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DYNAMIQUE ET GENETIQUE DES POPULATIONS DE CISTUDE D'EUROPE *EMYS ORBICULARIS*

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La science pour nous c'est bien, mais POUR TOUS, c'est encore mieux.

À un moment donné de leur vie, de nombreuses espèces doivent quitter le confort du cocon familial et par conséquent, se déplacer, pour aller fonder une famille ailleurs : c'est ce qu'on appelle la dispersion.

Chez de nombreuses espèces, de vertébrés notamment, c'est lorsque le duvet de moustache commence à pousser (dans le cas des mammifères !) qu'il est essentiel de disperser. Bien évidemment, la dispersion ne touche pas uniquement les mâles, mais concerne aussi les femelles, comme c'est le cas chez les oiseaux. Ce mécanisme essentiel évite aux individus de rentrer en conflit pour la nourriture et les partenaires reproducteurs. D'une manière générale, les individus d'une même espèce qui exploitent les mêmes ressources sont en compétition. Imaginons un monde où la dispersion n'existerait pas, les frères et sœurs d'une même portée se disputeraient les mêmes ressources. Et quoi de plus terrible pour des parents que de voir leurs deux enfants se crêper le chignon pour le dernier dessert qui reste sur la table ? Mieux vaut piquer le dessert du voisin que celui de la frangine ! De cette manière, éviter la compétition, c'est avoir de meilleures chances d'être dans de bonnes conditions pour survivre et se reproduire. Au final, les individus qui dispersent ont des petits en bonne santé qui ont de bonnes capacités à se reproduire et donc à perpétuer ce comportement. C'est ainsi que le comportement de dispersion peut se répandre dans une population. De la même manière, à travers la dispersion, la reproduction entre apparentés est minimisée. La reproduction entre individus génétiquement proches mène à une augmentation de la consanguinité se traduisant par l'accumulation de tares génétiques provoquant une diminution de la survie des individus.

Cependant, il n'est pas toujours évident de faire son baluchon pour quitter sa famille et construire sa propre vie, certains préféreront rester près de papa-maman alors que d'autre plus téméraires partiront. Il faut être conscient que la dispersion impose des coûts non négligeables. Quitter son domicile, c'est augmenter le risque de se faire manger par des prédateurs, de périr lors du déplacement (traversée de route par exemple), de ne pas trouver de coin où se loger et se nourrir, de ne pas se faire accepter par ses nouveaux congénères et de rester sans partenaire de reproduction. Mais, du point de vue des gènes et à l'échelle de la population, il y a bien souvent plus à gagner à voler de ses propres ailes !

La dispersion est un processus clef pour la survie des populations car elle permet leur connectivité. La dispersion est donc d'autant plus importante à maintenir lorsque les populations ont des effectifs faibles et fragmentés. C'est pourquoi il est essentiel de l'étudier afin d'établir des plans de gestions et de conservation les plus adaptés. Cette thèse s'inscrit dans cette démarche, mieux comprendre le déplacement des cistudes pour mieux les protéger ! En avant et Kawabunga !

Cha Ché POUR VOUS OT' mes ptits quinquins, mes ptits pouchins.

Si tô un Tio Biloute et que té comprind rin à chet' dispersion, bha jva t'expliquaï! Et pis t'inquiète po, y n'a d'autres qui sont po Ch'ti et qui comprenne'teu rin quin même !

Eul dispersion ché kôi ? Imaginons kt'es a't barak' avec eut famille, tes parents et tes frères et chœurs. Ché bien eud viv' chez ti comme cha, parce que t'as pô bsoin eu'd ker eun aut' barak, té dépinse rin, pis ya person' qui va eu't piquer tin chez tôi ! Eul problème, chi té part pô, ché que té va finir par faire des galipet' avec eu't chœurs, et cha ché po bin. Bha ouais, chinon t vas avoir des mongolito din 't famille ! Par contre, chi té part coquer ailleurs, bha là tes tio biloute y chront tout bieaux, y loucheront même pô ! Té vois, on comprind vite pourquoi ché importint de dispercher pour ti et seulement pour ti, tu tin fou ben des au't.

Bha chez les tio biloute cistudes, ché pareil ! Té peux avoir un avantach' à dispercher. Et pourquoi k'ché importin d'a'regarder cha té va mdire ? Bha ché bin din min métier de comprindre comment k'sa fonchionne chi on veut faire quekosse pour ché ptite tortues. Par eximple, si té recueilles at barak un cat, t va pas lui donner à bec'ter l'herbe, sinon i va claquer s'giff. T' lui donne po d'herbe parce que té sais, t'as appris, que cha maq' del viande. Bha ché pareil pour mi, chi jveux prindre des mesures efficace eud conservation, faut d'abord comprindre comment fonchionne ché populations et commint les individus y bougen't entre elles. Ché Comprindre pour mieux protéger.

Ta compri ? Té vois, té po un Boubourse ! Chinon, ché po grave, té viendra a'm barak, avec deux-trois picon bière, cha ira bin mieux pour comprindre !

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Résumé

La dispersion, caractérisée par les mouvements d'individus dans l'espace conduisant à la production d'un flux de gènes, permet la connectivité des populations. L'étude de la dispersion est devenue d'une importance primordiale pour prédire les conséquences des changements globaux sur la structure et la dynamique des populations. Les espèces à dispersion limitées, comme les chéloniens, sont particulièrement menacées par ces phénomènes. Cette étude se propose d'analyser la dispersion chez la Cistude d'Europe (*Emys orbicularis*), en régression en Europe, dans un contexte de fragmentation d'habitats et de déterminer les causes de ce comportement via l'analyse de la dynamique et de la génétique des populations. Nos résultats montrent, d'une part, que les temps de générations lents chez les cistudes (environ 12 ans) peuvent ralentir les phénomènes d'érosion génétique par dérive. Cette érosion lente est accentuée en présence de grandes populations même en milieu très fragmenté. D'autre part, la sélection aurait favorisée la philopatrie chez les femelles cistudes dans les milieux peu riches en site de ponte et de faible densité d'individus car elles ont un avantage à la territorialité. A l'inverse, le coût à la dispersion diminuerait pour les mâles car ce comportement éviterait la consanguinité. Les cistudes semblent donc très sensibles à la compétition intra-spécifique. En effet, la relaxation de la densité-dépendance des adultes permet un recrutement important de juvéniles. Cette dynamique favoriserait une récupération rapide des effectifs après une importante perturbation, ce qui est surprenant pour une espèce longévive dont les temps de résilience sont supposés lents.

Mots clefs

Conservation, capture-marque-recapture, densité-dépendance, dispersion, dynamique, *Emys orbicularis*, fragmentation, génétique, metapopulation.

Abstract

Dispersal, characterized by the movements of individuals in space leading to gene flows, allows populations to connect. The study of dispersal has become of essential importance to predict the consequences of global changes on the population structures and dynamics. Species with limited dispersal, such as chelonians, are particularly threatened by these phenomena. Our study aimed at analyzing the dispersal of the European pond turtle (*Emys orbicularis*), in decline in Europe, in a habitats fragmentation context and determining the causes of this behavior through analysis of population dynamics and genetics. Our results show, firstly, that the slow generation time in *Emys orbicularis* (about 12 years) may slow the genetic erosion by drift. This slow erosion is accentuated with large populations such as Kerkini populations, even with a strong fragmentation. On the other hand, selection would have favored philopatry in females in habitats with few nesting site and deers, because they have the advantage of territoriality. In contrast, the cost of dispersal decreases for males because this behavior allows inbreeding avoidance. The European pond turtles seem very sensitive to intra-specific competition. Indeed, the relaxation of adult density-dependence allows for a significant recruitment of juveniles. This dynamic promotes an unexpected rapid response of the population after a major disturbance, because chelonians are long-lived animals with a late age of first reproduction and very high generation time, therefore, the time of resilience to perturbations is also expected to be high.

Keywords

capture-mark-recapture, conservation, density-dependence, dispersal, dynamics, *Emys orbicularis*, fragmentation, genetics, metapopulation.

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Introduction

Introduction générale

La dispersion est définie par les mouvements d'individus (ou de propagules) dans l'espace qui conduisent à la production d'un flux de gènes (Ronce, 2007 ; Clobert, 2001). La dispersion permet donc la connexion des populations vivant dans des habitats favorables, encore appelés patchs, et séparées par une matrice qui leur est défavorable. Ce comportement est donc un ingrédient essentiel de la dynamique des métapopulations. La métapopulation se définit alors comme une population structurée spatialement en unités distinctes (sous-populations) connectées par des mouvements de dispersion (Levins, 1969 ; Hanski and Gilpin, 1997). Sous l'influence de la stochasticité environnementale et démographique, les sous-populations subissent tour à tour des extinctions locales et des recolonisations (Levins, 1969). Ainsi, les métapopulations ne peuvent que persister du fait de l'équilibre entre extinctions locales et recolonisations (Hanski, 1998), à condition que les échanges d'individus disperseurs entre sous-populations soient suffisamment fréquents.

