 2006 (82 among 212 ponds) and from the physiological state dataset (17 ponds) at the three investigated spatial scales, and analyses of correlation (Spearman test) between these two variables for the total ponds from the occurrence dataset (212 ponds) and from the physiological state dataset (17 ponds).

<i>Approaches</i>	<i>Forest availability</i>			<i>Forest fragmentation</i>			<i>Correlation</i>	
	<i>Median</i>	<i>Min.</i>	<i>Max.</i>	<i>Median</i>	<i>Max.</i>	<i>Min.</i>	<i>p</i>	<i>Rho</i>
Occurrence								
2 km	445 +/- 202	71	1107	489 +/- 493	1078	69	0.374	-0.061
1 km	112 +/- 74	12	398	117 +/- 136	934	8	0.476	-0.049
500 m	26 +/- 18	1	101	33 +/- 18	193	2	0.222	-0.084
Healthiness								
2 km	444 +/- 166	171	745	504 +/- 260	1078	227	0.002	-0.177
1 km	96 +/- 64	34	232	81 +/- 85	297	40	6.10^{-11}	-0.358
500 m	18 +/- 18	2	62	28 +/- 23	88	13	3.10^{-7}	-0.283

Table 2. Repartition of sampled individuals between studied variables, sexes and populations.

<i>Populations</i>	<i>Basal UCMc</i>		<i>Stress-induced UMCc</i>		<i>Body condition</i>
	<i>females</i>	<i>males</i>	<i>females</i>	<i>males</i>	<i>males</i>
B1	-	-	-	-	21
B2	-	-	-	-	19
B3	-	-	-	-	10
S1	8	5	8	2	15
S2	13	9	12	6	13
S3	3	4	3	2	4
S4	13	22	9	12	22
B4	-	-	-	-	25
S5	8	5	7	2	20
B5	-	-	-	-	21
S6	7	17	4	12	18
S7	13	12	7	7	26
B6	-	-	-	-	24
B7	-	-	-	-	20
B8	-	-	-	-	24
S8	9	7	3	5	11
B9	-	-	-	-	22

2.2. Evaluation of corticosterone concentration

We determined the corticosterone level (i.e. the main glucocorticoids released by amphibians; Homan et al., 2003) in urine samples to assess the physiological response to stress. Unlike blood concentration that delivers information on quasi-immediate stress and is potentially modified by animal handling in a few minutes (Romero, 2004), urinary dosage allows to assess both medium and long-term experience of stress (Queyras and Carosi, 2004). Furthermore, this method conforms to conservation standards in avoiding invasive sampling. Additionally, urine sampling is relatively easy since the toads respond to handling by a micturition reflex. Each animal was sampled two times to obtain both basal Urinary Corticosterone Metabolites (UCM) and stress-induced UCM. For estimating basal UCM, urine was sampled immediately after capture by placing the toad in a plastic box for a maximum of 45 min. As the time delay of metabolites excretion is of about hours for diverse species (Queyras and Carosi, 2004), individuals for which urine was not collected within 45 min after capture were discarded to avoid measuring stress due to animal handling. Toads for which we obtained a urine sample in time (81 males and 74 females) were then transported to the laboratory and placed in stressful conditions in order to evaluate their capacity to respond to a new stress event reflecting the existence of a chronic stress if the responsiveness of the hypothalamic-pituitary-adrenal axis function is suppressed (Romero, 2004). For this purpose, toads were confined in small individual boxes (15x10x10 cm) at 4°C and disturbed twice a day by lightly shaking their boxes. After 72 hours the second urine sample was collected. We thus successfully collected urine for 49 males and 52 females. All these animals were then released at their capture site. Urine samples collected *in situ* were kept on ice during transportation. All samples were stored in microcentrifuge tubes placed at -80°C until the measurement of UCM concentration was performed. We used corticosterone Enzyme-Immuno Assay (EIA) kits (N° 500651, Cayman Chemical) to measure UCM concentrations.

We followed the Cayman Chemical protocol for corticosterone EIA. We thus determined both basal and stress-induced UCM concentrations for each toad. To control for differences in urine concentration, we also evaluated the creatinine (C) concentration in urine and used it as a reference measure for UCM concentration (Queyras and Carosi, 2004). C concentration was determined with standard creatinine assay kits (N° 500701 Cayman Chemical). We thus obtained the corrected UCM (UCMc) concentration by the ratio $[UCM]/[C]$.

2.3. Statistical Analyses

In landscape studies, mensurative experiments can take advantages from different conditions in space to evaluate effects of area and fragmentation (McGarigal and Cushman, 2002). However, since habitat loss and fragmentation are often concomitant processes, the dependency between the two variables used to depict these processes should be controlled before model handling in order to avoid ambiguous conclusions (Fahrig, 2003). Nonetheless, methods to estimate relative effect for correlated landscape predictors, like residual regression, were biased by correlations among predictors (Smith et al., 2009). Consequently, we first performed a preliminary analysis to evaluate correlations between the area of forest and the number of forest fragments. A separate analysis was performed at each spatial scale on both the occurrence dataset and the subsampled ponds used to study the physiological state. Although the correlation between the two variables was significant within the subsample, it remains weak at each spatial scale and for both occurrence and physiological state datasets (Table 1) (Hinam and St. Clair, 2008). Second, we used a deviance analysis allowing to separate the variance contribution of the forest availability from the one of the number of fragments (Smith et al., 2009) (see below). The non-sequential F test (type III analysis) used to estimate the significance of the different effects included in the model best supported by AIC_w (McCullagh and Nelder, 1989) also allows to separate the contribution of each landscape variables in the inference analysis (see below).

Several studies already reported a negative influence of agriculture on toad occurrence (Piha et al., 2007; Janin et al., 2009). We thus tested the correlation of both landscape descriptors (forest availability and forest fragmentation) with the prevalence of crops in the landscape matrix (the area around ponds for which landscape elements differing from forest) (ratio crops/matrix) at the best supported spatial scale. Moreover, we verified the correlation between forest fragmentation and the percentage of crops area in the whole landscape (ratio crops/whole landscape). If forest availability is mechanically associated with the crops area in the whole landscape under focus (a circle centred on ponds of 500, 1000 or 2000 m), forest fragmentation reflecting only the allotment of forested grounds is not expected to be specifically associated with the crops/whole landscape ratio. Moreover, beyond crops, matrix is also composed for a large part of meadows and in a smaller part of residential area, roads and scattered urban area, specification association between forest availability or fragmentation and the crops/matrix ratio is unexpected unless that forest modification systematically emerges from conversion of natural areas into crops, condition that is not verified in our study area. This analysis was performed on the 17 ponds used to monitor toad physiological state and on the 142 ponds of the occurrence dataset in which females were present (detailed analyses of the landscape structure in the occurrence dataset are provided in Janin et al., 2009).

We used general linear models with binomial errors to assess the influence of forest availability and fragmentation on occurrence data. We first constructed for each spatial scale (i.e. 500 m, 1 and 2 km) a global model including both the two main effects and their first-order interactions. All reduced models were then considered, leading to a whole set of 5x3 models for which the AIC weight (AIC_w) was computed. The relative support of explanatory terms, as well as the one of each spatial scale, corresponding to different hypotheses, was estimated by a "chance" parameter (relative importance/(1-relative importance); relative

importance computed on the basis of evidence ratios; see Burnham and Anderson, 2002): the summed AIC_w of the models set including that effect (or that spatial scale) relative to the one of the models set that does not include that effect (or that spatial scale). Since deviance contributes to the AIC_w upon which evidence ratios are calculated, the evidence ratio values associated to a particular hypothesis reflect its "chance" to contribute to variance since they take into account the contribution of this hypothesis to the likelihood function allowing a statistical partitioning between the different sources of deviance. Thus, hypothesis supported by its "chance" parameter highlights the explanatory terms which significantly contribute to the deviance (or variance). We then used a non-sequential F test (type III analysis) to estimate the significance of the different effects included in the model best supported by AIC_w (McCullagh and Nelder, 1989).

Linear mixed models with normal errors and REML (Restricted Maximum Likelihood) estimations were used to assess the effects of the forest availability and fragmentation on the two physiological state indicators. We performed an analysis of covariance (ANCOVA) to examine the effect of both landscape variables on the body condition of toads. Females were excluded from this analysis since their body mass also depends on their reproductive investment. Body mass (log transformed) was treated as dependent variable and body length (log transformed) was introduced as adjustment covariate. A global model including all first-order interactions was constructed for each spatial scale. Moreover, in all models, the correlation of the dependent variable among animals belonging to the same population was taken into account by introducing the population of origin as a random effect on the mean value of the dependent variable. The relative support of each explicative term (as well as the spatial scales) was examined in the same manner than for the analysis of occurrence data. We also used a non-sequential F test (type III analysis) to assess the predictive power of the different effects included in the model best supported by AIC_w .

We additionally verified the impact of landscape on male toad size treating landscape variables and population effect as above. Similar models were also used to assess the effects of sex, population and landscape (forest availability and fragmentation) on basal UCMc concentrations and stress-induced UCMc concentrations. The UCMc concentrations were square-root transformed to improve the normality of residuals. All explicative terms were treated in the same way than in the body condition analysis. Moreover, to assess the global stress response of toads, we compared basal and stress-induced UCMc concentrations by variance analysis on repeated measures (ANOVA with mixed effects).

Finally, we examined whether the predictive power of landscape variables on toad occurrence could be improved given the mean population value of physiological state indicators. For this purpose, we used linear mixed models to test whether the standardised Pearson residuals of the regression of the landscape variables against the occurrence data could predict body condition as well as stress level. All analyses were performed with R 2.6. (R development team, Vienna, Austria).

3. Results

3.1. Evaluation of collinear landscape effects

For the 17 ponds for which physiological state indicators were investigated as for the 142 ponds in which females were present, we detected, as expected, no direct correlation between crops areas in total landscape of the 500 m circle (ratio crops/whole landscape) and the forest fragmentation ($r = 0.19$, $df = 15$, $p = 0.48$ for the 17 physiological state ponds and $r = 0.12$, $df = 140$, $p = 0.16$ for the 142 females' occurrence ponds). Moreover, results showed correlation of percentage of crops in matrix (ratio crops/matrix) neither with forest availability ($r = -0.20$, $df = 15$, $p = 0.44$ for the 17 physiological state ponds and $r = -0.11$, $df = 140$, $p = 0.19$ for the 142 females' occurrence ponds) nor with forest fragmentation ($r = -0.09$, $df = 15$,

$p = 0.73$ for the 17 physiological state ponds and $r = -0.1$, $df = 140$, $p = 0.25$ for the 142 females' occurrence ponds) as we could suppose.

3.2. Occurrence (scenario1)

Females were present in 142 of the 212 ponds surveyed for occurrence. Models including forest availability were the best supported by the chance parameters (Table 3). Among the three spatial scales, the best supported was the largest one (2 km) (Table 3). The presence of toads is thus significantly positively associated with the surface of forest within a circle of 2-km radius centred on ponds (coefficient = $2.251e-07$, deviance₂₁₀ = 8.886, $p = 0.003$). Identical results were obtained at the two other spatial scales although these latter are less supported by model selection (1 km: coefficient = $5.288e-07$, deviance₂₁₀ = 6.530, $p = 0.011$; 500 m: coefficient = $1.928e-06$, deviance₂₁₀ = 5.550, $p = 0.018$).

Table 3. Values of relative importance (on the basis on evidence ratios) of each hypothesis for both scale and landscape components according to response variables.

Type of response variable	Type of explanatory variable	Hypotheses	Chance parameter	
<i>Occurrence</i>	Scale	2 km	1.78	
		1 km	0.23	
		500 m	0.21	
	Landscape components	Habitat availability	4.17	
		Fragmentation	0.54	
		Interaction	0.22	
<i>Physiological state</i>	Body condition	Scale	2 km	0.04
			1 km	0.16
			500 m	4.60
	Landscape components	Habitat availability	2.11	
		Fragmentation	2.81	
		Interaction	0.22	
	Stress hormone	Scale	2 km	0.55
			1 km	0.18
			500 m	1.00
Landscape components		Habitat availability	2.14	
		Fragmentation	1.34	
		Interaction	0.42	

3.3. Physiological state indicators (scenarios 2 and 3)

Both forest availability and fragmentation, included as additive factors, were supported by the chance parameters for both body condition (Table 3) and corticosterone concentration (Table 3). For the two physiological state indicators the best supported spatial scale was the lowest one (500 m) (Table 3). The F tests applied to the best supported models showed first that body condition was significantly positively associated with forest availability and negatively correlated to forest fragmentation (Intercept: $F_{1,297} = 39.42$, $p < 0.0001$; Length: $F_{1,297} = 877.22$, $p < 0.0001$; Forest availability: $F_{1,14} = 4.66$, $p = 0.0486$; Fragmentation: $F_{1,14} = 5.92$, $p = 0.0290$; Fig. 1a). However, we did not detect any association between body length and forest availability or fragmentation (Intercept: $F_{1,298} = 345.91$, $p < 0.0001$; Forest availability $F_{1,14} = 0.25$, $p = 0.6278$; Fragmentation: $F_{1,14} = 2.81$, $p = 0.1160$). Additionally, and similarly to what was found for body condition, the F tests applied to the best supported models showed that UCMc concentration was negatively related to forest availability and positively to the number of forest fragments (Intercept: $F_{1,147} = 70.80$, $p < 0.0001$; Forest availability: $F_{1,5} = 10.38$, $p = 0.0234$; Fragmentation: $F_{1,5} = 6.36$, $p = 0.0530$; Fig. 1b). However, the elevated basal UCMc level associated to scarcity in forested grounds or to forest fragmentation did not reach the highest levels since the exposition to new stress events induced higher hormone concentrations in urine (Intercept: $F_{1,99} = 731.57$, $p < 0.0001$; Treatment: $F_{1,99} = 8.55$, $p = 0.0043$). Moreover, contrary to the levels of basal UCMc, the levels of stress-induced UMCc depend neither on forest availability nor on forest fragmentation but only on sex (Intercept: $F_{1,92} = 447.05$, $p < 0.0001$; Sex: $F_{1,92} = 8.75$, $p = 0.0039$). Although we did not detect differences of basal UCMc concentrations between sexes, our results also showed that the increase of corticosterone level induced by stress was stronger in males than in females (Intercept: $F_{1,99} = 731.57$, $p < 0.0001$; Treatment: $F_{1,99} = 8.55$, $p = 0.0043$; Sex: $F_{1,92} = 0.29$, $p = 0.5912$; Treatment*Sex: $F_{1,99} = 4.76$, $p = 0.0315$).

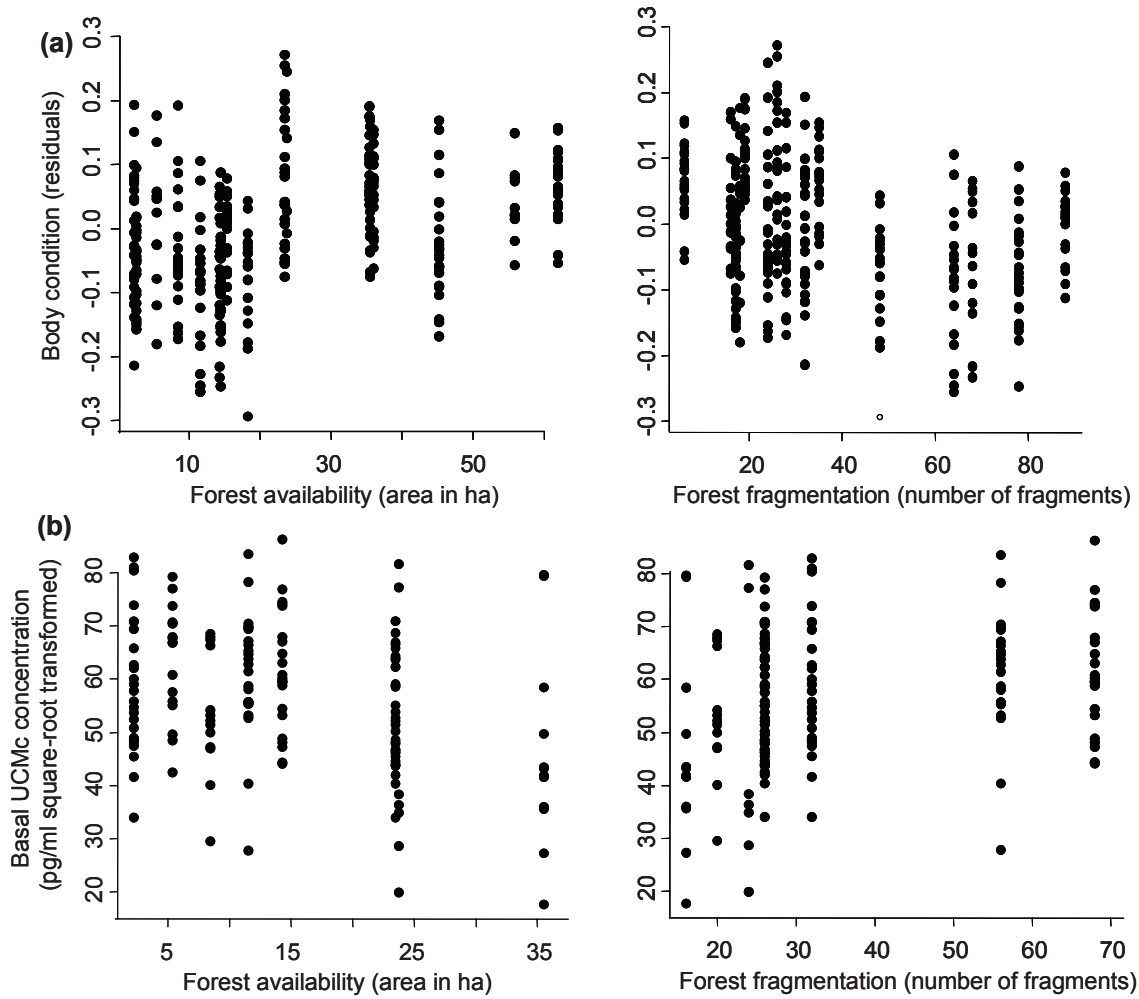


Fig. 1. Correlation between (a) body condition (represented by the residuals of the regression between the log-transformed body length and the log-transformed body mass), (b) UCMc concentration and the landscape variables (forest availability and fragmentation) in a 500-m radius circle around ponds in the different populations sampled for physiological state.

3.4. Improvement of the occurrence prediction by physiological state indicators

Finally, at the spatial scale of 500 m, the residuals of forest availability and fragmentation on occurrence tended to explain body condition (Intercept: $F_{1,297} = 27.56$, $p < 0.0001$; Length: $F_{1,297} = 873.19$, $p < 0.0001$; Residuals (Occurrence ~ forest availability + fragmentation): $F_{1,15} = 4.01$, $p = 0.0638$) but failed to predict UCMc concentrations (Intercept: $F_{1,147} = 1.19$, $p = 0.2767$; Residuals (Occurrence ~ forest availability + fragmentation): $F_{1,6} = 2.73$, $p = 0.1494$) (Fig. 2).

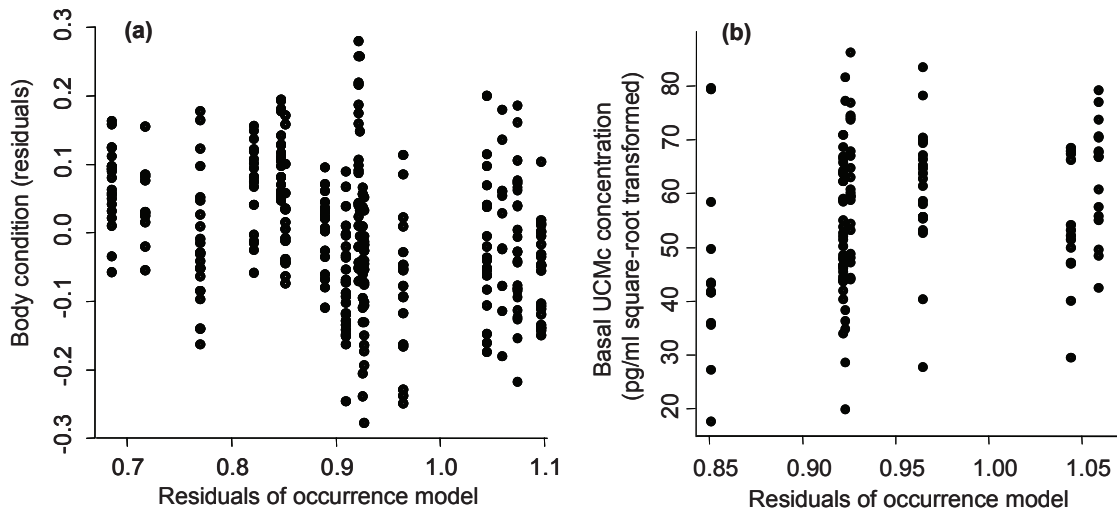


Fig. 2. Relationship between the residuals of the forest availability and fragmentation on occurrence and (a) body condition, (b) UCMc concentration.

4. Discussion

Our study shows that physiological state indicators such as body condition and corticosterone level are significantly altered by both habitat availability and fragmentation (Fig. 1). Similar results have been previously documented in other vertebrate groups (Cardozo and Chiaraviglio, 2008; Hinam and St. Clair, 2008), but to our knowledge, our study is the first that demonstrate such an influence at a population level. Our study also highlights that landscape effects on physiological state indicators substantially differ from those detected with an approach on the basis of occurrence. Indeed both the impacts of landscape components and spatial scales at which they are detected diverge between the two approaches (Table 3). Thus, the three evaluated methods using different proxies can clearly not be considerate as equivalent and the scenario 2 can be recommended to conservationists. Titeux et al., (2007) have previously highlighted that distribution modelling are improved by the addition of fitness-related parameters since occurrence does not necessarily reflect success of reproduction in birds and thus provided an important contribution to conservation management guidelines. Our results clearly support and generalize these findings by

providing additional proxies to evaluate habitat suitability, easier to obtain than reproduction parameters at least in amphibian species.

4.1. Impacts of forest availability and fragmentation on physiological state indicators

First, landscape alteration did not appear as a drastic deregulator of the responsiveness of the hypothalamic-pituitary-adrenal axis function (Romero, 2004) as some harsh and chronic stressors can be since it did not suppress the corticosterone response of individuals to new stress event. However, although the hormonal system was not totally disrupted by landscape alteration (the hypothalamic-pituitary-adrenal axis remains able to respond to a new stress event), we detected a worse toad condition, i.e. low body condition and elevated basal corticosterone level, in landscapes characterized by both low forest availability and high forest fragmentation in a 500m radius circle around the ponds (Table 3; Fig. 1). Although we did not provide evidence that reproductive success and survivorship are affected by reduced body condition and elevated corticosterone rate, our results strongly suggest that the mean fitness at population level is likely to be lowered in altered landscapes. First, worse body condition bears upon performances all the more that toads are capital breeders for which body condition is a strong determinant of the reproduction success and survival (Reading, 2007). Besides, common toads are motivated in undertaking costly migration to found high-quality habitats (Sztatecsny and Schabetsberger, 2005). Second, although the nature of the relationship between fitness and corticosterone in amphibians is complex (Moore and Jessop, 2003), the verification of the fitness-corticosterone relationship (Bonier et al., 2009) is expected provided non-linear relationships are considered (Bonier et al., 2009; Bush and Hayward, 2009).

However, one may ask whether such a relationship actually reflects the effect of forest availability and fragmentation rather than an effect of another landscape component or a confounding effect correlated to the landscape pattern. In particular, Janin et al., (2009)

highlighted the negative effect of crops within the matrix on toad occurrence. Nevertheless, it is unlikely that such a negative impact explain the detected differences of toad physiological state. Indeed, neither forest availability nor fragmentation was related to percentage of crops in the matrix (ratio crops/matrix) and fragmentation was also not related to the percentage of crops in the whole landscape (ratio crops/whole landscape). Considering confounding effects correlated to landscape structure, we could suspect variation in water quality among studied populations. Indeed, tadpoles in ponds situated in agriculture landscapes could be exposed to pesticides that exhibit a growth deficit (Boone et al., 2001) impacting definitively adult size (Altwegg and Reyer, 2003). Nevertheless, we minimized this risk of confounding factors by excluding ponds immediately surrounded by crops. Moreover, we detected no difference in body size among ponds despite this would be expected if larval development was altered by pesticides. Nonetheless, to definitively avoid confounding factors when controlled manipulative experiments can not be considered, there is a need for replicated mensurative studies (McGarigal and Cushman 2002). This work is a first step in this direction.

Two hypotheses may explain why unfavourable landscape structure around the pond induces a degradation of population physiological state: (i) a reduced connectivity between reproductive sites and terrestrial territories hinders the complementation process, and (ii) a food restriction within the terrestrial territories.

(i) As suggested by Homan et al., (2003), the distance covered and the types of habitat crossed during breeding migrations are likely to impact corticosterone level in amphibians. Although costly breeding migrations may well explain changes in the corticosterone basal level, this does not explain why body condition is also impacted. Indeed, body condition usually reflects long term effects of environmental conditions such as food intake during the preceding months (Sztatecsny and Schabetsberger, 2005) especially for capital breeders.

(ii) According to this hypothesis, the spatial scale of 500 m around ponds is likely to correspond to the residence area of most of the toads since landscape effects were clearly more striking at this scale than at larger ones (radius of 1 km and 2 km). This is also consistent with radiotracked summer migration distances of 55-1600 m (Sinsch, 1988) and classical home ranges in amphibians (Semlistch, 2008). Nutritional stress within the terrestrial residence could thus be responsible for both lowered body condition and increased stress hormone levels as previously illustrated (Kitayski et al., 1999; Suorsa et al., 2003). Besides, prey density was identified as an important determinant of home range of common toads (Indermaur et al., 2009).

Several causes could explain the association between nutritional stress and both forest availability and fragmentation. First, low body condition could result from a suboptimal density with respect to the reduced carrying capacity of forested grounds. In groups with complex life cycles that use two habitats (such as the amphibians), population size is regulated by the habitat with the lowest carrying capacity (Halpern et al., 2005) (Fig. 3).

Weak forest availability in the surroundings of a pond could induce strong competition in this habitat, which are expressed through low body condition. This process can emerge either in a static landscape in which the population is regulated by the terrestrial compartment through a stable demography, or in a dynamic landscape, which implies that a lag time exists between the dynamics of habitat loss and density adjustment (Fig. 3). Secondly, nutritional stress could also result from the enhancement of supplementation processes and/or edge effect consecutive to forest fragmentation (Fahrig, 2003; Fahrig and Nuttle, 2005). Edge effect can directly result in drastic food shortage by reducing density and quality of surface-dwelling invertebrate preys (Zanette et al., 2000) which compose the diet of common toads (Sztatecsny and Schabetsberger, 2005). Edge effect also induces indirect nutritional stress through human disturbance (Thiel et al., 2008) and enhancement of predation pressures by generalist

omnivorous predators (Storch et al., 2005; Eilam et al., 1999). Hence, toads inhabiting fragmented forest may be forced to supplement their resource acquisition by the use of multiple neighbouring patches, which may result in extended feeding trips through inhospitable matrix that drastically lower the quantity of foods gained per time.

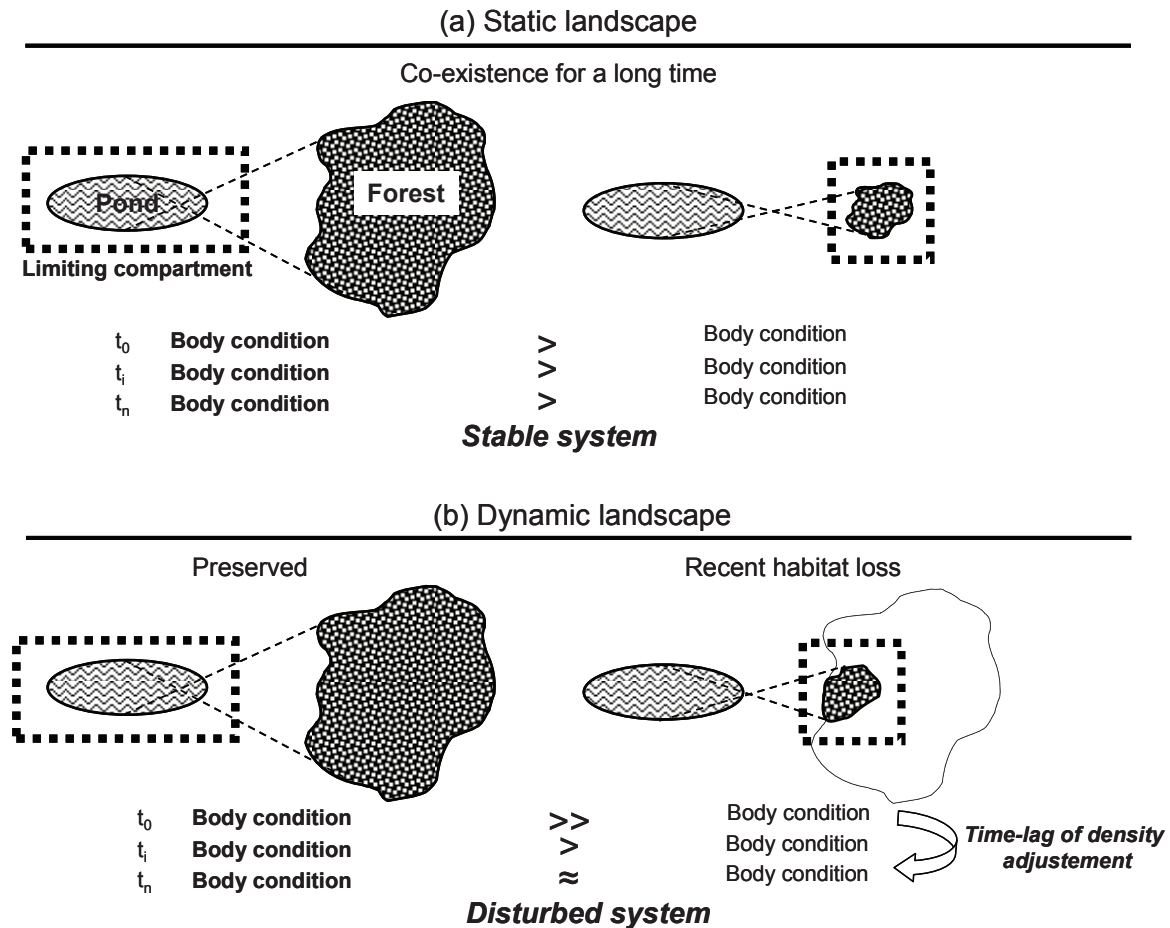


Fig. 3. Processes likely to cause positive association between body condition and forest area. In (a) static landscape, when forest area is weak (on the right part), the limiting compartment is constituted by this terrestrial habitat: each year a number of juveniles greater than the forest area can sustain is recruited, which creates a high competition for food detrimental for individual body condition. However, this system remains stable only if body condition does not greatly affect the investment in reproduction. In (b) dynamic landscape, where forest is lost (on the right part), the limiting compartment is also the remaining terrestrial habitat but

the competition is caused by the brutal reduction of its carrying capacity. The adjustment of the population size to the new carrying capacity is then a time-lagged process and the altered body condition in the disturbed system will progressively return to equilibrium.

4.2. Discrepancy between approaches on the basis of occurrence and physiological state indicators

If physiological state data suggest a negative impact of fragmentation on local populations, occurrence data fail to reveal such an effect although the sample size was higher for occurrence. Overall, if both the two approaches point out the end product of forest availability, they also differ in the spatial scale at which landscape variables are best selected. As the landscape pattern of ponds sampled to study toad physiological state was representative of the one observed at occupied ponds within the occurrence dataset, these differences are unlikely to result from contrasted landscape situations (Table 1). Moreover, as one approach tracks landscape effect on pond occupancy whereas the other investigates its effect on the relative fitness among occupied ponds, such discrepancies should be expected. Indeed, it is very likely that landscape patterns exert a different leverage on the population processes that determine respectively mean physiological state status and toad occurrence, still all these processes underlie population decline.

The negative effect of forest availability highlighted by both approaches is well in agreement with several previous studies that underline the role of landscape composition for toad population persistence (Scribner et al., 2001; Piha et al., 2007). Moreover, the non significance of fragmentation effect on toad occurrence is well consistent with the classical suggestion that the impacts of habitat loss outweigh those of fragmentation (Fahrig, 2003; Fahrig and Nuttle, 2005). Nevertheless, the magnitude of fragmentation effects, that should strongly depend on the biology of the focal species (Fahrig and Nuttle, 2005), was

considerable in regards of physiological state indicators reported at occupied ponds in our study (Table 3). Other elements of landscape structure, such as configuration of the habitats and nature and configuration of the matrix, can influence population persistence, notably for animals with complex life cycle (Joly et al., 2001; Guerry and Hunter, 2002). Above all, applying percolation models on the same occurrence dataset, Janin et al., (2009) pinpointed that population persistence depends crucially on landscape configuration regulating complementation processes. Using simplified descriptors of landscape patterns, such as forest area and numbers of forest fragments, suffers from a lack of power when applied to explain occurrence data. In this respect, it is interesting to note that Janin et al., (2009) failed to find any significant compositional effect on half of the sampled ponds used to calibrate percolation models (Janin et al., 2009). However, this is not to say that landscape configuration exert the same effects on both occurrence and physiological state of reproductive adults. In particular, hindering the complementation process of newly metamorphosed toads or reducing the connectivity between reproductive sites, which is not integrated in our measures of landscape in this article, could drastically alter toad occurrence at ponds.

Both approaches also differ in the spatial scale at which landscape variable were best selected: the larger spatial scale (radius of 2 km) is selected for occurrence data, while the smaller one (radius of 500 m) was retained for physiological state data (Table 3). One may remark that the larger scale correspond to genetic isolation distance (Scribner et al., 2001) whereas the smaller one relates the mean area of residence (Sinsch, 1988; Semlistch, 2008). This therefore suggests that toad physiological state mainly depends on within population processes while occurrence data are also heavily influenced by inter-population processes through dispersal and rescue effect taking place at larger spatial scale. It is also likely that such a discrepancy results, at least partly, from a delayed response to landscape alteration that is inherent to occurrence data. Indeed, some adults can subsist during several years in taking

advantage of low density in terrestrial habitats (Harper and Semlitsch, 2007) located at great distance from the pond (more than 1 km) compensating for high migration costs, despite the landscape alteration that should drive population to extinction. They thus contribute to the system inertia. In a complementary way, decreasing mean population physiological state may reveal a population decline largely before local extinction. Our results consolidate the hypothesis of the alteration of physiological state before population extinction since they showed that body condition tends to be negatively associated with the residuals of the regression of the landscape variables against the occurrence data (Fig. 2).

We propose, as evoked by Compton et al., (2007) and Gardner et al., (2007) a complete framework for population conservation at multiple scales integrating both ultimate causes and proximal mechanisms of population decline (Fig. 4) in order to better capture the properties of population responses to the change in landscape mosaic (Bennett et al., 2006). Probably larger scales than those under focus in this work should also be investigated, notably to integrate broader metapopulation (or patchy-population) dispersal-recolonization processes (Cushman 2006). Our study constitutes a first step to take into account the different processes that determine population persistence. Further developments are required, notably additional landscape studies, to separate the different effects of landscape configuration on population decline particularly with respect to the complementation process at the juvenile stage.