L'importance de l'étude de la dispersion a été démontrée dès la fin des années 1960 par Charles Krebs et al. (1969). Ces auteurs ont isolé des populations de campagnols par une barrière les séparant de populations naturelles. Dans un premier temps, les effectifs des populations captives se sont accrues de manière très importantes jusqu'à des valeurs non rencontrées dans les populations naturelles adjacentes. Dans un second temps, ces effectifs se sont écroulés jusqu'à l'extinction des populations captives. Krebs et al. ont ainsi montré que la dynamique d'une population de rongeurs pouvait être gravement impactée lorsque les individus ne peuvent pas se déplacer (l'effet Krebs ou l'effet barrière). La dispersion a alors commencé à susciter un intérêt grandissant, qui a conduit à la caractérisation de deux types de dispersion liés à l'âge des individus : la dispersion natale et la dispersion de reproduction

(Greenwood and Harvey, 1982). Chez certaines espèces, comme les oiseaux coloniaux par exemple, les individus prospectent parmi différentes populations (i.e. colonies) avant leur première reproduction, choisissant ensuite de se reproduire soit dans une nouvelle population (i.e. dispersion natale), soit de retourner nicher dans leur population natale (i.e. philopatrie natale). Chez d'autres espèces, c'est au stade adulte que les individus dispersent, le plus souvent après un échec de reproduction (après une année où les ressources alimentaires ont été insuffisantes pour nourrir les jeunes, ou après la prédation des œufs, par exemple) et vont ainsi rejoindre une nouvelle population (i.e. dispersion de reproduction).

L'étude de la dispersion s'est généralisée et l'ubiquité de ce comportement parmi les organismes laisse supposer que ce mécanisme apporte d'importants avantages sélectifs (Greenwood, 1980 ; Clobert, 2001). Aujourd'hui, les facteurs qui favorisent l'évolution de la dispersion peuvent être groupés en 3 catégories de facteurs : écologiques, génétiques et sociaux (Clobert et al., 2004). Le facteur habitat spécifique (i.e. écologique) regroupe l'ensemble des paramètres biotiques et abiotiques pouvant motiver la dispersion comme la disponibilité de nourriture et/ou la présence de prédateurs, de parasites ou encore de compétiteurs inter-spécifiques. Ainsi, la capacité de disperser peut s'avérer essentielle aux individus pour persister dans un environnement changeant. Lorsque les conditions climatiques sont variables, ou lorsque l'habitat est particulièrement fragmenté, les espèces capables de disperser ont l'avantage d'avoir une probabilité plus forte de retrouver un habitat de meilleure qualité (Clobert et al., 2008). Le second facteur favorisant la dispersion est lié au choix du partenaire et a donc des causes génétiques. En effet, la dispersion permet dans un système trop fermé d'éviter la consanguinité. Le flux génique généré par la dispersion permet d'augmenter la diversité génétique à l'intérieur d'une population (Greenwood and Harvey, 1982). Le dernier facteur, social, regroupe tous types d'interactions intra-spécifiques (coopération, compétition intra-spécifique asymétrique,…) qui peuvent être considérées comme des sources de la dispersion densité-dépendante. En effet, l'évitement de la compétition intra-spécifique est avancé comme l'un des principaux bénéfices de la dispersion. Ainsi, les individus qui dispersent d'un site fortement peuplé à un site où la densité est plus faible sont susceptibles de trouver un lieu avec des ressources plus abondantes et peu de compétiteurs, ce qui leur confère un avantage certain en termes d'aptitude phénotypique (Clobert et al., 2008). Bien sûr ces catégories ne sont pas cloisonnées et il est important de considérer que des interactions existent entre ces 3 facteurs. De même, la capacité à disperser ne peut être considérée comme un trait fixé, c'est-à-dire que tout individu de la métapopulation aurait la même probabilité de disperser avec succès. La dispersion varie avec certains aspects de la condition individuelle, comme l'âge (vu précédemment) ou le sexe.

La dispersion biaisée pour le sexe, c'est-à-dire que ce sont soit les mâles ou soit les femelles qui dispersent dans de grandes proportions, est observée chez un grand nombre d'espèce (Clobert et al., 2004). Parce qu'ils ne sont pas sujets aux mêmes pressions sélectives (notamment par sélection sexuelle), mâles et femelles se différencient, en plus de leur genre, à de nombreux égards : morphologie, physiologie et comportement (Gross, 1996). En particulier, les femelles ont une production de gamètes limitée et par conséquent vont maximiser la qualité des jeunes à travers le nourrissage et/ou le choix du partenaire. À l'inverse, les mâles sont plus limités par le nombre de partenaires disponibles (Andersson, 1994). Le type de régime d'appariement (monogamie, polyandrie,…) pourra restreindre la façon dont la sélection sexuelle agira sur chaque sexe et, par conséquence, influencera sur l'investissement respectif dans l'acquisition des ressources et des partenaires. Sur la base de ces hypothèses, Greenwood proposa en 1980 que le biais de dispersion selon le sexe observé chez les oiseaux et les mammifères soit lié à la territorialité, fondée sur les ressources, et uniquement présente pour l'un des deux sexes. Autrement dit, pour Greenwood, le sexe territorial serait le sexe philopatrique. Ainsi, les jeunes du même sexe que le parent philopatrique qui ne dispersent pas ont une probabilité importante d'hériter du territoire de ce parent. En ce sens, les avantages du sexe philopatrique sont importants : connaissance de l'habitat et évitement de la compétition pour un territoire. La progéniture de sexe opposé aurait également des avantages dans de telles circonstances, mais la probabilité de s'accoupler avec un proche parent serait importante et par conséquent ces jeunes devraient payer le coût de la dépression de consanguinité (Ebert et al., 2002). Ces jeunes auraient donc un avantage à disperser. Sujet à controverse, l'évitement de la consanguinité serait de nos jours le principal moteur permettant d'expliquer la dispersion biaisée par le sexe. Il faut toutefois préciser qu'une large majorité des espèces ont une proportion de disperseurs appartenant aux deux sexes suggérant que d'autres facteurs, comme l'évitement de la compétition entre apparentés, entrent aussi en jeu (Perrin and Goudet, 2001).

L'étude de l'évolution de la dispersion se dirige donc de plus en plus sur l'action combinée de plusieurs facteurs pouvant expliquer ce comportement (Baguette and Van Dyck 2007 ; Baguette et al., 2013). Perrin and Mazalov (1999 ; 2000) ont considéré par exemple que l'évolution de la dispersion biaisée par le sexe est dûe aux effets joints de l'évitement de dépression de consanguinité et de la compétition locale pour la ressource et le partenaire reproducteur. De nombreuses études empiriques analysées par Clobert et al. (2001) montrent que la décision de quitter sa population locale dépend de la qualité de l'habitat en termes de ressources alimentaires, de la quantité de refuges, des prédateurs, des parasites et des compétiteurs intra et inter-spécifiques. La plupart des espèces étudiées semblent avoir une réponse de dispersion état-dépendante aux changements de la qualité de l'habitat. Ainsi, la dispersion dépendrait des décisions d'individus dans un espace donné, à une période donnée.

Cette hétérogénéité peut conduire à la différenciation morphologique entre les individus disperseurs et philopatriques. Par exemple, chez le lézard à flancs maculés (*Uta stansburiana*), les individus ont des couleurs de gorge qui diffèrent selon leur schéma de dispersion (Sinervo and Clobert, 2003). Cependant, chez la majorité des espèces, les différences entre individus disperseurs et non disperseurs sont bien plus subtiles et sont d’ordre physiologique ou comportemental. Chez les campagnols par exemple, les individus asociaux sont prédominants parmi les disperseurs (Ims, 1990). Cette disparité nous montre que ces deux stratégies, disperseurs ou philopatriques, peuvent être sélectionnés. Par conséquent, la dispersion implique des coûts et peut être contre-sélectionnée. L’individu disperseur s’expose, lors de la phase de transition entre sites, à la prédation (Ims and Andreassen, 2000) et aux autres dangers liés au déplacement (Ronce, 2007) : naturels (conditions climatiques extrêmes…) ou artificiels (écrasement par les véhicules, noyade dans des filets…) augmentant considérablement la mortalité. De plus, le milieu colonisé peut se révéler sans ressources ou sans partenaires reproducteurs compatibles risquant de rompre des complexes de gènes coadaptés (Wiener and Feldman, 1993). Par ailleurs, dans certains cas, la philopatrie permet d’augmenter les chances d'hériter d'un territoire parental de haute qualité favorisant l’accès au partenaire reproducteur par exemple, comme c’est le cas chez les espèces se reproduisant en lek (Petrie et al., 1999). En conclusion, l’évolution de la dispersion, comme tout comportement en écologie, est dépendante de la balance entre coûts et bénéfices permettant dans certains cas la sélection de ce comportement et d’en autres, la philopatrie (Fig 1).