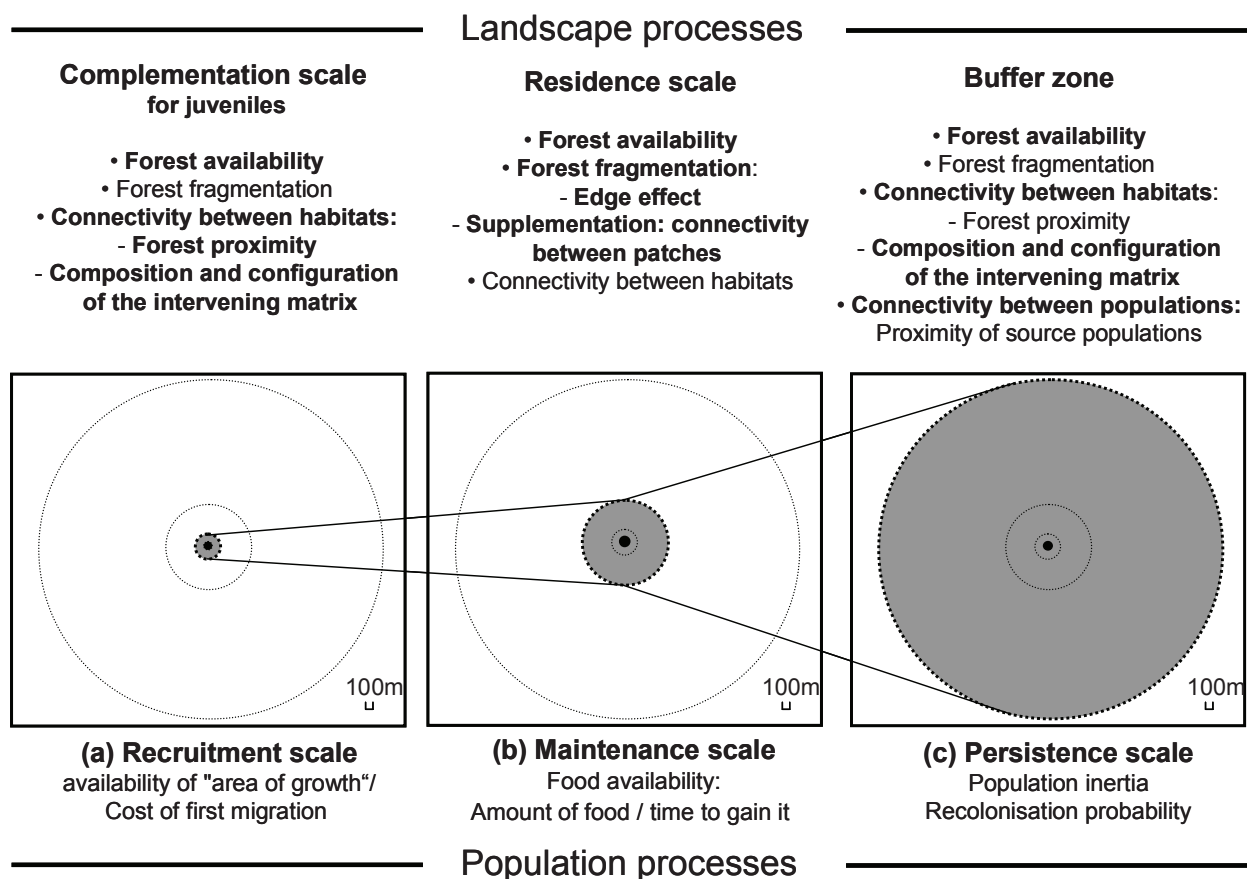


Fig. 4. Different scales of landscape management involved in amphibian conservation at a single pond. From the left to the right, the scales of (a) juvenile recruitment very close to the pond at which early growth and first migration go off (precise scale undetermined, beyond the scope of the present study), (b) maintenance area within which most of the individuals of the population live (defined by a 500-m radius disk centered on the pond), and finally (c) persistence area that includes habitat terrestrial patches that are distant from the pond (with high migration costs) where a restricted part of the population can live and that can constitute sources allowing individuals from distinct local population to contribute to rescue effects (defined by a 2 000-m radius disk centered on the pond in our study but probably larger). Although very likely, the role of the matrix was beyond the scope of the present study. Only the impacts of forest area and fragmentation were clearly integrated here.

5. Conclusions

To avoid spurious conclusions about landscape effects and to adequately manage habitats, it becomes crucial to work not only on occurrence but also with data reflecting more accurately immediate responses to landscape alterations. In this respect, physiological state indicators constitute a complementary approach, allowing an early warning of population decline and to intervene before population crash. Several arguments speak in favour of the usage of body condition (scenario 2) in management practices: this parameter can be easily obtained with a limited cost and is sufficiently powerful to track landscape effects on a limited population sample. We thus propose two nested scales of management: a narrow scale as core protection zone inhabited by a large proportion of individuals to maintain suitable residential conditions for a wide part of population, embedded in a larger buffer zone that can increase the inertia of population dynamics and offers recolonisation opportunity.

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Partie 3 : Estimer le coût de la traversée de la matrice via le stress

Avant-propos

Afin de comprendre également quel est le rôle joué par la fragmentation au cours des migrations saisonnières, cette troisième partie se focalise sur une mesure indirecte du coût lié au passage dans différents types d'habitat pour les crapauds communs adultes (femelles) et juvéniles. Les mesures directes de coût, tels que les estimations de dépenses métaboliques, demeurent très difficilement accessibles. Pour circonvenir ce problème, nous proposons une évaluation intégrative du coût par la mesure du taux d'hormones de stress libéré en fonction des types d'occupation du sol utilisé. L'avantage de cette évaluation indirecte réside dans le fait qu'elle nous renseigne plus sur le "ressenti" de l'animal dans les différentes conditions de paysage, nous donnant ainsi accès à la résultante de plusieurs mécanismes liés au paysage (déshydratation, coût énergétique, perception de la vulnérabilité ...) mais dont l'expression est immédiate.

L'évaluation d'un "coût" par une entrée plus physiologique permet de confronter des résultats expérimentaux en milieu semi-naturel aux valeurs de résistance calibrées par l'approche corrélative basée sur la modélisation. Ainsi, le recours à ces deux méthodes complémentaires offre la possibilité de 1) comparer la portée des différentes approches et de 2) proposer un travail permettant de cerner plus complètement la question de l'estimation des valeurs de résistance 3) de comprendre les mécanismes sous-jacents à ces valeurs de résistance en fonction du stade (comparaison des stades juvéniles et adultes).

Using physiological indicators of stress to assess landscape resistance in an amphibian.

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Abstract

The influence of matrix heterogeneity on movements has been largely evidenced. Multiple methods have been developed to assess the resistance of the different land uses composing the matrix: correlative methods on the basis of distribution and genetic patterns or behavioural ones on the basis of radiotracking and experimental studies. Nonetheless, the usage of physiological parameters to evaluate resistance has been poorly investigated although this method is able to provide an integrative estimation. We evaluated the level of stress hormone, corticosterone, of female toads and toadlets migrating across three types of grounds in semi-experimental arrays: arable, meadow and forest ground. Moreover, we tested the behavioural preference of females and toadlets by exposing them to different ground types. We predicted that the less stressful environment is the forest ground since it is their usual habitat. Meadow grounds are expected to be more stressful than forest ones, but to be less stressful than arable grounds. In the same way, we expected preferences for forest ground. The stress level of adult females was higher in arable ground than in forest and meadow ones but no difference of corticosterone was detected between forest and meadow grounds although no significant variation of temperature and humidity were detected. The corticosterone secretion in toadlets was not associated to the type of grounds but rather to the humidity rate in arrays. Whereas adult toads seem able to associate ground types with potential unsuitable conditions since they increased their level of corticosterone in arable ground, toadlets responded only to direct change in physical parameters (rate of humidity). Female toads selected preferentially forest and meadow grounds but avoided arable one, contrary to toadlets which avoided meadow grounds. Our results showed first that cross arable ground could be costly for both adults and juveniles and second that toadlets could be unable to respond to ground type as long as physical parameters are not affected, which makes them particularly sensitive to landscape fragmentation since they can not benefit from indirect cues to avoid some land use types.

Introduction

Current conversions toward more intense land use lead to habitat fragmentation, which is recognized to be one of the major threats to the long-term persistence of biodiversity (Fahrig 2003). In a fragmented landscape, the dependence of population viability on functional connectivity (i.e. the permeability of the landscape matrix to movement) grows in proportion of habitat loss since the success of migration and dispersal becomes a key factor of population dynamics (Joly *et al.* 2001, Baguette 2004). The most often in man-impacted landscapes, the matrix is a complex tangle of land uses that each can facilitate or impede individual movements (Taylor *et al.* 1993). Both composition and configuration of landscape matrix have been proved to clearly impact population occurrence (Janin *et al.* 2009). Therefore, considering displacement across different land uses improves our ability to capture the real complexity of landscape utilisation exhibited by many species (Wiens 2001, Drielsma *et al.* 2007).

Several methods have been used to assess the resistance to movement of different kinds of land use, and identify the ones that can be considered as impeding movements. First, indirect calibration methods at the landscape scale allow to assess landscape resistance in an efficient way either using occurrence data (Janin *et al.* 2009) or relying on analyses of genetic markers (Stevens *et al.* 2006). Additionally, numerous studies used direct observations of the responses of animals to habitat characteristics. The oldest ones used mark-recapture method to measure the relative resistance that different grounds oppose to animal movements (Ricketts 2001, Rothermel and Semlitsch 2002). This first attempt to derive resistance values from actual behaviour in real landscape was completed by the use of radio-telemetry data (Driezen *et al.* 2007). Additionally, experimental investigations of both locomotion performances (speed and linearity) (Stevens *et al.* 2004) and habitat selection (propensity to cross boundary between land uses) (Stevens *et al.* 2006) have been performed under controlled conditions.

The first correlative approaches provide powerful insights on landscape connectivity but require huge data sets to compensate for the inaccuracy of occurrence information (detectability, population inertia). The second based on behaviour raise the problem of the scale at which processes are investigated and of the accuracy of the extrapolation to real landscapes (but see Stevens *et al.* 2006). Given these difficulties, our aim was to explore another ways of connectivity expertise by assuming that resistance to movement, defined by the resultant of both willingness and cost of movement (Adrianensen *et al.* 2003, Drielsma *et al.* 2007) should be detected through variation of physiological parameters. Since direct measures of metabolism are rarely possible in the field, other variables could be measured such as water loss (Rothermel and Semlisch 2002) or level of stress hormones (Homan *et al.* 2003).

We propose here to evaluate the difficulty for an amphibian to cross different ground types through the measurement of the level of stress hormones coupled with behavioural experiments. Stress level has been previously used in several species to assess the global quality of residence area (Hinam and StClair 2008, Janin *et al.* submitted). Here we illustrate the use of stress hormones to assess the immediate response to challenging events experienced during migration (see Homan *et al.* 2003 for a first attempt of this method) since the perception of harsh environmental conditions (aridity, exposition to pollutants) and mortality risks (absence of shelters against predation) could induce the secretion of stress hormones (Bonier *et al.* 2009). For a migrating toad, we hypothesized that the less stressful environment is the forest ground since it is their usual habitat. Meadow ground is expected to be more stressful than forest one because of the absence of shelters, but to be less stressful than arable grounds because it provides better water resource and lower concentration of toxic chemicals. For identical reasons, we expected that facing to select between grounds, toads would prefer forest ground rather than meadow and meadow than arable ground. We have tested these

hypotheses in both adult female toads and toadlets by exposing migrating individuals to several environmental conditions in experimental corridors. With adult females migrating toward a pond for breeding, we have investigated stress level while they were exposed to the three ground types previously described. With toadlets migrating toward terrestrial habitats after metamorphosis, we compared the impact of four environments (shaded litter forest, open litter forest, open meadow ground, open arable ground) using a similar protocol.

Materials and methods

Experiment on adults

Physiological indicators of resistance

Study site

We conducted this experiment in the surroundings of a pond used as breeding site by a large population of common toads (*Bufo bufo*) (Ain department, 45°5083N, 5°6625E, France). This pond is embedded in a mosaic of second growth and primary forests interspersed with open grasslands and crop fields. Our experimental migration arrays were situated on the southern side of the pond that offers the possibility of implanting parallel arrays in different habitat types only separated by a few meters.

Experimental migration arrays

Three migration arrays of 50 m x 0.3 m were delimited by a 0.4 m high fence. They were oriented perpendicularly to the pond bank in order to allow a toad placed at the point the most distant from the pond to move in the direction of the pond. These arrays were spaced out by 10 m. For the arable ground condition, we installed the array within a corn field not yet sowed with trails of ploughing involving ground irregularities. For meadow condition, we implanted an array in a natural grassland that adjoins the corn field. Finally, the forest ground corridor was implanted along a natural grove and its ground was covered with fresh forest

litter sampled in the close forests. The three corridors were closed at their extremities. Temperature and humidity were recorded in each corridor during the experiments for controlling for potential disparities.

Tests of hormonal stress in response to land uses

Female toads in amplexus were captured near the pond (terrestrial habitat) or already in water (aquatic habitat) provided they did not begin to lay their eggs. In order to assess the initial stress level, a first sample of saliva was taken for each female with a oral swab constituted of a small cotton ball beforehand weighted. The female was authorized to chew the cotton ball for 15 s. The cotton ball soaked with saliva was then removed from the mouth and placed in a microtube also beforehand weighted and numbered. All samples were stored in ice during the field experiment (before later being frozen at -80°C in the lab). After saliva sampling, each female was immediately placed in a transport box containing wet forest litter and transported to the starting point of a migration corridor (the transportation distance was always less than 200 m). This run (capture, saliva collection and transport) lasted less than 6 min. Each female was kept in amplexus and all manipulations set with the male clung on its back. Before release females, we equipped each of them with a numbered elastic belt. The position of each female in the corridor was monitored each half-hour. To standardize the time spent in the experimental arrays, the females were recaptured after three hours and a final saliva sample was taken and stored with the same protocol as the initial one. Females were then freed from their belt, the amplexus was disjoined and both snout-urostyle length (SUL) and body mass (BM) of both male and female were measured. Experimented males and females (amplexed) were finally placed in permeable cases half-immersed in the pond and equipped of branches for egg laying to avoid capturing them again. The lays were adequately replaced in ponds at the end of the experiment.

Captures took place between the 26th February and the 12th March 2008. If one of the two cotton balls used to take saliva samples was contaminated with blood, we did not retain the sample since blood contamination alters the measurement of stress hormone in saliva (Cross et al. 2004; Mormède et al. 2007). Finally, we obtained exploitable data for 33 females.

Measure of corticosterone level

Cotton bowls were weighed in order to determine the amount of collected saliva in each sample. Each cotton bowl was transferred in microtubes equipped with large filters to retain cotton during centrifugation. We added 100µl of UltraPure water to dilute saliva. Cotton bowls were then centrifuged at 8,000 rpm during 5 min at room temperature. The pellets containing diluted saliva were collected and were directly used as samples in corticosterone determination kit. We used corticosterone Enzyme-Immuno Assay (EIA) kits (N° 500651, Cayman Chemical) to measure UCM concentrations. We followed the Cayman Chemical protocol for corticosterone EIA. We obtained for each female an initial and a final saliva sample and thus the stress augmentation related to both handling and migration within a given corridor.

Statistical analyses

Linear models were applied to corticosterone concentration data corresponding to the difference of corticosterone levels between the beginning and the end of the experiment. The capture place (on land or at pond), the distance achieved by females during experiment time (three hours), the weight of amplexed male and the kind of ground experienced by migrating female were set as explanatory variables. Neither temperature nor humidity were used as explanatory variables because they did not differ significantly among treatments. In order to work with residuals normally distributed, corticosterone values were square-root transformed. ANOVA with F-tests was then applied to the constructed linear model. We tested only first-

order interactions and we undertook a process of selection model to identify explanatory variables which significantly contribute to explain variance: each non significant term was removed from the models.

Ground type preferences

Study site and experimental design

In 2003, the behaviour choice of fifty eight female toads that were placed in the middle of rectangular arrays (7m x 7m) composed of two types of ground was investigated in semi-natural conditions (A natural population in reserve area of Crépieux-Charmy, Rhone, department, 45°5083N, 5°6625E, France). Eleven females had to choose between forest litter and meadow ground, twenty-one females between forest litter and arable ground and twenty-seven females between meadow and arable grounds. The position (summarized by the type of chosen ground) of each female was recorded each hour during four hours.

Statistical analyses

We used generalized linear models to evaluate the impact of the ground of start (first choice after the first hour in the array) on probability to change of ground type between the first and the last record. We used F-test of to asses the significance of explanatory variable.

Experiments with juveniles

Physiological indicators of resistance

Experimental site and arrays

The toadlets come from tadpoles rearing in the laboratory (35 individuals in 10 L aquariums, 18-22°C, aerated water, weekly cleaning with water renewing, feed *ad libitum* with spinach) originating from Rhone-Alps populations. Experiments took place between the 7th and the 16th July 2008, corresponding to the emergence period for common toads in the Rhone-Alpes region. The experimental devices were installed in the outdoor experimental area of our laboratory on the campus. Four small enclosures (40 cm x 40 cm x 5 cm) were build in four

different types of land uses created beforehand: arable ground, meadow ground, soil covered with forest litter exposed to sunlight (open forest ground) and soil covered with forest litter prevented from exposition to sunlight (shaded forest ground).

Tests of hormonal stress in response to land uses

Toadlets were exposed to a given experimental condition for fifteen minutes. Environmental variables (temperature and humidity) during this experimental time were recorded. After this time, toadlets were immediately recaptured and euthanized by an overdose of anaesthetic (Isoflurane). The entire body of each toadlet was placed in microtubes temporally stored in ice before being frozen at -80°C .

Measure of corticosterone level

The entire body of each toadlet was crushed just after defrosting. We added $500\mu\text{l}$ of UltraPure water to dilute the obtained homogenate and centrifuged the content of microtubes at 8,000 rpm during 5 min at room temperature to separate the liquid phase from the solid phase (small fragments that could prevent from accurate corticosterone determination). Fifty μl of the obtained liquid phase were then used as samples in corticosterone determination kit. To determine the corticosterone concentration in the body, obtained values were weighed by the mass of each individual. We thus obtained values reflecting corticosterone concentration per g of body. We used corticosterone Enzyme-Immuno Assay (EIA) kits (N° 500651, Cayman Chemical) to measure UCM concentrations. We followed the Cayman Chemical protocol for corticosterone EIA.