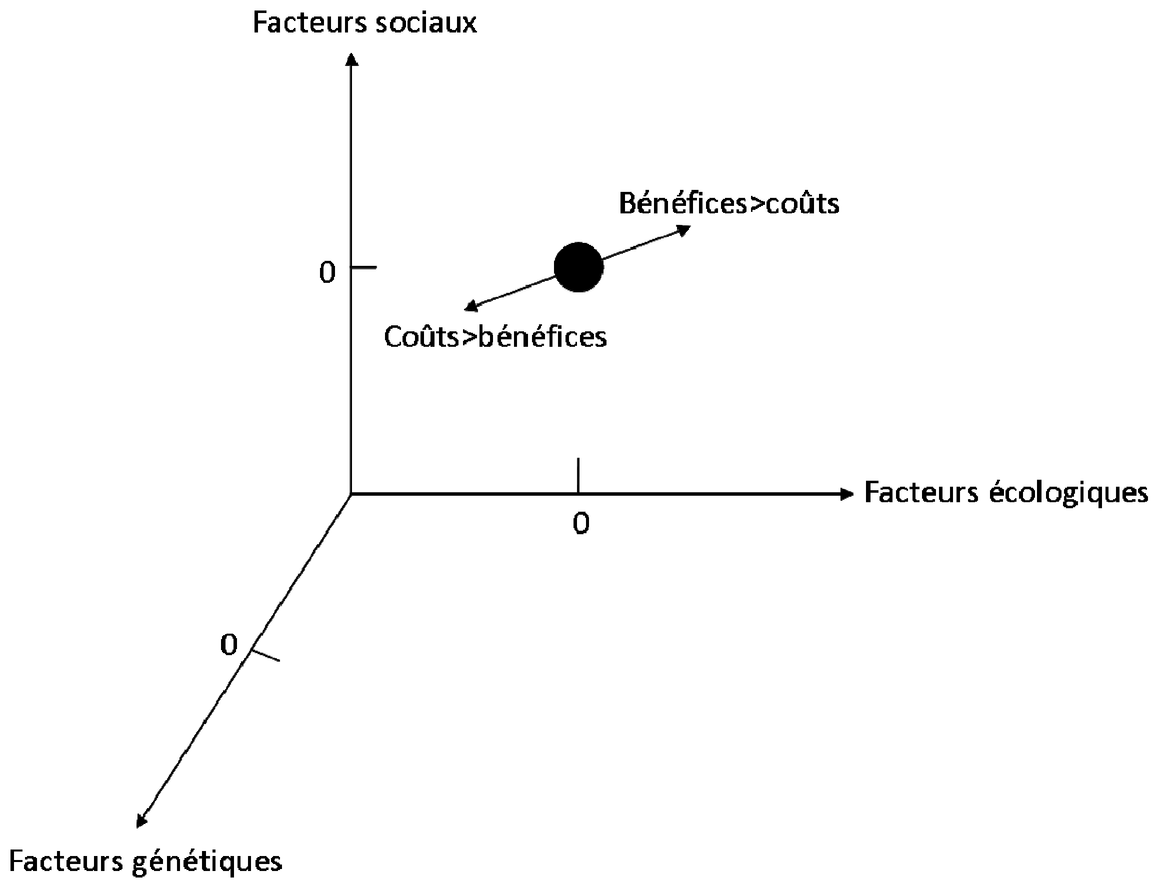

Figure 1. Multiples causes qui agissent sur l'évolution de la dispersion. Chaque cause est associée à des coûts et des bénéfices. Le zéro sur les axes symbolise le point où les coûts et les bénéfices sont contrebalancés et le point noir l'équilibre global entre coûts et bénéfices. L'évolution de la dispersion peut résulter de toutes ces pressions de sélection agissant ensemble, selon la manière dont la dispersion peut être considérée comme un trait bien défini ou au contraire, révéler de comportements distincts sous le contrôle de différents mécanismes. Modifié à partir de Hanski and Gaggiotti (2004).

Depuis le milieu des années 1990, l'étude de la dispersion a subi un nouvel engouement. En effet, comprendre pourquoi et comment les animaux et les plantes se déplacent est devenu d'une importance primordiale pour prédire les conséquences des changements globaux et de la fragmentation artificielle des habitats sur la structure et la dynamique des populations (Bowler and Benton, 2005). La fragmentation d'origine anthropique constitue une des plus grandes menaces actuelles pour la biodiversité. Elle a pour effet de réduire la capacité des organismes à disperser entre les différents patchs. Par conséquent, elle conduit à isoler des populations, et donc à augmenter le risque d'extinction par dépression consanguine ou par stochasticité démographique (Harrison, 1991). Les espèces ayant des capacités de dispersion limitées sont d'autant plus menacées car incapables de réagir à une telle dégradation de leur environnement. Une barrière géographique, telle qu'une matrice d'habitat infranchissable, peut constituer un réel obstacle à la reproduction et à la survie des individus. Dès lors, on comprend mieux l'intérêt croissant pour cette problématique en biologie de la conservation. En effet, l'enjeu de cette discipline dépend de la compréhension du fonctionnement et de la structure des populations qui permettent de tirer des conclusions éclairées sur l'échelle à laquelle protéger les espèces ainsi que les écosystèmes dont elles dépendent.

Les zones humides comptent parmi les écosystèmes les plus fragmentés et les plus dégradés à l'échelle mondiale (World Commission on Dams, 2000), ils sont donc fortement menacés. Depuis 2008, un groupe d'experts sur les écosystèmes au sein de l'UICN s'attache à développer les fondements scientifiques pour une Liste Rouge des Écosystèmes sur laquelle seraient inscrites les zones humides (Keith et al., 2013). L'objectif est de coordonner un processus de consultation menant à l'élaboration d'une méthodologie mondiale pour l'évaluation de l'état des écosystèmes, de manière analogue à la Liste rouge des espèces menacées de l'UICN (www.iucnredlist.org/). Parmi les espèces vivant dans les zones humides, de nombreux reptiles, amphibiens et poissons ont des capacités de dispersion limitées (Knutson et al., 1995). On considère que 46 à 57 % des tortues d'eau douce sont hautement menacées par la destruction des zones humides (Böhm et al., 2013). La connectivité de ces milieux fragmentés est donc d'une importance cruciale pour la conservation de ces espèces. Déterminer le degré des échanges entre patchs et l'échelle spatiale à laquelle ces interactions ont lieu est fondamental pour une gestion efficace.

L'étude de la dispersion chez les tortues d'eau douce (comme chez la plupart des espèces) peut être considérée sous deux aspects principaux : la connectivité démographique, prenant en compte les flux de disperseurs entre populations par analyse de données de capture-marquage-recapture (CMR) ou de radiotacking, et la connectivité génétique, prenant en compte les disperseurs efficaces, c'est-à-dire ceux qui se sont effectivement reproduits dans la population d'accueil.

Les études de dynamique des populations montrent que les tortues aquatiques sont très fidèles à leur site et ont des mouvements limités (Mitro, 2003 ; Roe and Georges, 2007 ; Roe et al., 2009). Cette philopatrie peut être considérée comme stable et durable. Une étude de capture-marquage-recapture (CMR) s'étalant sur 16 années montre ainsi que l'Emyde à dos en diamant (*Malaclemys terrapin*) restent fidèles à leur site (Gibbons et al., 2001), et parfois même, pour certaines espèces, à une superficie de quelques m² (Roe and Georges, 2007). Les femelles semblent montrer un degré de fidélité plus important que les mâles, notamment lié à la disponibilité et à la qualité des sites de ponte (Bowne et al., 2006) . Une étude montre que 33 à 40 % des femelles ont été trouvées à moins de 50 m de leur lieu de première capture au cours de deux saisons de reproduction, leur seul déplacement étant lié à la recherche de site de ponte (Szerlag-Egger and McRobert, 2007). Les mâles ont des comportements exploratoires plus prononcés, notamment pour la recherche de partenaire (Bower et al., 2012 ; Chelazzi et al., 2007). D'une manière générale, la majorité des déplacements pour les deux genres sont provoqués par la recherche de zones plus favorables en termes de ressources (alimentaires, sites de refuges et de thermorégulation ; Cosentino et al., 2010 ; Roe et al., 2009) et de disponibilité en eau (Roe and Georges, 2008). Par conséquent, les tortues peuvent se déplacer souvent à l'intérieur d'un domaine vital qui reste toutefois restreint (Bowne et al., 2006).

Cependant, il faut noter que les mâles et les femelles sont capables d'effectuer d'importants déplacements en milieu aquatique comme en milieu terrestre pour trouver un site de qualité (Herman et al., 1995 ; Stone, 2001). Ainsi, de façon très ponctuelle, on peut relever la transition entre deux sites d'une femelle sur plus de 5 km (Gibbons et al., 2001) ou des déplacements par les mâles de plus de 25 km (Bower et al, 2012)

En raison de la forte fidélité globalement observée, les échanges entre populations sont supposés faibles. La fragmentation artificielle, très présente chez les tortues d'eau douce, ne fait qu'accentuer ce schéma. Plusieurs études mettent en avant l'augmentation de la mortalité des femelles due à l'écrasement par les voitures lors de la recherche de site de ponte (Steen and Gibbs, 2004) et leur impact sur la structure des populations (Laporte et al., 2013). Ainsi, de plus en plus de populations ont des sexe-ratios biaisés par une majorité de mâles. Actuellement, de nombreuses populations fragmentées de tortues n'ont plus une dynamique de population suffisamment forte pour permettre la recolonisation des sites voisins (Tucker et al., 2001). Cette absence de connectivité est ainsi mise en avant dans plusieurs études de génétique des populations (Escalona et al., 2009 ; Souza et al., 2002). Elles montrent indirectement, par l'utilisation de marqueurs génétiques, que les flux de gènes sont limités par les barrières induites par la fragmentation (Mockford et al., 2007). La fidélité au site est également mise en avant génétiquement et le plus souvent associée à des modèles d'isolation par la distance (Escalona et al., 2009). Dans des paysages moins fragmentés, l'homogénéisation de la diversité génétique est provoquée par la dispersion des males (Sheridan et al., 2010). Ainsi, les biais de dispersion observés pour les mâles (Hart, 2005) et la forte fidélité au site pour les femelles (Freedberg et al., 2005) dans les études démographiques sont également révélées par les marqueurs moléculaires. D'autres études concernant les tortues aquatiques montrent au contraire de faibles niveaux de différenciation suggérant d'importants flux de gènes par une dispersion plus importante qu'elle n'est supposée chez les tortues aquatiques (Castellano et al., 2009; Lamb and Avise, 1992 ; Silva et al., 2011). Cependant, certains auteurs comme Hauswaldt and Glenn (2005), expliquent que l'absence de différenciation dans ces populations est due à des agrégations de reproduction où mâles et femelles se retrouvent dans un milieu favorable aux accouplements à une période très précise de l'année. Ainsi, ces regroupements peuvent expliquer à la fois la philopatrie observée et l'absence de différenciation génétique. Ces mêmes auteurs supposent également une dispersion natale des tortues aquatiques non négligeable. Pour expliquer l'homogénéité génétique, Sheridan (2010) et Davy (2013) mettent en avant l'importance de la taille de la population et du temps de génération important chez les tortues d'eau douce dans le phénomène de différenciation. En effet, ces deux paramètres ralentissent le phénomène d'érosion génétique par dérive. Par conséquent, l'impact de la fragmentation sur les flux de gènes peut être masqué (Shanas et al., 2012). En conclusion, les modalités de la dispersion chez les tortues d'eau douce sont presque aussi variées que peut l'être la dispersion dans sa globalité. Néanmoins, un schéma de dispersion semble se dégager dans la majorité des publications : une forte fidélité au site, notamment de la part des femelles, dépendante de la qualité du milieu, et un flux de gènes assuré par les mâles. Cette absence de grandes capacités de dispersion est renforcée par la fragmentation artificielle qui isole de plus en plus les populations. Nous pouvons également noter que l'analyse de la dispersion chez les tortues d'eau douce peut se faire par l'analyse de la génétique des populations de leurs parasites (Verneau et al., 2009) ou par l'analyse des graines qu'elles propagent (Burgin and Renshaw, 2008).

La Cistude d'Europe (*Emys orbicularis*) est une espèce emblématique des zones humides européennes. C'est certainement l'espèce de tortue (avec la tortue d'Hermann) la plus étudiée en France et en Europe. Il faut toutefois préciser que la Cistude est la seule espèce de chélonien largement répartie sur toute l'Europe, ce qui suscite de nombreuses études. Cependant, le vif intérêt pour ce chélonien vient aussi probablement du fait que c'est également le reptile qui a le plus régressé entre 1970 et 1990 avec une réduction de plus de 2 % de son aire de répartition (Servan, 1999). Cette régression d'aire reflète l'impact de toutes les perturbations anthropiques contemporaines sur une espèce à dispersion limitée : disparition et altération d'habitat, pollution, destruction directe, prélèvements d'individus, fragmentation… De nombreuses études se sont penchées sur la phylogéographie de cette espèce (Fritz et al., 2007 ; Lenk et al., 1999; Pedall et al., 2011 ; Sommer et al., 2009), offrant aujourd'hui une connaissance accrue de la répartition de l'espèce et de sa dynamique temporelle sur toute son aire de répartition en Europe mais aussi au nord de l'Afrique et au Moyen-Orient (Fig. 2).

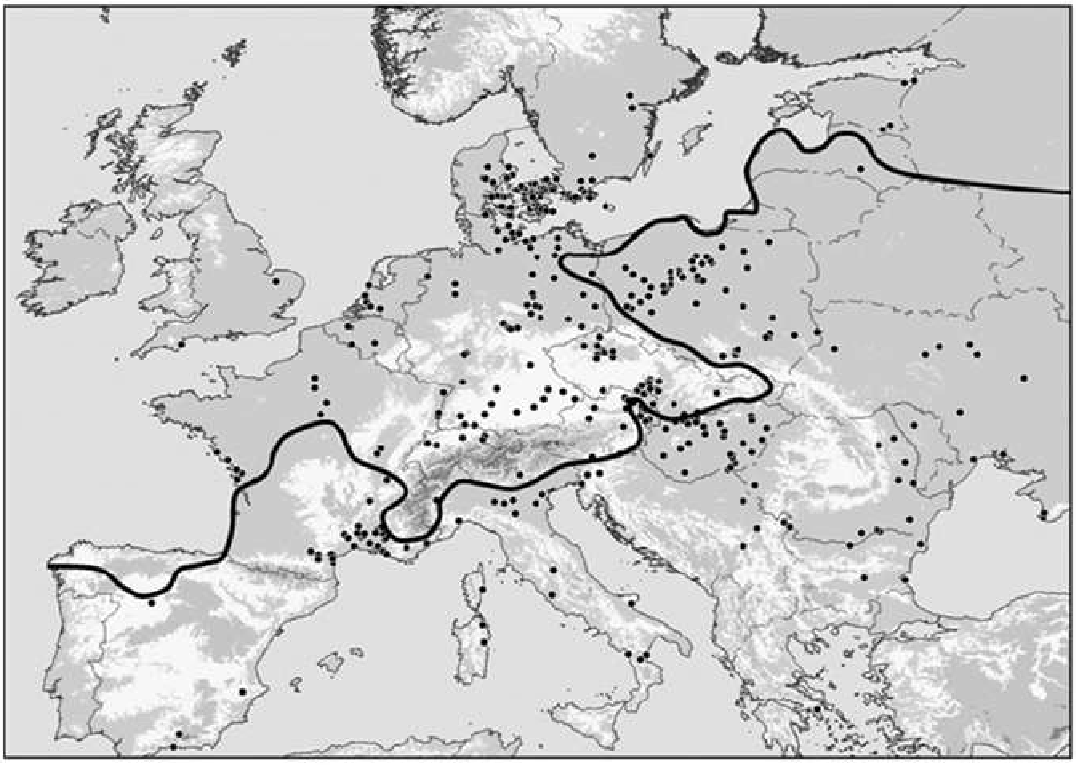

Figure 2. Aire de distribution actuelle des populations naturelles *d'Emys orbicularis* (au sud de la ligne noire) et localisation d'échantillon subfossiles datant de l'Holocène (points noirs). D'après Sommer et al. (2007).

La caractérisation des haplotypes chez des individus actuels, mais aussi subfossiles, a permis d'identifier les refuges glaciaires et de comprendre les voies de recolonisation de l'Europe, notamment après la dernière grande glaciation (Fig. 3). La répartition prépondérante de la lignée haplotypique II (associé à la sous espèce *E. orbicularis orbicularis*) sur tout le continent indique un épisode majeur de recolonisation à partir d'un refuge glaciaire situé au niveau de la Grèce. Les cistudes auraient utilisé les rivières et les étangs à travers les Balkans pour de grandes dispersions (Sommer et al., 2007) facilitant la colonisation des pays Baltes et du nord de l'Europe (Allemagne, Danemark, Norvège, Angleterre) et aurait ensuite colonisé la France par le nord (Bourgogne, Auvergne) par de petits déplacements (50 km parcourus pour 100 ans ; Sommer et al., 2007) jusqu'au Pyrénées en passant par la Camargue. D'autres refuges glaciaires ont été déterminés, caractérisés par la présence de lignée haplotypique bien définie, en Espagne (lignée VI, *E. orbicularis fritzjuergenobsti*), au sud de l'Italie (comprenant la Corse, la Sardaigne et le sud de la France, lignée V, *E. orbicularis galloitalica*), au niveau du littoral Adriatique (lignée IV, *E. orbicularis hellenica*) et au nord du Moyen-Orient (lignée I, *E. orbicularis orbicularis*).