Statistical analyses

Linear models were applied to corticosterone data (square-root transformed to obtain residuals normally distributed) with types of land use, temperature and humidity as explanatory variables provided these variables were not correlated (correlations between

variables were first explored) and present significant variance. We used F-test of type I (order-dependent test) to assess the significance of each explanatory variable.

Ground type preferences

Study site and experimental design

In 2003, the behaviour choice of sixty juveniles (from the same population that females used for behavioural experiment) that were placed in the middle of a rectangular array (70cm x 70cm) composed of two types of ground was investigated in laboratory conditions. Twenty juveniles had to choose between forest litter and meadow ground, twenty juveniles between forest litter and arable ground and twenty juveniles between meadow and arable grounds.

Statistical analyses

We used permutation tests (10,000 simulations) to assess the probability of obtaining the observed choice. All statistical analyses were performed with R 2.5.0 (R Development Core Team, Vienna, Austria).

Results

Physiological indicators of resistance

Initial stress of adult female toads tended to be dependent of capture location ($F = 3.41$; $Df = 1$; $p = 0.073$): females captured near the pond (on land) had higher basal corticosterone levels than females captured already at pond. The increase of stress during the experiment (difference between final and initial stress) was neither related to capture place, distance, nor to the weight of the mated male but was significantly affected by ground type in the array ($F = 4.46$; $Df = 2$; $p = 0.018$). Stress augmentation did not differ between meadow ground and forest litter. In contrast, arable ground induced significantly more stress than the

two other ground kinds (difference forest litter/meadow ground: $t = 0.067$; $p = 0.95$; difference forest litter/arable ground: $t = 2.59$; $p = 0.014$) (Figure 1).

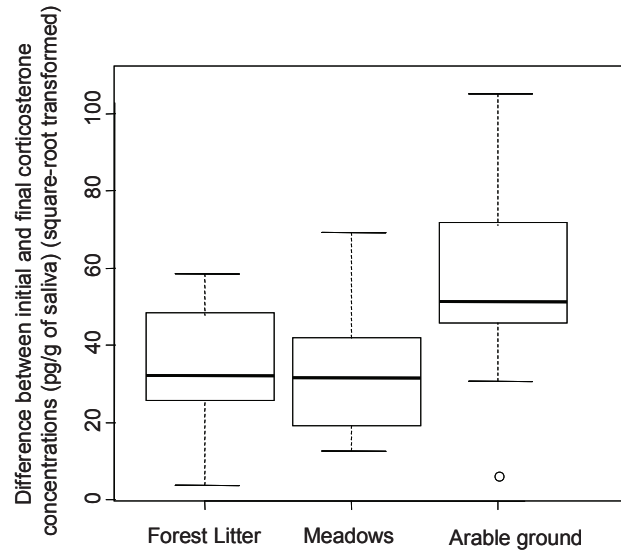


Figure 1: Differences between initial and final stress hormone levels in adult females according to ground type.

In experiments with juveniles, the environmental variables temperature and humidity were highly correlated ($r = -0.79$, $t = -14.24$, $df = 126$, $p < 3.10^{16}$). We thus only retained humidity and ground type as possible explanatory variables in the linear model. Results showed that humidity affects significantly the stress level of juveniles ($F = 6.40$; $Df = 1$; $p = 0.013$, Figure 2). The type of ground did not provide additional information about stress (non-significant p -value in F -tests).

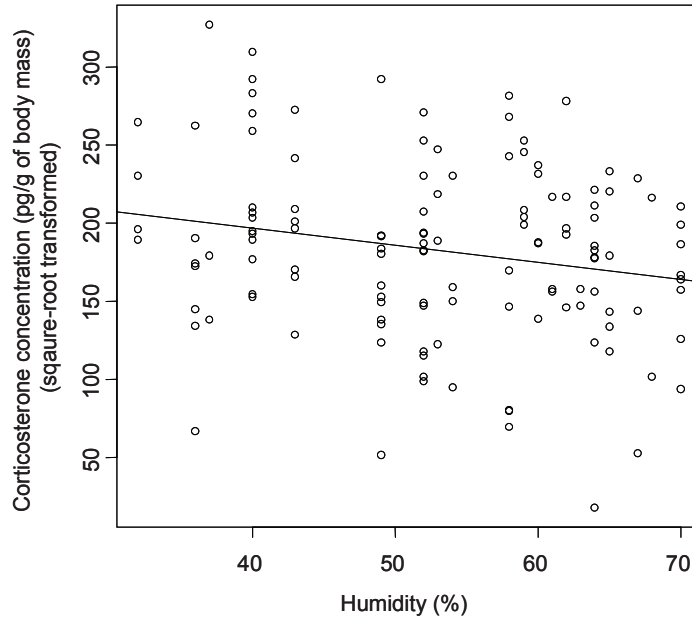


Figure 2: Relationship between humidity of ground and corticosterone level in toadlets.

Ground type preferences

Females exhibited significant preference for forest litter and for meadow ground but avoided arable ground (Ground of start as explanatory variable: $Df = 2$; $F = 10.019$; $p = 0.007$; arable ground-forest litter: $z = -3.101$; $p = 0.002$ / arable ground-meadow ground: $z = -2.252$; $p = 0.024$; forest litter-meadow ground: $z = 1.161$; $p = 0.2457$).

Juveniles exhibited strong preference for arable ground when they had to choose between arable and meadow grounds ($p = 0.007$) but no preference when they had to choose between arable ground and forest litter ($p = 0.12$) or between forest litter and meadow ground ($p = 0.89$).

Discussion

Our results show first that adult female toads present higher corticosterone levels when they encounter arable land during their pre-breeding migration. However, we did not detect any difference of stress between females migrating on forest litter or meadow ground. Second,

this work highlights that corticosterone levels of juveniles were negatively associated with humidity but we did not observe significant relationship between stress levels and ground types, contrary to our hypotheses. In the same way, toadlets exhibited no preference between forest and meadow but surprisingly selected preferentially arable ground to meadow ground and chose indifferently forest and arable grounds. Migration through other potential unsuitable environments such as pavements has previously been identified as stressor for male salamanders (Homan *et al.* 2003). Such a fully artificial ground characterized by very detrimental humidity condition in absence of rain seems likely to elicit corticosterone secretion even if it crossed on short distance (Homan *et al.* 2003). Our study shows that arable grounds which cover very large areas in most agriculture landscapes are also stressful for toads.

Arable land has been proved to be detrimental for amphibian populations either through correlation studies (Joly *et al.* 2001, Janin *et al.* 2009, Greenwald *et al.* 2009) or behavioural studies (Rothermel and Semlitsch 2002). Nonetheless, the way by which arable land affects amphibian populations and migrations remains unclear. It could create local drastic environmental conditions through low humidity rates and high heat, costly movements (movement through drills and tacky substrate hindering displacement), exposure to chemicals such as fertilizers or pesticides, and to predation. These factors could contribute to both a "physiological barrier" and a "behavioural barrier". The physiological barrier corresponds to high cost of movement and high mortality risks when crossing unfavourable grounds whereas the concept of behavioural barrier assumes reluctance to cross these grounds resulting from cognitive association between the appearance of a ground and the negative feeling got from a previous experience of moving across it, or from a spontaneous shunning (selected behaviour). We can notice that these two types of barriers, physical and behavioural, are not exclusive. Preceding works have identified arable land as both physiological barrier through

desiccation indices (Rothermel and Semlitsch 2002) and behavioural barrier through shunning experiments (Rothermel and Semlitsch 2002; Stevens *et al.* 2006). In our case, we show higher levels of corticosterone in adult toads crossing arable grounds than in toads crossing meadow grounds or forest litter although any variation of humidity nor of temperature were detected between the three ground types. Consequently, we consider that arable land does not constitute only a physiological barrier but also a behavioural one, at least for adult individuals since females present higher stress levels when crossing this ground without experiencing overheating nor aridity. The existence of a behavioural barrier is well supported by preference experiment in adult females which preferentially chose forest and meadow grounds. Nonetheless, we did not detect any relationship between ground type and corticosterone levels in toadlets, while stress hormones levels were inversely related to humidity. Thus, for juveniles, the role of physiological barrier seems predominant. This discrepancy between juveniles and adults is unlikely to be due to corticosterone measure since the use of the entire body of juveniles precludes from problems of detection that could be encountered with the sampling of biological fluids of very small animals. If behavioural barrier results from an associative learning, this is more likely that inexperienced juveniles are not able to respond by anticipation through shunning behaviour of potentially unfavourable grounds contrary to adult who are likely to have experienced several ground types while migrating. This hypothesis of the paucity of the role of behavioural barrier in toadlets is well supported by the results from preference experiments since no clear preference for ground was established on the basis of toadlets choice behaviour.

Juveniles are thus a really vulnerable stage. Indeed, they are first expected to be more likely to suffer from drastic environmental conditions because of their small size but they seem also not able to shun land uses that can reveal unsuitable under some weather conditions. The mechanism of behavioural barrier seems ineffective. Their movements are

probably guided by microclimatic conditions perceived at low distance such as humidity (Rothermel 2004). Juveniles start their migration during summer. In general, they are able to wait at pond banks for suitable weather conditions before starting migration under rainy weather. Nonetheless, the absence of behavioural barrier against arable ground makes juveniles likely to be trapped in unsuitable conditions after meteorological change.

Besides the greater vulnerability of juveniles, the consequences of higher stress level in adult toads migrating through unsuitable landscape can be costly and could impact population dynamics. High level of corticosterone is, for example, likely to suppress amplexic clasping behaviour in males (Moore *et al.* 2007) and thus may be responsible for direct fitness alteration. Even if amplexus is achieved, males can be disrupted enough to not fertilize laid eggs, this behaviour being frequently observed in captivity when males are under stress (personal observations). We can also suppose that breeding behaviour of females is likely to be altered by high corticosterone levels notably the delicate process of stretching and hanging the clutch in the vegetation essential to egg development, although in other species with high reproductive cost, females seem exhibit adrenal desensitization to prevent disturbances interfering with reproduction (Jessop 2001). But stress felt during pre-breeding migration may also play a favourable role as a cue to cope with habitat degradation (corticosterone-adaptation hypothesis, Bonier *et al.* 2009). Since juveniles are vulnerable to desiccation, females facing bad condition during pre-breeding migration can be encouraged to disperse to look for another pond offering better migration conditions to emerging juveniles.

The nature of the relationship between fitness and corticosterone in amphibians is complex (Moore and Jessop 2003). Indeed, if negative impact of stress is well supported in amphibian species, it is evident that during reproduction many amphibians display concurrent elevations in plasma levels of corticosterone with no suppression of reproductive behaviour or physiology, which is expected to actually facilitate specific aspects of reproduction (Moore

and Jessop 2003). In our study, females exhibit a tendency for lower corticosterone level when the pond has been reached. Corticosterone could thus play an important role during the pre-breeding migration period to mobilize energy and awareness. However, the relationship between fitness and corticosterone is rather quadratic than linear (Busch and Hayward 2009), suggesting clearly that if corticosterone secretion could be beneficial at first, exceeding a threshold become detrimental to reproductive success and/or survival. Under this proposition, the crossing of particularly stressing grounds during migration is likely to tip up the positive influence of moderated corticosterone into a detrimental influence of high corticosterone levels.

Despite relationship between fitness and corticosterone level variation requires further investigation than current knowledge, the results obtained highlight that modification of land uses can clearly affect the physiological state experienced by toads during migration. All the toad populations will not respond in the same way to this stress increase: some could be not affected by this additional pressure, and could even take advantage of stress during migration (for example when adult experiencing harsh migration condition will initiate dispersal event), whereas the persistence of others could be at stake according to population context (dynamic, history and the resources provided by the living habitat). Conservation biologists concerned about consequences of land use modifications on amphibians currently facing great challenges for which using corticosterone determination through non invasive way could bring precious help to understand processes, propose and evaluate conservation measures even at short time-scale.

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Partie 4 : Sélection d'une préférence d'orientation héritée en paysage fragmenté

Avant-propos

Les trois premières parties de ce travail ont été dévolues à l'estimation de l'influence des différentes composantes du paysage sur les populations de crapauds communs et à l'évaluation conjointe de la résistance de matrice. Elles constituent ainsi un premier volume s'attachant à identifier clairement comment le paysage peut altérer le fonctionnement et donc la persistance des populations. Les parties 4 et 5 nous permettent d'aborder une seconde thématique complémentaire qui approfondit nos connaissances sur les processus en jeu lorsque la fragmentation du paysage intervient en tant que force de sélection. En effet, la variabilité du comportement et d'autres traits d'histoire de vie liés au mouvement entre populations suggère l'existence de pressions évolutives capables de modifier ces caractéristiques. La fragmentation, déterminant en grande partie les mouvements des organismes, s'érige donc comme force évolutive capable d'induire des réponses adaptatives dans de nombreux cas soit au travers de processus de micro-évolution (sélection d'une gamme précise de traits héritables) ou de plasticité phénotypique (sélection en faveur d'un génotype permettant l'expression d'une gamme de phénotypes correspondant à différents environnements).

Dans la partie 4, nous nous intéressons à la réponse de populations ayant subi une fragmentation ancienne et stable depuis environ deux siècles, pouvant donc s'adapter au travers de processus de sélection portant sur les traits liés au mouvement. La fragmentation aboutit dans les cas sélectionnés à une dichotomisation de la structure du paysage autour du point : seule une des rives se situe à proximité d'un forêt, la rive opposée ne donnant accès qu'à des champs cultivés. Ainsi, la situation paysagère contraint les juvéniles à choisir le "bon

côté" du point d'eau lors de l'émergence pour maximiser leur probabilité de survie.

L'utilisation de signaux directs permettant aux juvéniles de détecter ce "bon côté" étant limitée tant par leur taille que par la discontinuité entre point d'eau et forêt (distance de quelques dizaines de mètres entre les deux habitats), nous faisons l'hypothèse de l'utilisation de signaux indirects, basés sur la détection du champ magnétique fréquemment identifiée chez les amphibiens, permettant aux individus de s'orienter spontanément dans la direction la plus favorable sous la pression de sélection que représente la fragmentation.

Evolving in an anthropogenic world: inheritance of vector navigation in common toads migrating in fragmented landscapes and its alteration by an herbicide

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Abstract

Movement behaviour is of crucial importance for animals at different key-steps of their life. However, decision rules regulating movement often remains obscure particularly in changing landscapes. Landscape fragmentation has been largely suspected, sometimes clearly incriminated, in the induction of drastic adaptive changes in movement traits. Moreover, whereas spontaneous orientation in animals during movement was historically interpreted as noise, a growth of evidence calls for new interpretation of this behaviour. We identified inter-generation transmission of spontaneous preferred orientation on the basis of the Earth's magnetic field allowing to optimize migration in response to landscape fragmentation in juvenile common toads. Without any stimuli, the direction selected by newly naïve metamorphs at the emergence could be predicted by the geographical position of forest remnants according to the pond in which their parents breed. However, this capacity to spontaneously make the good choice is altered by the presence of pesticide during the larval stage. These findings illustrate the crucial need to clearly understand the influence of fragmentation on movement characteristics in order to adapt landscape management to issues actually encountered by populations.

Introduction

Contemporary land transformation often involves the concomitant effects of reduction, degradation and fragmentation of habitats. If land transformation is notoriously acknowledged as a main driver of population extinction (Brook *et al.* 2008), it is also expected to give rise to contemporary evolution (Palumbi 2001, Carroll *et al.* 2007). Indeed, since first works on industrial melanism, micro-evolution of resistance to agrochemical toxins such as herbicides or pesticides, with other anthropogenic disturbances, constitutes well known sources of

contemporary evolution (Palumbi 2001; Rice and Emery 2003; Hendry *et al.* 2006; Strauss *et al.* 2006; Carroll *et al.* 2007). How habitat fragmentation can drive contemporary evolution is more controversial because the direction of selective forces depends on the way it alters the outcome of dispersal (Van Dyck and Matthysen 1999). On one side, habitat fragmentation may impede movements that occur through dispersal episodes between populations. Both theoretical and empirical studies suggest that a reduced dispersal propensity as well as a lesser dispersal ability should be selected within such a situation because of the too high dispersal cost as long as patch occupancy does not decrease (Dempster 1991; Heino and Hanski 2001; Cheptou *et al.* 2008). Since such a phenomenon is usually associated to a reduction of population size, dragged by genetic erosion in an extinction vortex is likely to be the ultimate evolutionary outcome (Saccheri *et al.* 1998; Templeton 2001). On the other side, habitat fragmentation may also impede movements within populations in regard of resource exploitation. Since these movements are not optional at the individual scale, the response to fragmentation should be an increase of movement capabilities. There is now accumulating evidences that micro-evolution of a higher movement efficiency took place in some flying-insect populations exposed to this situation. In particular, habitat fragmentation has been found to select for higher fly performance (Taylor and Merriam 1995; Merckx *et al.* 2003; Hanski *et al.* 2004) and a higher sensitivity to target habitat has been reported to occur in fragmented landscape although its determinism is not yet known (Merckx and Van Dyck 2007). Beyond flying species, ground dwelling ones undergo substantial constraints on locomotion and sensory system in regards of the movement range needed to reach target habitat, particularly for small animals. Visual cues may be rapidly out of range because of vegetation masking and hazardous climatic conditions make olfactory gradient alone not sufficient to localise target habitat (Rothermel 2004). At worst, animals crossing the matrix could fall in an ecological trap if available cues are confounded with those of the target

habitat (Kokko and Sutherland 2001). Navigation systems based on indirect cues such as geomagnetism may be a convenient alternative for this task. Provided animals have some information about the target position, they could also reach it more successfully. Magneto-reception is widespread in animals (Deutschlander *et al.* 1999) but vector navigation features such as a genetically-based orientation (Able 2000) are especially well documented in long distance migratory species for which naive animals cannot gain direct information about the target location (Wiltschko and Wiltschko 2005). Moreover, microevolution of migratory orientation has been found in response to range expansion of bird populations (Berthold *et al.* 1992). Landscape fragmentation could act similarly on short migratory episodes achieved by some species undergoing ontogenic habitat shifts. In these species, animals switch habitats during their successive life cycle stages (complementation process: Dunning *et al.* 1992) so that the disconnection of these habitats could operate a strong selective filter on migratory vectors. This is likely to be the case of semi-aquatic amphibian for which Becker *et al.* (2007) recently emphasized the importance of the fragmentation impacts on connectivity between complementary habitats and their consequences on population persistence. Non-random shoreward migration of newly metamorphosed juveniles towards forest area has been reported in several species (DeMaynadier and Hunter 1999, Rittenhouse and Semlisch 2006, Walston and Mullin 2008). Although direct cues and particularly olfaction are often advanced to explain such directional behaviour (Walston and Mullin 2008), there is still no clear indication that juveniles can rely on such cues to join target habitat when disconnection take place. Moreover, translocation experiments performed at the larval stage indicate that metamorphosed animals are poorly efficient to locate the target habitat even on a short distance (Rothermel 2004, Patrick *et al.* 2007). Magnetic compass is well known in amphibians particularly in homing behaviour (Sinsch 1987). It has been reported in y-axis navigation at the larval stage (Diego-Rasilla and Phillips 2007) and it has been also suggested

to play a role in shoreward migration (Patrick *et al.* 2007). Micro-evolution of vector navigation could therefore take place provided that the selective gradient resulting from land transformation is consistent over a sufficient evolutionary time (Miaud *et al.* 2004).