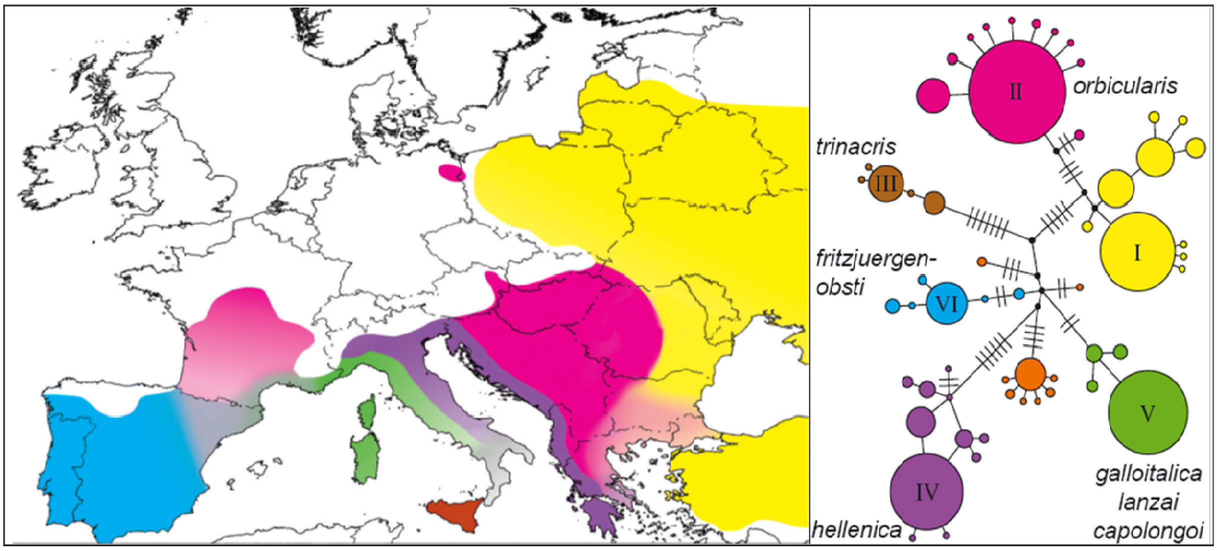

Figure 3. Réseau haplotypique (ADN mitochondrial, cytochrome b ; à droite) et distribution des lignées haplotypiques (à gauche) chez *Emys orbicularis*. D'après Fritz et al. (2007) et Pedall et al. (2011).

D'autre part, de plus en plus d'études de dynamique et de génétique des populations nous montrent le fonctionnement des populations, nous renseignent sur leur structure et leur connectivité et, qui par conséquent, analysent la dispersion, le plus souvent dans un contexte de fragmentation (Pereira et al., 2011). La cistude ne semble pas échapper au schéma que nous avons défini précédemment. En effet, l'espèce semble très fidèle à son site (Ayres and Cordero, 2007 ; Cadi and Miquet, 2004 ; Lebboroni and Chelazzi, 1991) et possède de faibles capacités de dispersion (Sommer et al., 2009). Les femelles sont caractérisées par une plus forte philopatrie (Bona et al., 2012) et on recense des mouvements de dispersion plus important de la part des mâles (Olivier et al., 2010). La fidélité des femelles est également dépendante des sites de pontes, mais peut varier selon les individus (Mitrus, 2006).

La quasi-totalité de ces découvertes vient de l'étude de populations de faibles effectifs et vivants en zone protégée (Réserve Naturelle, Parc Naturel Régional,…). Très peu d'études ont examiné l'impact de la fragmentation sur la structure des populations non protégées et non gérées. Par le biais de ce travail de thèse, nous avons souhaité aborder ce point. Ainsi, et ce sera l'objet du premier chapitre, nous avons déterminé l'impact de la fragmentation sur des populations de grands effectif, dans une zone Ramsar non gérée, en Grèce au niveau du lac Kerkini. Cette zone a subit la construction de deux barrages (en 1932 et 1982) favorisant la rétention d'eau pour l'agriculture et provoquant la disparition de la zone humide précédemment présente au profit d'un grand lac profond. Cette rétention s'est suivie d'un intense endiguement. Nous supposons que la plaine d'inondation représentait un environnement très favorable aux cistudes et que sa disparition a provoqué un éclatement des populations dans les refuges favorables (mares, rivière) situés uniquement en quelques points autour du lac de barrage. Aujourd'hui, nous pensons que ces populations ne sont plus connectées et que cette fragmentation a un impact sur la structure génétique des cistudes. Pour répondre à notre hypothèse, nous avons étudié la connectivité des populations situées autour du lac Kerkini, par l'utilisation de marqueurs génétiques mitochondriaux (cytochrome b) et nucléaires (microsatellites). Par ailleurs, nous avons proposé un modèle de l'évolution de la structure génétique de ces populations grecques pour les 100 prochaines générations.

D'autre part, comme nous l'avons vu précédemment, l'étude de la dispersion ne peut être dissociée de l'étude des métapopulations. Pourtant, peu d'études sur les chéloniens d'eau douce (Gibbs, 1993) prennent en considération la dimension métapopulationelle. Dans le chapitre 2, nous avons souhaité étudier la dispersion démographique et génétique de trois noyaux de populations de cistude en Camargue, sur la réserve Naturelle Régionale de la Tour du Valat, suivies par capture-marquage-recapture depuis 1997 (pour deux d'entre eux). Nous avons supposé que ces deux noyaux suivies par CMR, distants d'1,5 km et connectés par de nombreux corridors biologiques, étaient liés par une importante dispersion provoquant une homogénéisation génétique. Pour répondre à cette question, nous avons utilisé des modèles de dynamique des populations permettant de quantifier les mouvements de transition entre deux noyaux. Par ailleurs pour déterminer si ces mouvements amenés à la production d'un flux de gènes entre les noyaux, nous avons utilisé des marqueurs génétiques nucléaires (microsatellites) et mitochondriaux (cytochrome b).

Pour finir, en plus de ces deux objectifs concernant l'analyse de la dispersion chez la cistude, nous avons souhaité développer une étude plus appliquée, exposée dans le 3ème chapitre et montrant la façon dont la dynamique des populations peut être utilisée par des gestionnaires d'espaces protégés dans un contexte de gestion intégrée. L'objectif de la biologie de la conservation étant de fournir des principes et des outils pour la préservation de la diversité biologique (Besnard, 2013), nous montrons comment estimer, par l'utilisation de modèle CMR, l'impact des changements des plans de gestion d'eau et d'intensité du pâturage sur les tailles de populations de cistudes de la Tour du Valat. Cette démarche alliant sciences et gestion, nous a permis en parallèle d'estimer la résilience des populations longévives face à une perturbation.

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Chapitre 1

Impacts of dam construction on the genetic structure of a long-lived species, the case of the European pond turtle (*Emys orbicularis*) in Kerkini (Northern Greece)

Impacts of dam construction on the genetic structure of a long-lived species, the case of the European pond turtle (*Emys orbicularis*) in Kerkini (Northern Greece)

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Abstract

The European pond turtle *Emys orbicularis* is a long-lived freshwater turtle species, characterised by a large generation time and low dispersal abilities, is affected by home range reduction due to loss of favorable habitats, competition with invasive species and landscape fragmentation. We investigated the impact of dam constructions in Kerkini (Northern Greece) using both mitochondrial DNA and microsatellite markers to assess and compare genetic diversity levels between populations around the retention lake, infer past and present connections, and we tried to determine evolution of the genetic structure of populations through simulations for the next 100 generations. We found high level of private allelic richness, and no genetic differentiation for population around the lake. We supposed that these populations are originating from a large historical population present before the dam construction in a favorable floodplain. The population size was kept sufficiently large that the changes in allelic frequencies due to the genetic drift are not sufficient to reduce genetic diversity in spite of no gene flow and the beginning population isolation. We estimated tens of generations, depending of the population size, are necessary to obtain a start of differentiation.

Key words

Conservation genetics, *Emys orbicularis*, dam, genetic differentiation, long-lived species, freshwater turtle.

Introduction

Currently many species of freshwater turtle are alarmingly declining (Buhlmann et al., 2009). The destruction or degradation of wetlands habitats is considered as one the major causes of the decline and habitat fragmentation through its effects on population size and connectivity is playing a key role (Dodd, 1990; Williams and Osentoski, 2007). Freshwater turtles require both aquatic (feeding) and terrestrial (nesting) habitats to complete their life cycle and the impact of global change on these habitats needs to be quantified in order to understand the ultimate causes of population decline (Ficetola et al., 2004).

Recently, several genetic studies have compared genetic diversity among “isolated” or “fragmented” populations and “continuous” or “unfragmented” populations (e.g. Richtsmeier et al., 2008; Pittman et al., 2011; Banning-Anthonysamy, 2012). Other studies have investigated the effects of predefined barriers to gene flow such as dams or urban development (e.g. Bennett et al., 2010). These studies show that anthropogenic activities have consequences on the genetic structure of the population and produce important population differentiation associated with restricted gene flow (e.g. Sethuraman et al., 2013; Richtsmeier et al., 2008, Selman et al., 2013), low genetic diversity (Gonzalez-Porter et al., 2011), morphological variations between populations (Clavijo-Baquet et al., 2010) and population bottleneck (Escalona et al., 2009). However, other studies produced contradictory results with reduced effects of wetland destruction resulting in low intra-specific differentiation in several turtle species (e.g. Amato et al., 2008; McGaugh et al., 2008).

Population genetics response of freshwater turtles to fragmentation is poorly understood and difficult to estimates (Alacs et al., 2007). Freshwater turtles are long-lived organisms with delayed sexual maturity, low reproductive success and large generation time

(Couturier et al., 2011). These life-history characteristics may delay the genetic erosion due to genetic drift. In addition, many species are often characterized by low dispersal abilities (Couturier et al., 2011) that might induce restricted gene flow and differentiation even in absence of anthropogenicaly induced fragmentation. Hence, for a given effective population size *Ne*, more time is needed in order to detect the impact of both drift and reduced gene flow and modeling is often required predictive studies (Neuenschwander et al., 2008).

The European pond turtle (*Emys orbicularis*) is the reptile showing the most important range reduction in Europe since the 1970s (Servan, 1999) and the species is considered in decline (Cadi and Joly, 2004). The principal causes of pond turtle rarefaction is habitat loss and anthropisation (Rogner, 2009). Many human practices (water management, agricultural development, road network…) have an important impact on this freshwater turtle and produce population fragmentation (Rogner, 2009; Trakimas and Sidaravicius, 2008).

The phylogeography of *E. orbicularis* have been extensively studied allowing to identify glacial refugia, dynamic of inter-glaciation colonization and latitudinal gradient of diversity (Fritz et al., 2005, 2007; Lenk et al., 1999;Velo-Anton et al., 2008; Pedall et al., 2011). On the opposite, population and conservation genetics of this species was only recently approached. Population differentiation might occur at variable spatial scale, ranging from geographic distance to very local scale (Velo-Anton et al., 2008). Microsatellite loci proved useful in assigning ex situ individual to their native natural population (Velo-Anton et al., 2007). Corridors assumed to maintain gene flow between populations were identified as not as efficient as expected (Molnar et al., 2001). Reduced diversity and inbreeding associated with anthropogenic pressure was identified as increasing proportion of individuals with anomalous shell morphology (Velo-Anton et al., 2011).

In Northern Greece, the Kerkini area, a Ramsar site, historically characterized by a floodplain representing a continuous habitat for *E. orbicularis*, was modified since the early

30s by the construction of dams and associated dikes, as well as increasing use of water for irrigation associated with the expansion of rice culture. As a consequence, Kerkini wetland area has drastically reduced and has been heavily impacted with a consequent loss of biodiversity (Pyrovetsi and Papastergiadou, 1992; Crivelli et al., 1995a; Crivelli et al., 1995b). The distribution of populations is now scattered around some restricted part of the lake occupying now most of the original flood plain. Both the lake itself and extension of agriculture lands around it seem to be both detrimental for European pond turtles settlement and present reduced structural connectivity between populations. Dam lakes are known to be associated to reduced water velocity and increasing water depth, both and lower oxygenation, a factor known to impact diving ability in freshwater turtles (Clark et al., 2009). Agricultural areas are known to be less favored by freshwater turtles (Bodie and Semlitsch, 2000) and might reduce connectivity between ponds (Bowne et al., 2006). Agricultural activities (pesticide, harvesting) might be a source of mortality and nesting site perturbation (Steen and Gibbs, 2004). This Ramsar area have been extensively prospected in spring and summer over the last 14 years by one of the co-author (NT) allowing an assessment of the distribution of sites were turtle can be observed (feeding or basking). The number of sites harbouring turtle is limited and the remaining populations around the lake might be actually characterized by low functional connectivity. As approximately 8 generations (see Material and methods) have passed since the construction of the first dam this might have resulted in promoting genetic differentiation. In addition, if such fragmentation has been associated with a reduction in effective population size, both diversity and differentiation by also have been impacted by genetic drift. This situation provides an original and pertinent model to quantify for the first time the effect of dam-lake habitat fragmentation on the genetic diversity and spatial structure of *Emys orbicularis*.

Northern Greece is known to harbour two mtDNA haplotypic lineages (I and II; Lenk et al., 1999; Fritz et al., 2005) characterized by shallow divergence (0.53-0.95 %). Lineage II is considered as being present since a long time, while the presence of lineage I is thought to be associated with more recent post glacial colonization from the west (Lenk et al., 1999; Fritz et al., 2007). Both haplotypic lineages are expected to be present in the Kerkini area. The potential impact of the species phylogeographic history is therefore to be taken into account while studying contemporary population genetic structure. It has been observed in other part of *E. orbicularis* distribution contact zone with either asymmetric introgression (nuclear genome of one haplotipic lineage replace by the genome of another lineage) or to a lower extends individuals with mixed ancestry (Pedall et al., 2011). Alternatively, one could hypothesized that two lineages might have mixed for enough time to erase any differences.

The aim of our study is to investigate the impact of Kerkini's landscape modification; both agricultural extension and the construction of the dams and associated lake on the genetic diversity and its spatial distribution. First, we relied on both mitochondrial DNA and microsatellite markers to assess and compare genetic diversity between populations as well as its spatial structuration. Site along the river upstream of the dam-lake were sampled in order to evaluate the spatial at which differentiation might occur in the absence of anthropogenic action. The fact that the phylogeographic history of the area was not the factor explaining the spatial structure was taken into account. Second, we took advantage that one of the studied sites has been followed for more than 14 years by Capture-Mark-Recapture (CMR) and that over 1200 turtles were marked to estimate demographic size, generation time and compared genetic results about gene flow were to emigration rate from this site. This would allow testing if dispersal occurred over recent past. Finally, we used simulation to evaluate in a prospective framework the evolution of the genetic differentiation between populations through simulations for the next 100 generations.

Materials and methods

Study area

The Kerkini (41°12N, 23°09E) area is located ca 100 km north of Thessaloniki in northern Greece, close to the Bulgarian border (Fig. 1a, Chelazzi et al., 2006). This area was historically the flood plain of the Strymon River and its tributary, the Kerkinitis River. All the area (Fig.1b) was considered at that time as being a suitable continuous habitat for pond turtle (Crivelli et al., 1995a). Part of the land was transformed into an irrigation reservoir-lake by the construction of a dam and a dyke to the east in 1932 (Fig. 1c). Following major siltation and an increase in the area being irrigated, a new, higher dam was erected in 1982 (Fig. 1c), with a new dyke to the west, and a higher eastern dike causing further habitat and landscape disruption (Crivelli et al., 1995a, 1995b).

Sample collection

We collected blood samples from 212 Pond turtles from 5 sites during spring 2011 (Fig. 1d, Table 1). Three sites (L1-L3) are located around the Lake while two sites (L4 and L5) are located in a non-impacted area upstream the Strymon River. Turtles were caught with fish traps or directly by hand. Blood were sampled from dorsal tail vein (or dorsal coccygeal vein) and stored in preservative buffer (Seutin et al., 1991). We collected blood samples of up to 0.2 ml from terrapins aged above 3-4 years old. Genomic DNA was extracted from 15µl of blood, using a standard phenol-chloroform protocol (Hillis et al., 1996). The resulting DNA pellets were resuspended in 100 µl TE buffer, pH 8.

Population demographic analysis

In 1998, we started to mark terrapins individually on marginal scutes with hacksaw (Olivier et al., 2010) within the L3 site at sub-sites within 1200m from each other. From 1998 to 2012, we run a standardized monitoring program (Capture-Mark-Recapture, CMR) by trapping terrapins every two weeks, from April to mid-August, for two consecutive days (see Chelazzi et al., 2006). Morphological measures (length of the back and weight) were also noted. One thousand two hundred and twenty four different adult individuals of *Emys orbicularis* have been marked in 14 years (1998-2011). We took advantage of this 14 years CMR program to calculate demographic size using the CAPTURE program (Rexstad and Burnham, 1991) in MARK software (White and Burnham, 1999). To simplify the estimation of population size, we considered that the population is closed (no birth, death and migration). CAPTURE allows varying assumptions regarding capture probabilities including inter-individual heterogeneity of capture (M*h*), time variation in capture probability (M*t*), a behavioural response to the first capture (M*b)* and the different possible combinations of effects on capture probabilities. The best model is selected based on the maximum value (between 0 and 1) of a discriminate model selection criteria specific to CAPTURE. In addition, we calculated the capture densities for two consecutive days in the same period at L1, L2 and L3. Knowing the population size at L3, we roughly estimated demographic size in L1 and L2 by comparison of these capture densities.

Generation time, the average age at which the reproduction is ensured, was calculated as $g = \mu + [s/(1-s)]$, where μ is the average age at first breeding and s is the adult survival rate (Sæther et al., 2005). We considered a survival rate (s) of 0.89 for adult survival (Ficheux, unpublished data). The acquisition of sexual maturity in *Emys orbicularis* varies between geographical areas: in Northern areas pond turtles start reproduce being 8-12 years old (Schneeweiss et al., 1998), whereas in Southern areas the reproduction starts at 6-10 years old (Lebboroni and Chelazzi, 1991). Males attain reproduction two or three years before females (Olivier et al., 2010). The reproductive success does not seem to be dependent on individuals experience but conditioned by favourable meteorological conditions. Therefore, we estimated 9 years old as being the average age at first breeding (μ) and so generation time (g) was estimated to be 17 years. No other estimations were found in literature.