In the present study we examined whether across-generation transmitted vector navigation in naive juveniles of the common toad (*Bufo bufo*) has occurred in populations facing a restricted migration corridor since an ancient fragmentation process (since more than 200 years). For this purpose we take advantage of wetlands surrounded by harshly contrasted landscape where metamorphs only survive if they choose the "right" side of the pond . Current land transformation substantially differs from historical one notably through the change of agricultural methods such as the use of agrochemical toxins. Several evidences indicate that agrochemical contamination of ponds affect physiology, fertility as well as behaviour of non targeted species like amphibians (Brunelli *et al.* 2009). We therefore also examined whether amitrole, a pesticide known to alter tadpole antipredator behaviour (Mandrillon and Saglio 2007), could also alter the selective gradient exerted on vector navigation by habitat fragmentation when present during the larval stage at the usual level of environmental contamination.

Materials and methods

Spontaneous orientation in juveniles

Studied populations

We sampled adult toads in four ponds selected for having one side surrounded by an inhospitable matrix (croplands, urban area) and the opposite side by forest, which is the usual habitat of the species (Denton and Beebe 1994). We relied on older maps since those of Cassini (XVIIIth C) to verify that the organisation of local landscape is remained relatively stable over at least more than two centuries (40-50 toad generations). The four populations

were selected for a landward migration direction corresponding to each cardinal point (N, E, S, W). If migration direction is under selective forces of habitat structure, we thus predict that the spontaneous orientation of toadlets would be that of the current direction of the forest in the pond of origin.

Experimental design

In 2007, we captured between eight and ten males and females at each pond during the breeding period and brought them to the laboratory. We realized three types of mating: within-population mating (four replicates for each population); opposite between-population crossing with parents originating from populations with opposed expected directions (four replicates for North/South (2 NF/SM and 2 NM/SF) and Eastern/Western crossings (2 EF/WM and 2 EM/WF); intermediary between-population mating by crossing individuals from populations with expected directions differing of 90° (three replicates for each of the four potential crossings, N/E, N/W, S/E and S/W, with at least one alternation of the origin of male and female) (see Table 2 for details). We obtained a laying for each mating and raised approximately 500 tadpoles of each progeny tadpoles until metamorphosis in 60*40*40 cm tanks (18-22°C, aerated water, weekly cleaning with water renewing, feed *ad libitum* with spinach).

The orientation of newly metamorphosed juveniles was investigated in four experimental arenas installed in dark rooms. Each arena was constituted of plastic bowl of 0.9 m diameter the edge of which being equipped with a gently sloping immersed net to allow toadlet emergence. The bowl was inserted at the centre of a circular area of diameter 1.5 m that allows free movement on a circular band of 10 cm after which inner walls drove the animals to eight pitfall traps corresponding to cardinal and intermediary points. At night, a sample (five individuals) of metamorphosing animals (*i.e.* Gosner stage 44, Gosner 1960) of each sibling group was tested. Animals were kept in the dark until the completion of the

orientation test (all the toadlets caught in a pitfall trap). For each sibship, the first twenty juveniles that performed the test successfully were considered in the analyses in order to keep comparable sample sizes.

Spontaneous orientation after exposure to a pesticide

We focused on the South population (forest occupying the southern edge of the pond). In 2008, we mated in the lab ten pairs of adults sampled at that site. From each clutch, we reared two samples of 150 tadpoles in 40*30*30 cm tanks (18-22°C, aerated water, cleaning two times per week with water renewing, feed *ad libitum* with spinach), the rest of the clutch being released at the capture site. One of these samples was bred in conditions identical to those of 2007 (control group). The other sample was first reared under similar conditions than the control group before being exposed forty days after hatching to water contaminated with 0.1 mg.l⁻¹ amitrole (the lowest dose altering behaviour in Mandrillon and Saglio 2007) until metamorphosis (“pesticide” group). At metamorphosis, orientation of thirteen juveniles of each pair from each group (control and pesticide; total 130 individuals/group) was investigated using the same protocol as exposed previously.

Statistical analysis

We first tested directedness within each sibship. For within-population crossing, we performed a V-Test (Mardia 1972) to test whether the observed angles were clustered around the one expected given the migration corridor of the population. Since individuals of a given sibship cannot be treated as independent data, we also performed global V-Tests based on the mean angle of each family according to the expected azimuth from the population of origin. Because between-population crossing could give rise to a multi-modal circular distribution, we used a Watson U² test based on the empirical distribution of frequencies, which is more

suitable in such a situation (Steele and Chaseling 2006). Although this test is less powerful than a V-Test to detect departure from randomness when data come from unimodal distribution, we also performed this test for within-population crossings to allow comparison. Furthermore, we performed a Wilcoxon exact test to examine whether between-population crossing resulted in a lower mean vector length (i.e. a higher angular variance) than the one observed with within-population crossing. We also performed several tests to explore the underlying mechanisms of vector navigation inheritance. First, we examined whether the offspring vector navigation could result from a co-dominant effect of parental genotypes. In such a case, we could expect a higher angular distance between the parent's populations to give rise to a lower mean vector length if the offspring's vector navigation results from the expression of each parental genotype with an equal probability. We used a Wilcoxon exact test to perform this comparison. Co-dominance between parental genotypes could more simply be expressed through the equal contribution of parental genotypes to the offspring vector navigation. In this case, one expects the observed angles to be clustered around the mean angle of the interior arc formed between the two expected angles given the parental population of origin. However, if the expected azimuths of parental population are on opposed direction (i.e. opposite between-population mating), no interior arc can be defined so that the observed angles could be clustered around the mean angle of either both arcs formed between the two parental population azimuths. To test this hypothesis we performed V-Tests using the mean angle of the interior arc formed between the two parental population azimuths in the case of intermediary between-population crossing (i.e. for which the angular distance between the expected directions of parental populations was 90°). In the case of opposite between-population mating, we first doubled the angles to convert circular data as axial ones before applying the V-test. Finally, we also examined maternal inheritance using a V-Test according to the expected angle given the migration corridor of the maternal population.

We first tested whether amitrole altered the vector navigation of juveniles using a V-test for each sibship according to the treatment experienced during larval stage (*i.e.* Pesticide *versus* Control). We also performed a global V-Test within each treatment based on the mean angle of each family. We also compared the mean vector length using a Wilcoxon test for paired measures.

All tests performed were based on a permutation method (using 5000 randomised sampling) and adjusted for a discrete circular distribution. SAS program was used for all statistical analyses.

Results

Spontaneous orientation in juveniles

Migrating toadlets from within-population crossing exhibited directedness in accordance with the usual direction of postmetamorphic migration in the population of origin (Figure 1). Indeed, both the V-tests performed at the level of sibships and the ones performed at the level of populations using the mean familial angles were strongly significant (Table 1). These results therefore clearly support the existence of vector navigation within all the studied populations.

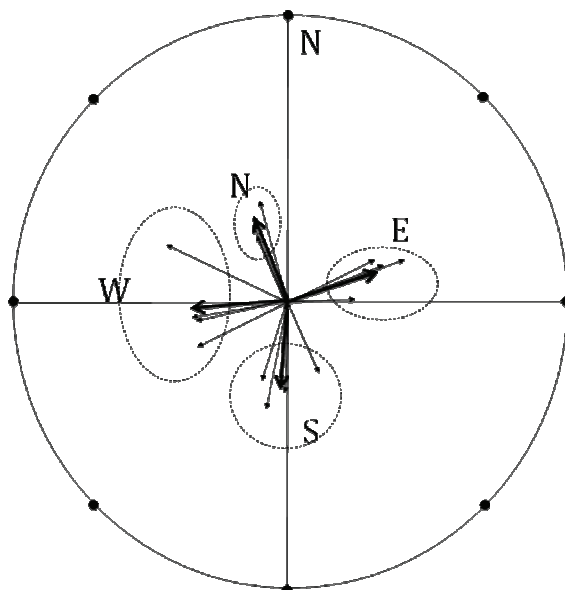


Figure 1: Directional preferences of juveniles from within-population crossings. The thin lines give the mean vectors for each progeny, while bold lines give the mean vectors for all the individuals of a population. The dot-lined ellipses surround vectors of groups of similar origin according to the direction of the forest. The plots on the circle correspond to the positions of the pitfall around the experimental arena.

Table 1: Sibship directedness of within-population crossings

θParental Population*	$\bar{\theta}_i$	\bar{R}_i	Sibship V-Test		Watson Test		Global V-Test
			V _{obs}	P-val	U ² _{obs}	P-val	
0	39.73	0.37	0.35	0.008	0.172	0.087	V _{obs} =0.12 P<0.0002
0	37.10	0.44	0.42	<0.001	0.294	0.011	
0	2.34	0.23	0.23	0.048	0.085	0.363	
0	47.95	0.34	0.31	0.015	0.177	0.083	
90	232.12	0.25	0.23	0.061	0.098	0.283	V _{obs} =0.29 P<0.0002
90	213.31	0.36	0.35	0.011	0.185	0.075	
90	225.00	0.34	0.31	0.020	0.154	0.124	
90	227.71	0.30	0.27	0.031	0.112	0.238	
180	51.06	0.35	0.32	0.024	0.135	0.184	V _{obs} =0.36 P<0.0002
180	19.92	0.35	0.34	0.013	0.169	0.100	
180	23.99	0.33	0.33	0.022	0.156	0.130	
180	312.29	0.49	0.44	0.003	0.247	0.048	
270	157.00	0.38	0.37	0.003	0.208	0.049	V _{obs} =0.30 P<0.0002
270	131.25	0.27	0.25	0.044	0.111	0.239	
270	141.06	0.30	0.28	0.029	0.121	0.209	
270	175.29	0.32	0.32	0.018	0.126	0.184	

Footnotes: *Expected angle (deg.) according to the azimuth position of the migration corridor around the ponds of the parental population. $\bar{\theta}_i$: mean vector angle, \bar{R}_i : mean vector length

Significant P-values at the 5% α level are indicated in bold.

According to the Watson U^2 tests, only one out of the twenty progenies from between-population crossing showed a significant departure from uniform circular distribution (Figure 3, Table 2). Nonetheless, Watson U^2 tests require large dataset to be significant and it is likely that our test suffered from a lack of power since the same test performed on sibship belonging to intra-population crossings also failed to reveal directedness in most of the cases although the associated P-values were lower (see Table 1). The mean vector length of progenies from between-population crossing exhibited, nevertheless, a significantly higher angular variance than that of within-population crossing (Wilcoxon Test: $n=36$, $Z=3.302$, $P=0.0005$). Conversely, the angular variance did not significantly differ between the “opposite direction” crossing and the “intermediate direction” crossing (Wilcoxon Test: $n=20$, $Z=-0.34$, NS). Only two out of the twenty progenies exhibited orientation in accordance with the mean angle between the directions of the parental populations, whereas five out of twenty showed orientation in accordance with the migration direction of the maternal population (V-tests, Table 2).

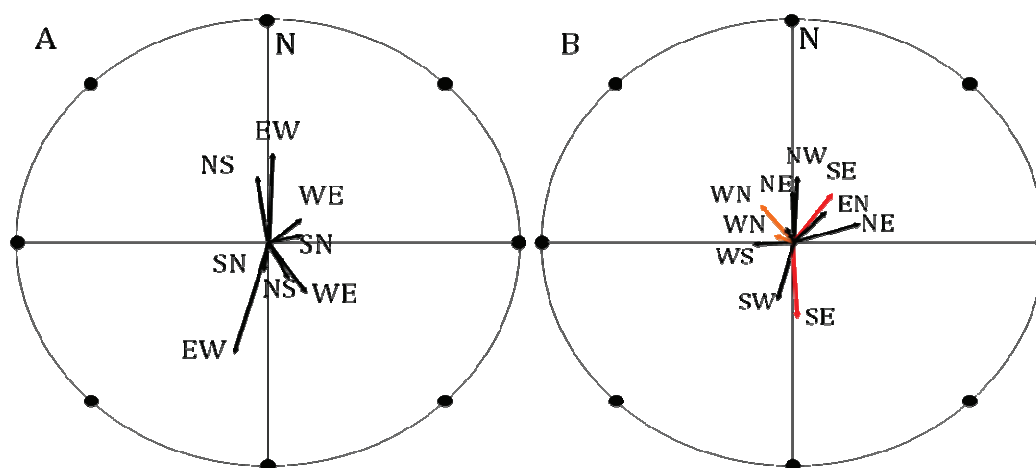


Figure 2: Directional preferences of juveniles within between-population crossing. A. Opposite directions. B. Intermediate directions. The mean vector for each sibship is labelled according to the parental populations, the first character corresponding to the maternal population). Other legends as in Figure 1.

Table 2: Sibship directedness of between-population crossings

$\theta_{\text{♀}}$ population*	$\theta_{\text{♂}}$ population*	$\bar{\theta}_i$	\bar{R}_i	Watson Test		V-Test ($\theta_{\text{♀}}$)		V-Test ($\theta_{\text{♀♂}}$)	
				U^2_{obs}	P-val	V_{obs}	P-val	V_{obs}	P-val
0	90	93.37	0.18	0.05	0.620	0.13	0.214	0.18	0.117
0	180	175.78	0.39	0.16	0.109	0.01	0.477	0.00	0.495
0	180	13.47	0.19	0.09	0.346	0.19	0.097	0.25	0.047
0	270	225.00	0.07	0.01	0.983	-0.03	0.593	0.07	0.399
90	0	180.00	0.23	0.09	0.301	0.23	0.069	0.16	0.155
90	0	34.28	0.27	0.10	0.296	0.08	0.286	0.24	0.062
90	180	172.04	0.30	0.20	0.055	0.30	0.014	0.20	0.077
90	270	128.45	0.18	0.05	0.650	-0.16	0.854	0.05	0.659
90	270	198.33	0.30	0.15	0.141	0.30	0.025	0.21	0.080
180	0	77.65	0.16	0.10	0.266	-0.13	0.806	0.09	0.360
180	0	114.87	0.27	0.14	0.145	-0.15	0.834	0.25	0.945
180	90	256.17	0.21	0.08	0.409	0.13	0.185	0.21	0.079
180	90	315.00	0.07	0.03	0.901	0.07	0.334	0.07	0.330
180	270	8.13	0.16	0.05	0.626	0.16	0.133	0.12	0.195
270	180	108.04	0.26	0.12	0.234	-0.21	0.913	0.04	0.082
270	180	173.81	0.34	0.15	0.137	0.34	0.012	0.25	0.206
270	90	25.53	0.14	0.03	0.914	-0.03	0.585	0.04	0.647
270	90	160.53	0.13	0.06	0.510	0.13	0.196	0.20	0.093
270	180	152.72	0.27	0.10	0.289	0.26	0.044	0.23	0.071
270	180	148.63	0.51	0.31	0.020	0.49	<0.001	0.45	0.003

Footnotes: *Expected angle (deg.) according to the azimuth position of the migration corridor around the ponds of the maternal (\ominus) and paternal (\oslash) population. $\bar{\theta}_i$: mean vector angle, \bar{R}_i : mean vector length, V-Test (θ_{\ominus}): result of the V-test according to the azimuth position of the migration corridor around the ponds of the mother, V-Test ($\theta_{\ominus\oslash}$): results of the V-test according to the mean angle within the interior arc between the maternal population angular expectations and the paternal one (if the parental population azimuths are not on a opposed direction) or both the two mean angles between the maternal population angular expectations and the paternal one (if the parental population azimuths are on an opposed direction).

Alteration of orientation by pesticide

Among the ten sibships of the “control” group, only seven showed a significant departure from uniform circular distribution at the 10% α level (Figure 3, Table 3). Although these results are slightly less significant than the ones reported in the experience on vector navigation given the reduced sample size of siblings used per sibship (i.e. 13 versus 20), the global V-test based on the mean angle of each sibship remains strongly significant ($n = 10$, $V_{\text{obs}} = 0.26$, $P < 0.0002$).

On the contrary, there was no evidence for directedness among the ten sibships exposed to amitrole since the lowest P -value reported by the V-test was 0.41 (Figure 3, Table 3) and the global V-test based on the ten mean vector angles was insignificant ($V_{\text{obs}} = -0.05$, $P = 0.42$). The angular variance of the mean vector length was clearly increased in the amitrole group compared to the one of control group (Wilcoxon paired test: $n = 10$, $S = 27.5$, $P = 0.002$).