Mitochondrial DNA data

We amplified by Polymerase Chain Reaction (PCR) 1428 pb of cytochrome b (Cytb) using newly designed forward EM-GluCytb-F (5'-CCACCGTTGTATTCAACTAC-3') and reverse EM-ThrCytb-R (5'-AGTCTTCAGTCTTTGGTTTAC-3'). These primers were designed from the *E. orbicularis* complete genome sequence available in GenBank (Accesion number JN999703) witin tRNA flanking cytb gene. These new primers were designed as already published primers (mt-A and CR12H, Lenk and Wink, 1997) did not amplify in most samples due to punctual mutations. Cytb is a classical marker to infer population genetics and phylogeography of pond turtles (Fritz et al., 2005; Lenk et al., 1999; Pedall et al., 2011). All PCR reactions were performed in a total volume of 20µl, including 2µl of 1/10 diluted DNA, 1X of HotMaster^{TM} Taq buffer (5PRIME®), 200µM of dNTPs, 200nM of each primers and 0.25 unit of Taq polymerase HotMaster^{TM} (5PRIME®). PCR conditions were 1 min 30 sec at 94°C followed by 35 cycles of 30 sec denaturing at 94°C, 45 sec annealing at 55°C, and 45 sec extension at 65°C. Final elongation step was at 72°C for 10 min. PCR yield and specificity was checked by running a 2 µL aliquot on a 2% agarose gel. PCR products were purified with Exonuclease 1 (2 units) and Shrimp Alkaline Phosphatase (1 unit) enzymes for 1 hour at

37°C. Sequencing was performed by MACROGEN® society in The Netherlands following a Big Dye sequencing protocol. Sequencing chromatograms were edited and aligned by manually with MEGA 5 software (Tamura et al., 2011). The haplotypes Ia (accession number: AJ131407), Ib (accession number: AJ131408) and IIa (accession number: AJ131411) were included as references.

Microsatellite DNA data

Fourteen microsatellites loci: msEo21, msEo41 (Pedall et al., 2009), Emys 1, Emys 8, Emys 11 (Ciofi et al., 2009), GmuD16, GmuD51, GmuD55, GmuD62, GmuD87, GmuD88, GmuD93, GmuD107, GmuD114 (King and Julian, 2004) were used following a M13 tailing strategy (Schuelke, 2000). PCR conditions were identical to mtDNA amplification except that 100nM of M13 fluorochrom (700 or 800) labelled primer and 0.45 unit of Taq polymerase HotMaster^{TM} (5PRIME) were used. PCR conditions were 5 min at 94°C followed by 35 cycles of 40 sec denaturing at 94°C, 30 sec annealing at 56°C, and 1 min extension at 65°C, followed by a final elongation step of 65°C for 5 min.

Molecular diversity data analysis

To estimate diversity at microsatellite loci we estimated the mean number of allele (A), the number of rare alleles (RA, frequency < 0.05 in the whole dataset), the allelic richness (Ar) and the number of private alleles (PAr). Both Ar and Par were corrected by the rarefaction method for a common size of 32 individuals (Kalinowski, 2004; Leberg, 2002). All these analyses were realised with HP-RARE 1.0 software (Kalinowski, 2005). We used rarefaction method because population sampling sizes have an impact of allelic diversity detection;

especially for microsatellites which show many rare alleles (ElMousadik and Petit, 1996, Petit et al., 1998). The general principle is to use allele frequencies observed initially in a sample to determine the allele numbers in a smaller sample (Kalinowski, 2004; Leberg, 2002). Differences between sites for Ar were tested with non-parametric Wilcoxon tests.

We estimated the Weir and Cockerham (1984) estimator of F_{IS} for each site from multiloci microsatellite data. Significant difference from zero of F_{IS}, indicating a deviation from Hardy Weinberg Equilibrium (HWE), was tested using 1100 random permutations of alleles between individuals. Linkage disequilibrium for each pair of loci was tested for each site. Both analyses were implemented with FSTAT 2.9.3. (Goudet, 2002). Nomimal significance level (5%) was adjusted with Benjamini–Yekutiely corrections for multiple comparisons (BY; Benjamini and Yekutieli, 2001). It was preferred to Bonferroni correction as this correction is regarded as being very conservative, whereas BY provides a better compromise between type I and II errors (Narum, 2006). Five loci were discarded from the analyses (Emys 11, GmuD16 GmuD51, GmuD55 and GmuD114) as genotyping errors (null alleles and/or stuttering) were detected while applying the program MICRO-CHECKER (van Oosterhout et al., 2004)

To detect recent genetic bottleneck, we tested the presence of an excess of heterozygosity in the population as expected after a bottleneck compared to a population at mutation-drift equilibrium with BOTTLENECK 1.2.02 (Cornuet and Luikart, 1996). After few generations this genetic signature tends to disappear as the population reaches mutation-drift equilibrium again. We ran BOTTLENECK for the two kinds of mutation models appropriate for microsatellite data: the stepwise mutation model (SMM; Luikart and Cornuet, 1998) and the two phase model of mutation (TPM; Di Rienzo et al.,1994). Following recommendations of Piry et al. (1999), we ran the TPM with 95% of single-step mutation and a variance of 12 among multiple steps, and used a signed-rank Wilcoxon test to assess significance of the heterozygosity excess.

Effective population size (*Ne*) was estimated for each site based the bias corrected version of the linkage disequilibrium method (LD, Waples and Do, 2010) using the NeEstimator V.2 (Do et al., in press). Only alleles with frequencies >0.05 were used. Confidence intervals (95% CI) were implemented.

Spatial genetic structure analysis

First, the potential impact of the species phylogeography history on contemporary population structure was studied. We hypothesized that lineage I and II are mixing for enough time to erase any such phylogeographic pattern or that complete introgression occurred. Therefore, the drivers of the actual pattern of differentiation would contemporary processes occurring over recent historical time scale. This hypothesis was tested in two ways. First, we checked that the two mtDNA lineages (I and II) were not spatially distributed according to a geographic pattern reflecting the suspected phylogeographic recolonisation pattern (e.g. Haplotype I only present in the estern part of the studied area). Second, we computed F_{ST} for microsatellite markers according to mtDNA lineage to check potential nucleo-cytoplasmic disequilibrium resulting in differentiation that would occur according to mtDNA haplotype lineages rather than another factor (e.g. geography).

Two different approaches were adopted to determine contemporary spatial genetic structure. First, differentiation between site pairs ("site centred approach") was tested. We first used conventional multi-loci F_{ST} by for microsatellites (Slatkin, 1995) and by θ_{ST} an analogue index for mitochondrial data (Excoffier et al., 2005) implementing ARLEQUIN version 3.5 software (Excoffier et al., 2005). As F_{ST} might have a negative dependence on within-sampling unit heterozygosity, causing them to approach zero when diversity is high (which is the case for most of our loci in the present study) we also applied the index developed by Jost (2008) as estimated for small samples by D_{est} using GNEALEX 6.5 (Peakall and Smouse, 2006, 2012). Statistical significance for both F_{ST} and D_{est} were tested using 9999 random permutations of individuals between sites and nominal levels of significance was adjusted with Benjamini–Yekutiely corrections for multiple comparisons. Second, an “individual centred approach” was applied based on microsatellite data; identify the most probable number of genetic units (K) in a Bayesian clustering framework grouping individuals into populations based on both HW and linkage equilibrium. We used STRUCTURE software, version 2.3.4, (Pritchard et al., 2000). Five runs (500 000 MCMC iterations with an initial burn-in of 100 000 steps) for each value of K (1 – 7) were performed to assess consistency of estimations of log-likelihood of the posterior probability of the data for a given K (ln Pr(X/K)). We chose the admixture model with non-independent allele frequencies. We then computed the posterior value of K (Pr(K/X)) following Eq. 3 in Pritchard et al. (2000) using R ver. 2.7.2 (Development Core Team, 2008). Following the recommendations of Waples and Gagiotti (2006) we used this statistic rather than Evanno’s test (Evanno et al. 2005), as the observed level of differentiation was low (see results).

Prospective genetic differentiation simulations

In order to simulate the possible consequences of the dam construction on the future genetic structure, we performed computer simulations with QUANTINEMO software (Neuenschwander et al., 2008). We simulated over 100 generations 5 times the same abstract model to evaluate the impact of drift on the evolution of pairwise differentiation (as estimated by F_{ST} values) for the L1 L2 and L3 populations, starting from the currently observed diversity within each site. We supposed no dispersion between site, no biased sex-ratio, no selection, random mating and mutation model by default.

Results

Population sizes and demographic connectivity

The most appropriate model was estimated to M*bh* (criteria of 0.61) corresponding to the Pollock and Otto (1983) model. Population size estimate at L3 was 1471 (95% IC: 1355-1684). For identical capture session, 222, 18 and 82 individuals were caught at L3, L1 and L2, respectively. Therefore, population size estimation were 543 (95% IC: 500-622) and 119 (95% IC: 109-137) at L2 and L1 respectively (Table 4).

Among the 1224 adults marked in the L3 site (all marked in this site), only 17 individuals were captured in the closest site, L4, during the blood sampling campaign (Table 5). No other European pond turtles were found at the other sites.