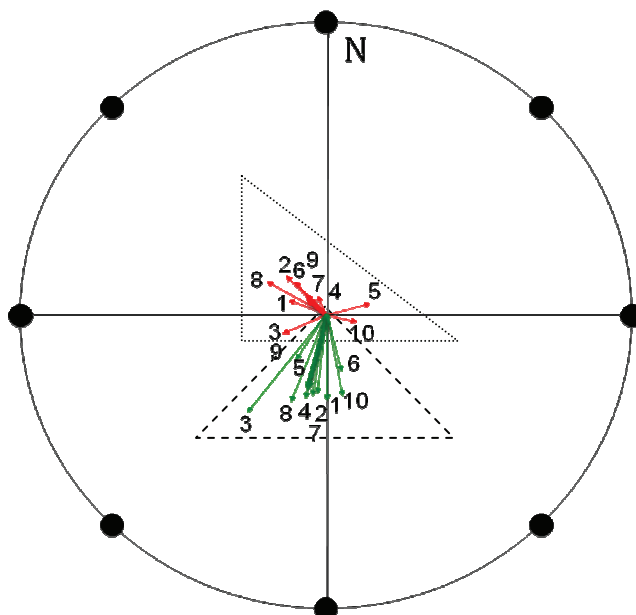


Figure 3: Effect of Amitrol on the directional preferences of juveniles. Vectors of each sibship is in thin line and labelled by a number. The general mean vector for all individuals is in bold. "Pesticide" group in dot-lined triangle and "Control" group in dash-lined triangle. See Figure 1 for other legends.

Table 3: Sibship directedness according to the treatment experienced during the larval stage

Sibship	Treatment	$\bar{\theta}_i$	\bar{R}_i	Sibship V-Test	
				V_{obs}	P-val
1	Amitrol	315.00	0.14	-0.05	0.609
	Control	180.00	0.29	0.29	0.079
2	Amitrol	270.00	0.19	-0.13	0.743
	Control	201.60	0.28	0.27	0.084
3	Amitrol	315.00	0.16	0.06	0.411
	Control	256.82	0.42	0.33	0.036
4	Amitrol	270.00	0.08	-0.06	0.609
	Control	209.28	0.29	0.28	0.075
5	Amitrol	32.65	0.14	-0.04	0.581
	Control	209.28	0.22	0.21	0.156
6	Amitrol	270.00	0.15	-0.10	0.670
	Control	152.72	0.20	0.19	0.175
7	Amitrol	225.00	0.07	-0.06	0.602
	Control	193.83	0.27	0.27	0.098
8	Amitrol	299.28	0.22	-0.11	0.706
	Control	225.00	0.32	0.29	0.089
9	Amitrol	270.00	0.09	-0.07	0.631
	Control	250.53	0.18	0.15	0.231
10	Amitrol	29.28	0.09	0.02	0.453
	Control	158.40	0.28	0.27	0.082

Discussion

Our results clearly demonstrate the existence of an across-generation transmission of vector navigation on the basis of magnetic field adapted to the local environment in common toads. Indeed, the juveniles originally from within-population pairs did not emerge at random but the mean vector angle formed by their orientation significantly corresponds to the optimum direction predicted by the analysis of landscape structure for each of the four tested populations (see Table 1., Figure 1). Although there is ample evidence of magnetic sensitivity in various taxa (Deutschlander *et al.* 1999, Wiltschko and Wiltschko 2005) and particularly in migrating amphibians (Urodeles: Phillips 1986, Diego-Rasilla 2003; Anurans: Sinsch 1987, Freake *et al.* 2002), rare are the studies that underline the existence of across-generation transmission preference for magnetic direction (but see Miaud *et al.* 2004; Schlegel and Renner 2007) and this is the first time to our knowledge that the landscape fragmentation is clearly recognized as a driver of this process.

Orientation mechanisms

Although it is a widespread phenomenon, the precise mechanisms involved in the use of Earth's magnetic field for navigation often remains obscure (Wiltschko and Wiltschko 2005). In our experiment, the navigation was tested in specific experimental arenas positioned indoor, in an isotropic light-deprived room common to all tested populations for which different orientations were identified. The obtained results thus clearly demonstrated the use of magnetic orientation by juveniles to express a direction although we did not artificially deviate the magnetic field vector. Until recently authors argue for a light-dependent compass (Deutschlander *et al.* 1999) based on a radical pair mechanism in amphibians (Wiltschko and Wiltschko 2005). Nonetheless, the persistence of orientation under light-deprived conditions rather supports the hypothesis of receptor mechanisms involving magnetite elements. The hypothesis implying magnetite is also supported by other studies (Gould 2008) run in total

darkness either focusing only on the spontaneous alignment with respect to the earth's magnetic field (Schlegel 2007, Schlegel and Renner 2007) or analyzing also the compass component (Diego-Rasilla *et al.* 2008).

Emergence of the phenomenon

Such navigation systems based on the Earth's magnetic field have been identified over a large range of spatial scales (Lohman *et al.* 2007), from local homing (sandhoppers, Scapini *et al.* 1985; amphibians: Sinsch 1987) to long-distance migration (Alerstam 2006; Lohman *et al.* 2008). However, only a small part of the usage of magnetic field detection seems to be fully understood and the interpretation of aimed orientation frequently remains interpreted as "noise" particularly at the local scale (Gould 2008). We hypothesize a variation of natural bias of orientation between individuals can persist within populations until selective pressure (for example fragmentation) is applied to this system, which results in the standardization of the bias leading to optimal orientation. The investigation of the existence of various orientation biases in populations breeding in continuous landscape (exempted from selective pressure due to landscape) could enhance our understanding of the underlying mechanism.

Processes of transmission of vector navigation

Although this study did not focus on the identification of the across-generation transmission process in play, the mean orientation of juveniles from crossbreed provides support or detracting to the three alternative hypotheses explaining apparent inheritance: maternal inheritance, epigenetic transmission or genetic transmission (according to the concept of inclusive heritability Danchin and Wagner 2010). First, only two out the twenty progenies exhibited orientation in accordance with the mean angle between the directions of the parental populations, which suggests that the hypothesis of a co-dominance effect of parental genotypes is weakly supported (V-tests, Table 2). Moreover, as the angular variance of progenies from between-population crossing is higher than that of within-population

crossing, there is no evidence supporting co-dominance between parental genotypes expressed through equal contribution of parental genotypes to the offspring vector navigation. Further, five out of the twenty progenies showed orientation in accordance with the migration direction of the maternal population (V-tests, Table 2). Once again, the alternative hypothesis of a maternal transmission of vector navigation is not fully supported. The results obtained for crossings do not allow to properly identify the mechanisms of vector navigation transmission but highlight that there are probably more than one mode of transmission and that the process is more complex than an oligogenic transmission (Ugolini *et al.* 2003) (Figure 2).

Viable mechanism in dynamic landscapes?

Relying on across-generation transmitted preference could be detrimental if landscape changes rapidly and in unpredictable way as it is the case with human modifications. Transmitted specific behaviour could thus become an ecological trap (discrepancy between the cues and the true quality of different habitats, Kokko and Sutherland 2001) consecutively to landscape modification since it no longer maximizes fitness (Schlaepfer *et al.* 2002). According to the process of transmission, the ecological trap could be more or less drastic. If transmission occurs through a parental (maternal) transmission or epigenetic, the behaviour can be rapidly modified according to the changes. Contrarily, a purely genetic transmission is a more inertial process that requires a sufficient number of generations to be modified and can block populations in behaviour inadequate to landscape a sufficient time to go to extinction. Nonetheless, it is likely that an interplay between genetic selection at the population level and learning and plasticity at the individual one (Scapini *et al.* 1995) could make the system more flexible. Vector navigation is information probably used by individuals when they lack from reliable direct cues in their environment at fragile life stages but this system is very likely to be supplanted by the use of suitable direct cues when they become available.

In anthropogenic agricultural landscapes, tadpoles frequently undergo the impacts of high concentrations of chemical agents. Besides the diminution of global activity in tadpoles due to pesticides (Brunelli *et al.* 2009), there is also a lot of evidence of the alteration of behaviour in amphibians (Park *et al.* 2001, Mandrillon and Saglio 2007). Moreover, pollution such as pesticides or metal contamination was previously identified as a disrupter of orientation behaviour in other species (Vyas *et al.* 1995; Ungherese and Ugolini 2009). In our case, whereas the rest of the siblings clearly oriented to the expected South direction, juveniles breed in the presence of amitrole had a random orientation although this chemical agent was introduced at common environmental concentrations and only at late stages (Table 3, Figure 3). Therefore, adaptations to local landscape structure could be invalidated by the presence of pesticides that is a frequent correlate of landscape fragmentation.

If the processes of across-generation transmission of the identified vector navigation remain under investigation, it is clear that the selection of specific fixed behaviour in response to landscape fragmentation has important implications in conservation biology since transfer of individuals to rescue population or compensatory measures modifying landscape organization are likely to be inefficient or even detrimental, notably if the response system is not much flexible. Where fragmentation intervenes, the investigation of body of consequences including adaptive modifications of movement traits is crucial to adequately manage altered landscapes.

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Partie 5 : Variation du comportement lors de l'émergence en fonction de la fragmentation du paysage

Avant-propos

A travers cette cinquième et dernière partie, nous explorons l'existence de variation de comportement lors de l'émergence qui constitue la première exploration de l'habitat terrestre des juvéniles, selon le degré de fragmentation de l'habitat forestier. En effet, on peut s'attendre à ce que les juvéniles devant faire face à un morcellement important de l'habitat forestier présentent un comportement d'exploration plus intense que les juvéniles émergeant directement dans de grands patches de forêts, ce qui maximise leur chance de trouver un patch favorable. Cette cinquième partie constitue une première approche de telles différences comportementales entre populations, ainsi que les mécanismes évolutifs sous-jacents (plasticité phénotypique ou transmission via un processus d'héritabilité inclusive). Les mécanismes adaptatifs en jeu sont encore en phase d'investigation bien que nos dernières expériences (non intégrées à ce document) aient permis d'identifier une partie de la réponse. Suivent donc ici, les résultats d'une comparaison de suivis comportementaux lors de la phase d'émergence d'individus juvéniles issus de populations se reproduisant dans des paysages pour lequel le degré de morcellement de l'habitat terrestre diffère.

Habitat fragmentation affects migration behaviour of juvenile common toads

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Abstract

Displacement characteristics are crucial drivers of the success of decisive movements (resources acquisition, migration, dispersal). As landscapes change, mobility patterns are likely to be altered. In species with complex life cycle that implies obligatory migrations between habitats, one can predict that movements would be promoted by fragmentation. Here, we compared the movement characteristics of naïve toadlets from a gradient of fragmented landscapes to test the hypothesis of a positive correlation between fragmentation and levels of mobility. We studied toadlet movement in experimental arena providing small patches of suitable conditions (forest litter, shelter, humidity). We recorded the use of these patches (*patch behaviour*) and its association with an explorative behaviour (*overtake behaviour*). The more fragmented is the originate landscape, the more toadlets were more prone to associate these two behaviours showing thus a higher motivation to explore. Moreover, when only one behaviour is observed during a run, the more fragmented is the originate landscape, the less toadlets exhibited the *patch behaviour*. Because the toadlets were reared in a common environment, the behavioural differences detected according to the level of fragmentation

result from inheritance. Our results thus illustrate that fragmentation is likely to create across-generation transmittable interpopulation variation in movement characteristics of amphibians.

Introduction

Although movements are key processes in dynamics and viability of subdivided populations and in biodiversity conservation, there is yet much to learn about the behavioural mechanisms that sustain movement efficiency such as orientation and habitat selection (Nathan *et al.* 2008). Beyond differences between species, interest for variation among populations is currently emerging, notably because of anthropogenic environment modifications leading to harsh local conditions are able to constitute important selective forces. Landscape fragmentation, habitat loss and patch isolation can act as an evolutionary driver of movement characteristics (Baguette and Van Dyck 2007). Adaptations in mobility behaviour would thus emerge in populations facing habitat loss and/or fragmentation. Nonetheless, although this hypothesis is well supported by theoretical models (decrease or increase of dispersal in response to fragmentation according to different conditions (Travis and Dytham 1999; Heino and Hanski 2001), evidences of changes in movement behaviour in response to landscape alteration in nature remain scarce (but see Merckx *et al.* 2003, Merckx and Van Dyck 2007). By focusing on flying insects, Van Dyck and Matthyssen (1999) have first synthesised the likelihood of a change of movement pattern and/or morphology in response to habitat fragmentation. They have argued that subtle changes could be tracked, and collected empirical studies illustrating such a continuous evolutionary response. Several works have indeed demonstrated that different components of movement behaviour can be effectively modified under landscape pressure: locomotor capacities (Taylor and Merriam 1995), illingness through flying propensity and boundary crossing in butterfly (*Pararge*

aegeria Merckx *et al.* 2003) and through tiptoe behaviour in spider (*Pardosa monticola*, Bonte *et al.* 2006), and navigation capacities through habitat detection ability (Merckx and Van Dyck 2007).

Most studies dealing with movement / landscape relationships focus on dispersal because of its ecological and evolutionary implications. Nonetheless, the achievement of smaller movements crucial for completing life cycle (migration between two different habitats: complementation processes, Pope *et al.* 2000) or repeated daily (movements between different patches of the same habitat to obtain sufficient amount of resources: supplementation process, Dunning *et al.* 1992) is also exposed to the selective forces determined by landscape structure. Particularly, complementation process implies the achievement of migration whatever the incurred risk. We can thus expect that movement patterns of species with complex life cycle should be greatly affected by the selective forces induced by habitat loss and fragmentation.

Although the direction of the response to the selective pressure resulting from fragmentation is complex to predict (selection promoting movement or on the contrary lowering it: Merckx *et al.* 2003, Bonte *et al.* 2010) because multi-factorial, populations facing an alteration of matrix connectivity are compelled to minimize migration costs and to favour behaviour that allow the more efficient movement in landscapes subjected to fragmentation. Indeed, seasonal migration, contrary to dispersal, can not be easily counterselected by fragmentation since they are not optional. One can also make the same prediction for supplementation movements.

Our study focus on a species with complex life cycle, the common toad *Bufo bufo*, to investigate responses of populations compelled to move frequently through fragmented landscapes. We investigated variation in obligatory-movement behaviour: we predicted an enhancement of movement efficiency in fragmented landscapes resulting from selective

forces due to higher landscape fragmentation (resulting from agriculture intensification). We adopted an experimental approach by comparing land-ward movement of toadlets originating from populations sampled along a gradient of landscape fragmentation (defined by the number of forest fragments surrounding pond). By a common garden experiment, we have exposed naive toadlets when leaving water after metamorphosis to a choice between entering or not a shelter habitat made of forest litter, mimetic of the target habitat of the postmetamorphic migration. Since toadlets were reared under controlled conditions in the laboratory, any difference in choice behaviour would be a consequence of transmissible variation (either genetic / epigenetic heritability or condition-dependent parental effect).

Materials and methods

Study design and sampled populations

In April 2008, seven toad clutches were sampled at ten ponds distributed along a gradient of landscape fragmentation. We investigated landscape structures with special emphasis to forested areas that constitute the usual terrestrial habitat of toads (Denton and Beebe, 1994). Among the multiple fragmentation metrics (Calabrese and Fagan, 2004), we chose the number of forest fragments that reflects the primary effect of fragmentation, *i.e.* the cutting up of habitat in smaller pieces. The cutting up of habitat is very likely to compel both the success of the first migration and, later, the movements between fragments to supplement resources (food, water, shelters). Because these movements are not optional, we consider that the number of forest fragments is a better integrative variable of fragmentation than forest area. Nevertheless, we measured overall forest area within the same area as complementary variable. For defining a forest fragment, we considered in a GIS each pixel or group of pixels of forested grounds completely surrounded by another landscape elements. The number of

fragments within a 500 m radius circle centred on the focal pond (this distance reflects probably the habitat area used by toads see part 2) is then counted.

Tadpole rearing

Each sample of clutch was housed in a 10 L aquarium in a temperate room (18-22°C). After hatching, the tadpoles were reared until metamorphosis in the same aquariums, at a density of 35 tadpoles per aquarium (aerated water, cleaning two times per week with water renewing, feed *ad libitum* with spinach). Aquariums were cleaned and water entirely changed two times per week.

Behavioural experiments

After metamorphosis, behavioural observations took place indoor by means of a 150 cm radius dodecagonal arena made of inert plywood. The arena was placed in a climate room with very few anisotropy sources. To make observation without disturbing the tested animals, the experimenter is placed above the experimental arena on a mezzanine equipped with small holes. In each of the twelve arena sectors, we installed a small patch of forest habitat (15 x 7.5 cm) at an intermediate distance between arena centre and arena border. This patch of forest habitat is constituted of forest soil and litter strongly dried after sampling to eliminate any potential prey. The sample was then moistened before being installed on the arena. For each arena sector, the forest patch occupied half the width of the sector, thus giving the toadlet an equal possibility of reaching the arena border without entering a forest patch. Furthermore, this disposition makes highly probable the detection of a forest patch by a moving toadlet, whatever the direction of its movement. Thus, we considered that each toadlet overtaking the ring of patches has detected the presence of at least one forest patch. Groups of 5 juveniles were released at the centre of the arena after an habituation period of five minutes in the release box (Plexiglas box of 10 cm diameter) and we recorded the behaviour of each of them during thirty minutes. We then measured body size and mass of each toadlet. We classified

toadlet behaviour according to three types: (i) *patch behaviour* when the animal entered a forest patch without explored the area beyond patches, (ii) *overtake behaviour* when the animal overtake the ring of patches without entering a forest patch, and (iii) *associate behaviour* when the animal combines the two former behaviour (Table 1). The *patch behaviour* reflects the motivation to experience the stimuli from the target habitat (Rothermel *et al.* 2004). The *overtake behaviour* reflects a dominance of the motivation to move over the motivation to experience target habitat. The associate behaviour reflects a global high motivation to explore the different possibilities offered by the experimental arena. We excluded from dataset toadlets that did not reach the test circle. We monitored the behaviour of five toadlets by clutch (i.e. 35 toadlets for each pond, and 350 in total).

Table 1: Description of the behaviour types.

patch	overtake	Behaviour type	Type of behaviour
1	0	Only <i>patch behaviour</i>	Exclusive
0	1	Only <i>overtake behaviour</i>	Exclusive
1	1	Sequence including <i>patch</i> and <i>overtake behaviour</i> (<i>associate behaviour</i>)	Associated

Statistical analysis

We used generalized linear models to compare the proportions of toadlets adopting each behaviour type according to landscape fragmentation experienced by their population. We have assumed a binomial distribution of errors and a logit link function for behaviour data. "Fragment number" and "forest amount" were implemented as landscape factors into models, and body size and the residuals of the linear model between body size and mass as

morphometric factors. First-order interactions were also investigated. We selected explanatory variables on the basis of the lowest AIC. In order to consider the association of two behaviours, we used logistic regression of explanatory variables (landscape and morphometry) on condition-dependent response variable corresponding to *associate behaviour* (probability of *overtake behaviour* given *patch behaviour* has also occurred during the run) and we evaluated the significance of the effect with F-tests. To evaluate the effect of explanatory variables on behavioural options in case of single behaviour, we also used logistic regression of explanatory variables on a response variable in which the response variable is composed of the two exclusive variables, one corresponding to success and the alternative to fails (*patch behaviour* corresponds to success and *overtake behaviour* to fails since the one excludes the other). All statistical analyses were performed with R 2.5.0 (R Development Core Team, Vienna, Austria).

Results

Among the 350 toadlets tested, 224 effectively left the release box and reached the test zone. Whatever the fragmentation level, the most frequent behaviour was the single *patch behaviour* (Figure 1). The proportions of the other behaviours varied according to fragmentation (Figure 1). For each type of models, the one with the lowest AIC was the simplest model including only one explanatory variable: the number of fragments. The ΔAIC between this model and all others of the same type exceeds 2. Neither body size, mass nor forest amount were factors retained on the basis of AIC. *Associate behaviour* is positively and significantly related to the number of forest fragments (see Table 2 and Figure 2a). In case of single behaviour, *patch behaviour* is inversely related to fragment number (and reciprocally, *overtake behaviour* is positively related to fragment number (Table 2; Figure 2 b).

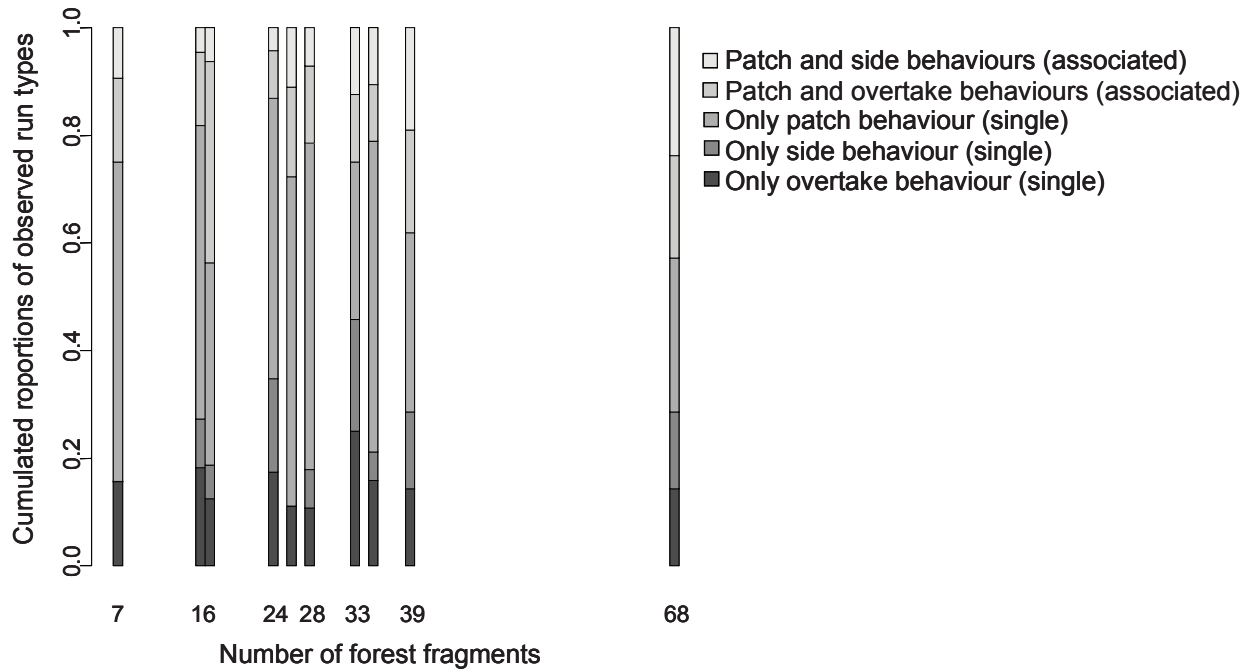


Figure 1: Repartitions of observed run types according to the number of forest fragments.

Table 2: Results of F-tests applied to logistic regressions on behaviour during runs.

Models	Deviance	<i>Df</i>	$P > \chi^2$
<i>Overtake</i> [<i>patch</i> = 1]~number of fragments	4.5	166	0.034
<i>Single behaviour</i> ~number of fragments	3.9	162	0.050

(1 = *patch behaviour* 0 = *overtake behaviour*)

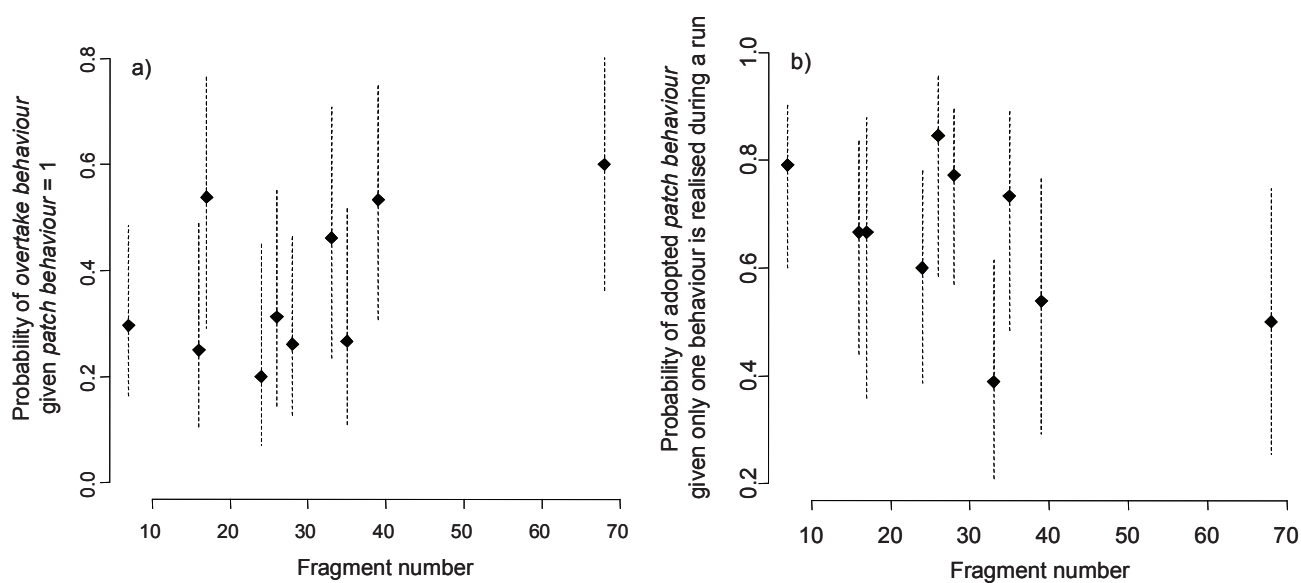


Figure 2: Observed probabilities for the different behaviours and their binomial confidence intervals (95%) for a) *overtake behaviour* given *patch behaviour* has occurred (associated behaviours) and b) *patch behaviour* given only one behaviour is observed during the run (exclusive behaviour).

Discussion

This work provides some interesting insights into the relationship between landscape context and movement behaviour of migrating toadlets. First, whatever the number of forest fragments the most frequent option chosen by a toadlet is the single *patch behaviour*, which corresponds to a risk-averse choice. Juveniles are thus clearly sensitive to favourable micro-conditions (shelters, humidity) provided by the patch. Our results thus match findings of other authors (Rothermel 2004). Nonetheless, the results also show that toadlets differ in movement behaviour according to the landscape from which their parents originate. Indeed, the most fragmented is the terrestrial habitat, the most frequent is the expression of associate behaviour. This result suggests that the motivation to move and explore *i.e.* their propensity to shift from one behaviour to another in a limited time increases with habitat fragmentation. Besides, when only one behaviour is expressed, the frequency of *overtake behaviour* is

positively related to fragmentation level. Toadlets from the more fragmented landscapes are thus less prone to stop moving for consuming suitable conditions than toadlets from the less fragmented landscapes. On the contrary, juveniles from more continuous landscapes were more inclined to opt for a risk-averse behaviour and were more frequently satisfied of this choice since they rarely associated the entrance of a patch with a riskier behaviour. Although we expected an impact on movement behaviour of both forest amount and number of forest fragments, and their interaction (corresponding to the mean size of forest fragments), only the number of fragments was identified as a significant explanatory variable. Rather than the average habitat area, and/or its variability, this is the intensity of the cutting out that appears as a determinant of population movement characteristics: a small but single forest fragment would not elicit change in population behaviours whereas a large forest amount but strongly divided would promote the motivation to move. Thus, movement behaviour are not modified in response to habitat availability but to fragmentation *per se*. Higher motivation to explore may be adaptive in fragmented landscapes in which the resource (forested habitat) is fractioned *i.e.* if multiple fragments increase the average length of migrations, provide exploration possibilities of multiple migration azimuths from pond and/or promote supplementation process (Dunning *et al.* 1992) to obtain a sufficient amount of resources are available. Modelling could help us to verify that a strong motivation to explore is able to enhance fitness outcomes in landscapes with highly fragmented habitat.

Although our data are not sufficiently numerous to investigate sequence organisation, we assume that choice order (first entering the patch or first overtaking it) supports information about motivation dominance (to move or to consume suitable conditions) that could be analysed by other experiments. Moreover, additional experimental designs should be realised to fully investigate variation of movement characteristics according to landscape fragmentation. Speed, ability to cope with ground heterogeneity or willingness to cross

different boundary types could be indicators of evolutionary responses to the degree of fragmentation of landscapes (Merckx *et al.* 2003, Stevens *et al.* 2004, 2006). Movement-related morphology could also be investigated since it has been shown to be impacted by landscape structure (Taylor and Merriam 1995 for flying species) or in response of invasion context (Phillips *et al.* 2006 for amphibians species). We strongly suppose that changes of behaviour particularly the ones promoting movement potential (distance covered without any stop), propensity to move or to explore, or boundary crossing must be associated with morphological and/or physiological modifications allowing to better resist to exigent movement conditions.

The behaviour and space-use of organisms are function of both the structure of landscape in which they move and the individual perception (functional grain, Baguette and Van Dyck 2007) which itself could be controlled by the landscape structure of origin. Landscape dynamics as evolutionary force could also select for phenotypic plasticity resulting in responses of phenotypes to variance of habitat through reaction norms (Merckx and Van Dyck 2006). However, rearing tadpoles in common garden excludes this hypothesis in our study. The observed relationships between behaviour and habitat fragmentation are thus expected to emerge from across-generation transmitted traits acquired in response to fragmentation. According to Danchin and Wagner (2010), different sources of variation, regrouped under the term of inclusive heritability, are likely to be responsible for across-generation transmitted traits in this study case: genetic variance but also epigenetic variance and parental effect. According to these different types of transmitted variance, the number of generations or time required to see emerging such a behavioural change in response to landscape fragmentation could be variable. It would be also very interesting to understand the behavioural mechanisms underlying the transmission of the information about landscape

structure. Simple mechanisms such as modulating the motivation to move and to explore could be a valuable candidate.

The surprising results of this work have important implications for our understanding of population response to landscape degradation through adaptations of movement behaviour. Although this study remains a preliminary analysis of this thrilling questioning that needs further investigations, it opens new insight in our perception of movement adaptation: it could exist mechanisms of trans-generational transmission of information even in species with long generation time. Nonetheless, a risk-prone behaviour should present costs such as higher predation pressure during movement, exhaustion of energy reserves, or faster desiccation that have to be compensated for maintaining population viability. Further investigations are necessary for fully understanding the balance between costs and benefits and the resulting trade-offs that should determine the long-term outcomes of this evolutionary pathway (Hanski *et al.* 2006).

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Perspectives

Synthèse des résultats :

- La composition et l'organisation spatiale du paysage jouent toutes les deux des rôles importants dans la persistance à long terme des populations :
 - la composition et la configuration de la matrice expliquent une part importante de la distribution des populations (chapitre 1)
 - la quantité et la fragmentation de l'habitat terrestre affectent l'état physiologique des individus (chapitre 2)
 -
- Des adaptations comportementales se mettent en place en réponse à la fragmentation du paysage :
 - adaptations au paysage local favorisant les mécanismes d'orientation spatiale qui maximisent la survie lors de la migration des juvéniles (chapitre 4)
 - adaptations à la fragmentation de façon plus générale : modifications des caractéristiques du mouvement pour rendre la migration efficace (chapitre 5)
- L'agriculture intensive (grandes cultures) est un type d'occupation du sol défavorable :
 - sa résistance aux mouvements est élevée comme l'a montré la modélisation (chapitre 1)
 - un paysage d'agriculture intensive induit un stress chez les adultes (chapitre 3)
 - ce type d'usage du sol peut constituer un piège pour les juvéniles chez lesquels l'étroitesse de la fenêtre de perception ne permet pas une évaluation globale du paysage ; guidés par des stimulus proximaux d'humidité (condition requise pour que les juvéniles débutent leur migration), ils peuvent subir un stress hydrique violent lorsque les paramètres hydrométriques deviennent subitement hostiles dans un champ de terre nue suite à un changement de conditions météorologiques (chapitre 3).

Bien que centré autour d'une seule question, ce travail décline plusieurs axes de recherche qui se complètent et reflètent chacun une des facettes du problème très complexe de l'impact de la perte d'habitat et de la fragmentation du paysage. En ce sens, les résultats obtenus ouvrent pour moi plusieurs perspectives. La plus évidente est de parvenir à intégrer de façon contrôlée

le fonctionnement en réseau de populations (métapopulation ou *patchy populations*) dans les modèles coût-distance. La prochaine étape est en effet d'approfondir notre compréhension des mouvements et de leurs conséquences sur les populations. Elle peut s'appuyer sur l'élaboration de modèles cette fois-ci individu-centrés, intégrant des facteurs démographiques et sélectifs. Deuxièmement, l'utilisation du dosage de corticostérone comme élément intégrateur de l'état interne nécessite tout d'abord que le lien entre stress et fitness, très complexe, soit plus précisément compris. Son usage pour répondre à diverses questions de connectivité fournit néanmoins d'ores et déjà des applications très prometteuses. Enfin, les résultats obtenus, qui mettent en évidence une canalisation des caractéristiques comportementales du mouvement en réponse à la perte d'habitat et à la fragmentation, n'est que l'ébauche d'une pleine compréhension des réponses évolutives à la dégradation du paysage. Ces résultats ouvrent grand la porte à de futurs travaux sur les mécanismes de transmission de l'information impliqués ainsi que la flexibilité du système en réponse à des variations rapides.

1. Ce que peut encore nous apprendre la modélisation.

1.1 Intégration du fonctionnement en réseau de populations (*patchy populations*)

La partie 1 de ce travail illustre très clairement l'intérêt de l'utilisation de l'outil coût-distance pour mettre en évidence l'importance de la connectivité à l'échelle d'un cycle de vie imposant un processus de complémentation, et donc des migrations saisonnières. Bien qu'embarquant de manière sous-jacente une partie des mécanismes propres au fonctionnement en réseau de populations (une aire de migration plus vaste confère plus de chance à la population focale d'être intégrée dans un réseau de populations locales fonctionnant en réseau), le protocole d'échantillonnage ne permet pas d'étudier directement l'impact du fonctionnement en réseau sur la distribution des populations de crapauds communs. Or la persistance de ces populations

dépend à la fois de la possibilité pour les individus de compléter un habitat par un autre mais aussi de la possibilité d'effets de rescousse et d'échanges génétiques, possibles dans les populations fonctionnant en réseau (Pope *et al.* 2000). Si la prise en compte de la complémentation, niveau de base auquel la connectivité intervient, est indispensable et un préalable à l'intégration d'autres niveaux dans la modélisation (Pope *et al.* 2000), incorporer l'effet du fonctionnement en réseau est un enjeu extrêmement important pour utiliser cet outil en conservation (Compton *et al.* 2007). Il faut alors également être dans la capacité de séparer les effets dus à la connectivité entre habitats (mettant en jeu les processus de complémentation) de ceux dus à la connectivité entre populations assurant le fonctionnement en réseau. Recueillir des jeux de données supplémentaires dont le design est conçu pour mettre en évidence les rôles respectifs de ces deux niveaux de connectivité emboîtés (travailler notamment sur l'intégralité des points d'eau dans une zone donnée pour prendre en compte l'ensemble des échanges possibles et acquérir des données relatives à la génétique des individus se reproduisant dans ces points d'eau) (Safner *et al.* sous presse) se révèle aujourd'hui nécessaire pour pleinement répondre à cette problématique de modélisation de la connectivité par l'outil coût-distance.

1.2 Développer une modélisation individu-centrée

Les modèles spatialement explicites basés sur des relations coût-distance ont à la fois un fort potentiel pour comprendre l'influence de la connectivité fonctionnelle et un intérêt appliqué direct, en particulier pour les espèces à valence écologique étroite (ayant un habitat privilégié) car les risques d'erreurs sur les coefficients de résistance sont minorés par les forts contrastes de coûts entre les différents habitats de la mosaïque. Néanmoins, ils sont insuffisants pour les espèces à forte valence écologique (utilisant potentiellement de nombreux types d'habitats) ou pour étudier les trajectoires des individus et leurs conséquences en fonction des choix de ces derniers face au paysage. Le développement de modèles individu-centrés, intégrant des

paramètres individuels (variation de l'état interne et des capacités de mouvement et de navigation, survie) en plus de la localisation et des facteurs sélectifs permettant de mesurer des coûts et des bénéfices en terme de fitness en réponse aux différentes trajectoires, devient alors nécessaire pour affiner notre compréhension des mécanismes de déplacement, de leur conséquence sur la dynamique des populations mais aussi de leur évolution. Néanmoins, cette étape nécessite un important travail de mise au point tant la modélisation individu-centré requiert un nombre important de paramètres correctement informés pour remplir efficacement son rôle. Ainsi, cette perspective nécessite l'acquisition de nouvelles données au travers de protocoles expérimentaux précis et exigeants et/ou d'outils de calibration puissants permettant d'estimer une gamme de valeurs traduisant correctement la réalité des caractéristiques de déplacement pour chacun des différents paramètres utilisés.

Ces deux volets demanderont dans tous les cas un important travail de mise au point tant du point de vue de la modélisation informatique qu'en terme de questionnements biologiques sous-tendant les règles imposées aux modèles.

2. Déclinaisons de l'usage de l'hormone de stress

Il subsiste le besoin de comprendre sans équivoque les interactions entre le taux détectable de corticostérone et la fitness (Bonier *et al.* 2009). Néanmoins, loin de remettre en cause l'existence d'un lien étroit entre corticostérone et fitness, les revues traitant de ce sujet illustrent plutôt la nécessité d'envisager différemment cette relation notamment en s'intéressant à l'existence de relations non linéaires (Bonier *et al.* 2009; Bush et Hayward 2009), car la variation du taux de corticostérone n'est elle-même pas linéaire en fonction du temps. Or cette dimension de la durée d'exposition au stress joue un rôle crucial dans la relation entre niveau d'hormones de stress et fitness. Il s'agit également de replacer au cœur de nos questionnements l'idée que la fonction première des hormones de stress et de faire face à

un défi environnemental, c'est à dire une situation inhabituellement difficile. Leur rôle est donc initialement prévu pour avoir une durée d'action limitée et doit permettre à l'animal de gérer cette situation en apportant des réponses comportementales et/ou physiologiques appropriées (qui peuvent être l'évitement de cet environnement). Un gain de fitness est donc attendu par rapport à une situation dans laquelle l'animal aurait subi le défi environnemental sans être dans la capacité d'apporter une réponse. Il est néanmoins vraisemblable que toute réponse a un coût qui pourrait être mis en évidence en comparant sur le long terme la survie et le succès reproducteur d'animaux selon qu'ils ont été exposés ou non à un (ou des) défi(s) environnemental(aux). De plus, si l'augmentation de la sécrétion d'hormones de stress ne permet pas de faire face au défi environnemental, on peut s'attendre à un effondrement du système de régulation. Avec la persistance du stress dans l'environnement, le taux de corticostérone peut s'effondrer et le système devenir insensible à un nouveau stimulus (Figure 8).

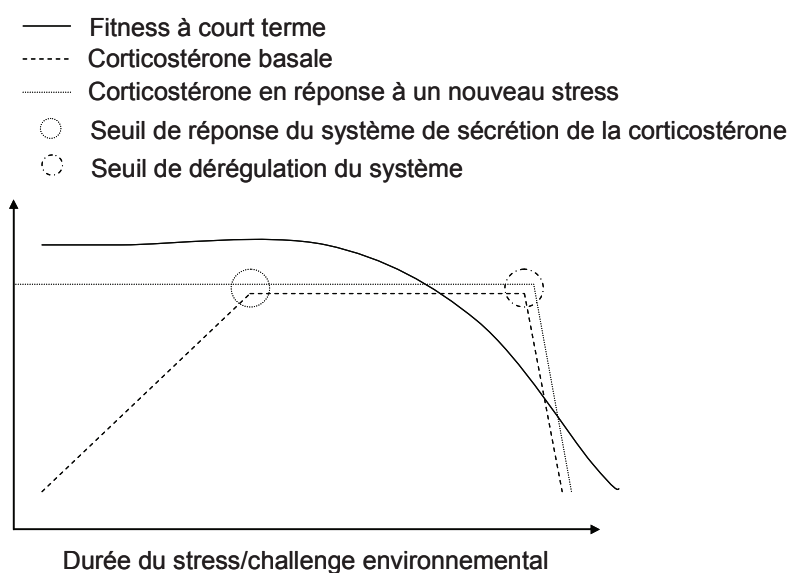


Figure 8 : Relations théoriques liant durée du stress, taux de corticostérone et fitness résultante. Aux points d'inflexion de la courbe des taux de corticostérone se situent des seuils dont le franchissement modifie le système de réponse au stress par sécrétion de

corticostérone. Le premier seuil correspond à la réponse maximale apportée par le système. Le taux de corticostérone n'évolue plus en fonction du temps (plateau). Puis au-delà d'une certaine durée d'exposition au stress, le second seuil est atteint à partir duquel le système s'effondre, l'exposition au stress ayant duré trop longtemps sans feed-back positif du à l'élévation du taux de corticostérone. Le taux de corticostérone chute alors très bas et le restera même si un nouveau stress intervient. La fitness est alors fortement impactée. Intégrer la durée du stress permet de mieux comprendre le lien complexe entre fitness et taux de corticostérone.

Deux expériences à échelles de temps différentes pourraient être mises en place pour approfondir cette relation entre stress et fitness. A échelle temporelle courte, il pourrait être envisagé de stresser artificiellement des femelles et mâles adultes juste avant un évènement de reproduction grâce à des patches délivrant des doses quantifiables de corticostérone (Meylan & Clobert 2005) et de mesurer l'altération du comportement de reproduction comme l'aptitude du mâle à maintenir l'amplexus (Moore *et al.* 2005) ou la qualité du comportement d'oviposition. Cette première expérience permettrait d'évaluer les conséquences du stress sur la fitness au cours des migrations. A échelle temporelle plus longue, il serait particulièrement pertinent de stresser, toujours de façon contrôlée, des femelles en automne avant la phase d'ovulation puis de mesurer l'investissement dans la reproduction (nombre des oeufs, taille, ...) et les performance de la descendance (survie, taille, poids, résistance à un stress des têtards ...) en fonction du taux de stress. Cette seconde expérience nous renseignerait quant à l'impact en terme de fitness d'un stress durant la période pendant laquelle les animaux se préparent à l'hibernation.

Cependant, même si les mécanismes ne sont pas décryptés avec précision, l'augmentation du taux d'hormones de stress reste un indicateur de modification de l'état interne qui se solde par

un coût si l'on se réfère à une situation sans stress. Le développement de méthodes de prélèvement non invasives au cours de ce travail fait du taux de corticostérone un outil fiable pour répondre à diverses questions liées à la perte d'habitat et à la fragmentation du paysage. Cette méthode trouve notamment des applications directes dans des problématiques de rupture de connectivité en permettant une évaluation fine des mesures compensatoires entreprises pour restaurer les échanges. Des protocoles d'évaluation avant, après et à différentes étapes d'un passage à faune pour amphibiens ont notamment été entrepris depuis deux saisons (Réserve Naturelle de l'Étang du Grand Lemps, Isère) et fournissent des résultats prometteurs en évaluant la perception que les animaux ont de l'équipement et en mettant en évidence quelles parties du passage déclenchent un stress. D'autres applications sont à envisager. Notamment l'outil d'évaluation du stress pourrait permettre d'étudier l'impact de l'écrasement routier sur le comportement des individus et le rôle de l'information publique. Les infrastructures routières constituant également un élément de fragmentation important dans le paysage, leurs conséquences, au-delà d'un simple point de vue démographique, sur les mouvements des individus s'avèrent particulièrement intéressantes. En effet, on peut s'attendre à ce que les comportements individuels associés à la migration soient modifiés en réponse à l'écrasement de congénères, la présence de cadavres étant supposée une source d'information publique. Des expériences préliminaires nous ont permis de mettre en évidence qu'un taux de stress plus élevé était associé à la proximité avec un congénère écrasé et que les individus se déplaçaient plus et plus vite sur des routes où le nombre d'écrasement était élevé. Il serait très intéressant de tester si 1) cette élévation de la corticostérone déclenche un comportement de fuite, et 2) cette réponse physiologique et comportementale est retrouvée chez l'ensemble des populations de crapauds ou si elle est dépendante de la présence de routes à fort trafic entre l'habitat terrestre et aquatique (adaptation locale).

3. La fragmentation : force évolutive des caractéristiques de déplacement ?

Ce travail a mis en évidence des spécificités transmissibles dans le comportement de déplacement du crapaud commun en fonction de la structure du paysage. Il permet donc d'illustrer que perte d'habitat et fragmentation constituent des forces évolutives en action dans la nature. En effet, une structuration fortement contrastée de l'habitat induit des préférences spontanées d'orientation chez les juvéniles des populations concernées. De plus, la fragmentation de l'habitat dans lequel les parents évoluent semble modifier le taux de mouvement de la descendance au stade juvénile et sa propension à explorer d'autre part. Ces résultats ne constituent néanmoins qu'une première étape dans la compréhension de l'action de la structure du paysage comme force sélective. D'un point de vue proximal, les variations des comportements de déplacement soulèvent la question de l'identification des canaux de perception, des capacités de navigation et des capacités locomotrices sur lesquels ces variations reposent. En effet, il apparaît important de mettre en évidence les différences physiques et/ou physiologiques responsables de variation dans les comportements de déplacement pour bien comprendre le processus de différenciation des mouvement des animaux selon le paysage d'origine. Un autre point crucial à élucider demeure la nature des mécanismes de transmission de l'information impliqués. Ils peuvent d'ailleurs être variables notamment selon l'histoire des populations, leur isolement, la rapidité des changements du paysage Il va s'agir désormais de déterminer si cette différence de comportement chez des animaux élevés dans des conditions identiques mais issus d'adultes provenant de paysages différents (structuration contrastée de l'habitat autour du point d'eau vs. habitat entourant complètement le point d'eau/ habitat fragmenté vs. habitat continu) peut résulter d'une transmission via un effet maternel (par exemple une femelle stressée dans un habitat fragmenté peut transmettre à ses œufs une information de type hormonal), d'un héritage génétique via l'émergence et la sélection de certains allèles codant pour un type précis de

comportement (canalisation) ou pour une plasticité phénotypique (normes de réaction si les signaux de l'environnement sont fiables), ou d'une transmission via un mécanisme épigénétique. La question du mécanisme de transmission soulève aussi celle, prépondérante, de l'échelle de temps à laquelle les populations doivent répondre à ces changements. Si les modifications du paysage sont très rapides, on attend une certaine labilité des mécanismes de transmission mais le rythme même des changements peut être variable et imprévisible, pouvant provoquer l'apparition d'un décalage entre perception du paysage par les individus et fitness résultante (phénomène de trappe écologique).

Plusieurs plans expérimentaux sont à envisager pour répondre à ces questions. Premièrement, l'analyse des mouvements de juvéniles issus de croisements entre populations provenant de paysage fragmenté et préservé pourrait permettre de tester l'hypothèse d'une sélection des comportements basée sur des mécanismes de transmission de gènes ou d'expression du génome (si on observe une réponse comportementale intermédiaire sous l'hypothèse de co-dominance des traits comportementaux) ainsi que l'hypothèse d'un effet maternel à l'origine de ces différences comportementales (si le comportement des juvéniles est prédit par le paysage d'origine de la mère). Ce type d'expériences a d'ores et déjà initié. Si un effet maternel était révélé, il pourrait être également intéressant de chercher à le mimer par exemple en comparant les mouvements de juvéniles issus de femelles dont les régimes alimentaires diffèrent en quantité. Le test de l'existence d'une plasticité phénotypique à l'origine de différences comportementales lors des mouvements nécessiterait d'établir dans un premier temps quels sont les indices environnementaux utilisés par les organismes pour exprimer tel phénotype particulier. Enfin, pour analyser le temps nécessaire à l'émergence de ces adaptations comportementales dans les populations, les mouvements de juvéniles issus de populations pour lesquelles l'historique de fragmentation diffère, fragmentation très récente

(depuis les années 50), fragmentation d'ancienneté intermédiaire (depuis le début du 20^{ème} siècle) et fragmentation ancienne (depuis le début du 19^{ème} siècle), pourraient être analysés.

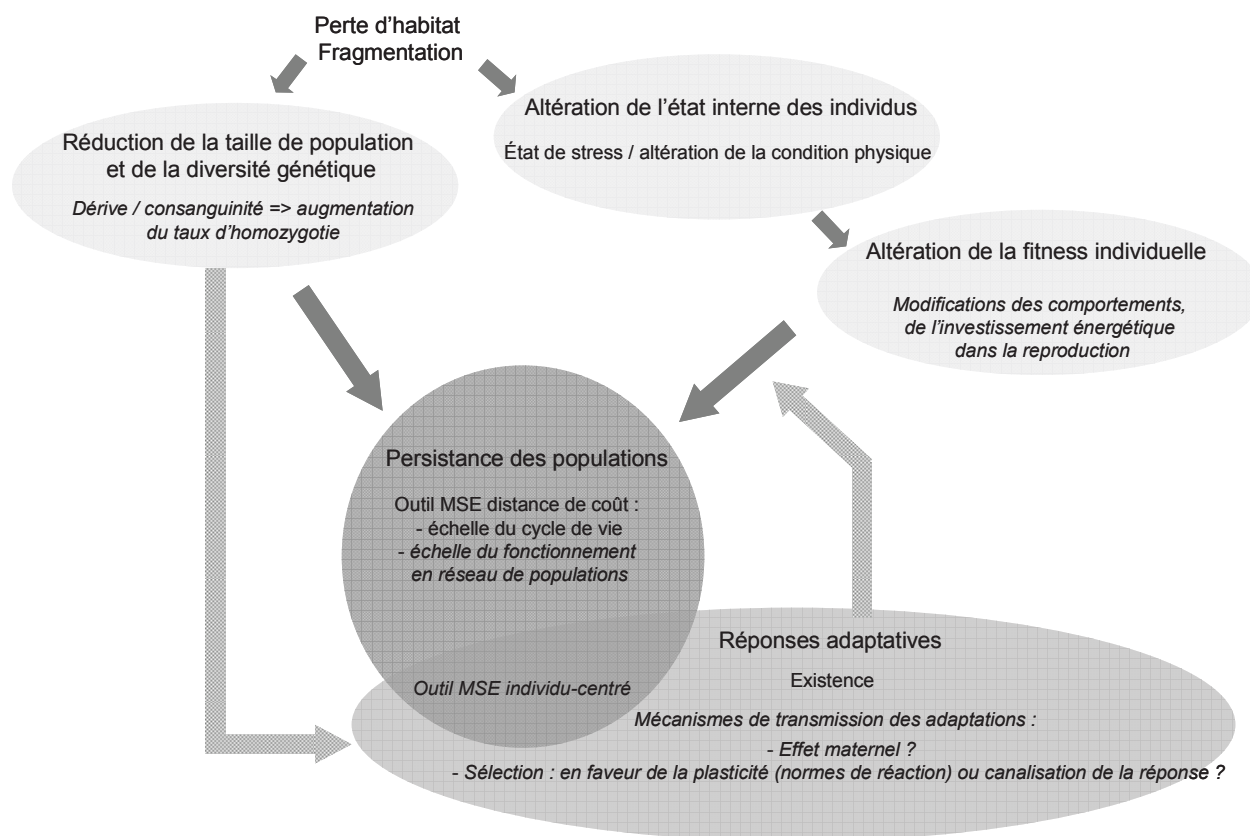


Figure 9 : Résumé de la problématique de l'impact de la perte d'habitat et de la fragmentation du paysage sur les populations et apport du présent travail. Les étapes en italique appartiennent aux perspectives et restent à réaliser. MSE = Modèle Spatialement Explicite.

La perte d'habitat et la fragmentation induisent une réduction de la taille des populations pouvant aller de paire avec une altération de l'état interne des individus et donc de leur fitness. Cette dégradation du paysage va en effet aboutir à une altération de l'état interne des individus se traduisant par un déficit en fitness. Il est alors possible que, dans ce contexte, des mécanismes adaptatifs soient mis en place au sein des populations pour répondre à ce défi que représentent perte d'habitat et fragmentation. Dans les populations de petite taille, la

variabilité génétique va être faible et le taux d'homozygotie fort suite aux processus de dérive génétique et de consanguinité. Néanmoins, les conséquences de cette homozygotie peuvent se traduire par de l'adaptation si les allèles sont sélectionnés par le contexte local. Ce potentiel adaptatif des populations vivant en milieu altéré va dans tous les cas jouer un rôle sur la possibilités de mise en place d'adaptations en réponse à la dégradation du paysage.

Ce dernier volet des perspectives se révèle particulièrement riche car à l'interface de l'écologie du paysage, l'écologie comportementale et écologie évolutive. De plus, si nous ne cherchons pas à décrypter ces mécanismes adaptatifs en réponse à la fragmentation, nous risquons de préconiser des solutions de conservation peu ajustées à la réalité des processus en jeu voire inefficients ou même néfastes.

Ainsi, les conséquences de la perte d'habitat et de la fragmentation du paysage constituent une question complexe et multi-facettes. Néanmoins, seule l'intégration de l'ensemble des résultats obtenus par différentes approches semble capable de fournir une image proche du fonctionnement réel des processus en jeu, image composite mais indispensable pour élaborer des mesures de conservation adéquates.

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