Mitochondrial & nuclear DNA diversity

Three haplotypes were observed out of a total of 187 individuals sequenced for Cytb from our 5 studied sites. Both haplotypic lineage I (haplotypes Ia and Ib) and II (haplotype IIa) were present (Lenk et al., 1999; Table 2). Haplotypes Ia and Ib differ on one nucleotide while a 6 nucleotides difference is observed between the haplotype IIa and the two other haplotypes corresponding to 0.4% divergence (Table 2). All haplotypes were present in all sites except IIa absent in the L5 site (Table 2). Haplotype Ia is the most frequent (50.8%). No haplotype can be considered rare, the frequency of any haplotypes being greater than 5% in the total pool of 187 individuals.

Genetic diversity was analysed for nine microsatellite loci for 212 individuals from the 5 sites (Table 3). Microsatellite loci appeared to be inherited independently of each other, as no evidence for linkage disequilibrium was found for any pair of loci. The genetic diversity was high with an average number of alleles per locus (A) of 11.82 (Table 3). Range of variation across sites was arrow (10.7-12.8) (Table 3). Allelic richness (Ar) corrected for a common size was also characterized by narrow range of variation across sites (9.5-9.8) and not different among sites (all Wilcoxon tests with p value>0.5). The private corrected allelic richness (PAr) was low in each site (0.37-0.60) and not different among sites (all Wilcoxon tests with p value>0.5). Average number of rare allele (RA) was high, ranging from 3.6 to 5.6. Expected heterozygoty was high in all population (0.85-0.87).

All populations showed no deviation to Hardy-Weinberg Equilibrium, all F_{IS} values being not significantly different from zero (Table 3).

Bottleneck was not evidenced in any site as no heterozygosity excess compared to mutation-drift equilibrium was detected, neither for the SMM nor for the TPM models of mutation (Table 4).

The effective population size (*Ne*) could be estimated for four sites. Also it any finite value could be produced for L4, the *Ne* of this site is thought to be very high. The four remaining sites were characterized by a large *Ne*, although variable according to the site they were all in the same order of magnitude (Table 4), ranging from 145 to 657 individuals.

Spatial genetic structure

Spatial distribution of mtDNA haplotypes indicated the presence of haplotypic lineage I in estern part of the studied area (see above). Microsatellites showed no genetic structure according to haplotypic lineage (F_{ST} = 0.0035, P=0.1613).

No genetic differentiation was observed between the populations pairs around the Lake (L1, L2 and L3), neither for microsatellite (both F_{ST} and D_{est}) nor for mtDNA (θ_{ST}) (Table 5). Other pairwise comparisons only revealed very few cases of significant differentiation, the level being always low (see Table 5 for details).

Bayesian clustering method found no genetic structure and indicated both maximum likelihood and highest posterior probability for only one genetic cluster (K=1; P (K=1) =1).

Prospective genetic differentiation simulations

The simulation of the evolution of future differentiation is approximately the same for pairwise L1-L2 and L1-L3 (Fig. 2) reaching a moderate F_{ST} value of ca 0.1 after 100 generations. For the pairwise comparison involving the largest populations (L2-L3), only a third of the previous value is reached after 100 generations (Fig. 2).

Discussion

In the present study we have investigated the impact of a major anthropogenic perturbation, the landscape modification through the emergence of a dam, on a historical population of the European pond turtle (*Emys orbicularis*). The consequences of the dam emergence were the fragmentation of the initial population and the isolation of the fragmented subpopulation due to the inappropriate habitats constituted by the dam lake and intensive rice cultures. Our aim was to quantify, with molecular markers, the emergence of a genetic differentiation among subpopulations due to slow permissive barriers to gene flow and to predict the evolution over 100 generation of the genetic structuration among subpopulations. However, in long-lived species, the generation time is an important parameter to take into account because it can delay the antagonist effects of genetic drift and reduced gene flow for small fragmented populations (Marsack et al., 2009, Kuo and Janzen, 2004, Velo-Antón et al., 2008, Bennett et al., 2010). Freshwater turtles are particularly affected by anthropogenic disturbance such as habitat fragmentation, degradation or destruction (Bohm et al., 2013). However, the consequences of habitat degradation on the population structure and survival of these animals are poorly understood and difficult to estimates (Alacs et al., 2007). Moreover, freshwater turtles are long-lived organisms with low dispersal abilities, delayed sexual maturity, low reproductive success and large generation time that may slowdown the genetic erosion due to genetic drift (Couturier et al., 2011).

As a preliminary step, we first showed that phylogeographic processes that took place along the Holocene are not any more a driving force that could explain the present day pattern of spatial differentiation at the studied local scale. In the part of the Balkans where the Kerkini area in located, Holocene colonisation of individuals of mtDNA lineage I in an area represented by mtDNA lineage II is known to have occurred (Lenk et al 1999; Fritz et al 2009; Sommer et al., 2009). We showed both lineages are indeed present in the Kerkini area but that the distribution of the lineages within of this area is not spatially structured in terms of presence/absence and that both lineages could be finding in almost all sites. More importantly, differentiation for microsatellite markers was not structured according to haplotypic lineage. This pattern is congruent with either symmetrical crosses leading to homogenization or asymmetric introgression occurring between lineages enough generations ago (along the Holocene). Indentifying which process occurred (introgression versus symmetrical crosses) is out of the scope of this study and would require extra sampling in the Balkans in areas where both haplotypic lineages are in allopatric distribution. Whatever the process which occurs during Holocene it is anymore a driving force nowadays.

Mitochondrial and microsatellites data felt to detect differentiation between the sites surrounding the lake (L3, L1 and L2) either estimated by θ_{ST}, F_{ST}, or by D_{est}, an estimator of Jost (2008) measure of differentiation although the set of markers was informative enough to detect even low level of differentiation (as observed for few sites combinations). Similarly, private allelic richness was high at these sites. In the literature, is reported a wide diversity of level of differentiation for *E. orbicularis*. Population differentiation was reported a geographic scale (10s to 100s of Km, Velo-Anton et al., 2008; Molnar et al., 2011). However, both Velo-Anton et al. (2008) and Molnar et al. (2011) showed with the same studies that differentiation could also occur within a given site. To explain the absence of genetic structure around the lake, one of the first hypotheses could be the presence of gene flow (Sheridan et al., 2010). The gene flow involves dispersal followed by reproduction of individuals between sites (Stevens et al., 2006). Even if the terrapins are able to disperse several kilometers, the majority of movement is below 1000 meters (Mitrus, 2010). However, the behavior of dispersal and use of landscape is very variable from one species to another or from one population to another (Ficetola and Bernardi, 2006). Indeed, in *Malaclemys terrapin*, Hauswaldt and Glenn (2005) have highlighted the lack of genetic differentiation over a distance of more than 30km despite strong site fidelity by females. The authors hypothesize aggregation of individuals on breeding sites promoting both genetic mixing and philopatry. We can imagine a similar system of mating aggregations to Kerkini. However, to our knowledge, no aggregation site is known. In addition, the lands surrounding the lake are used for agriculture, which is known to negatively impact the chelonians dispersal (Bowne et al., 2006; Rizkalla and Swihart, 2006). Furthermore, no turtles were captured within the lake over the years, notably by fishermen and or through biodiversity monitoring. It seems that the lake is too deep to be crossed. Clark et al. (2009) also reported that the water retention lakes are habitats unfavorable with a lack of oxygenation. Finally, since 1999, no turtles marked at L3 were found on any other site (except for L4). However, it is not possible to definitively rule out the presence of corridors, for example, the irrigation channels to the east of the lake might connect L3 and L1 sites. Indeed, Molnar et al. (2011) showed that even corridors with good potential structural connectivity and therefore assumed to maintain gene flow between populations were identified as not as efficient as expected.

The second hypothesis good explain the absence of between sites around the lake is the existence of a large historical population. Indeed, both allelic diversity and expected heterozygosity were high for microsatellite markers in all sites, including also high proportion of rare alleles. The observed level of diversity is in the upper range of what have been observed for other population genetic studies on *Emys orbicularis* (Pedall et al., 2011; Velo-Anton et al., 2007; Velo-Anton et al., 2008, Molnar 2011). However it is be kept in mind that comparison between studied for microsatellite can be highly biased by differences in the set of loci and sampling sizes used. It could be assumed that the actual lake populations emerged from a very large ancestral population. Indeed, before the construction of the first dam in

1932, the Kerkini area was a flood plain (Crivelli et al., 1995a, 1995b). This plain was probably a very favorable environment for feeding and reproduction for *Emys orbicularis*. It provided a continuous area between the Strymon and Kerkinitis rivers, with a carrying capacity probably allowing accommodating a large population. If the population size was kept sufficiently large relative to the time since the construction of the dam (approximately 8 generations ago), it is possible that the changes in allelic frequencies due to the genetic drift are not sufficient to reduce genetic diversity and that high diversity could be maintened (Hamilton, 2009) despite population isolation. Both demographic and effective population size of the current populations are large and are in agreement with the ability of maintaining high level of diversity. Demographic populations have been estimated for many *E. orbicularis* (Rivera and Fernández, 2004) and could be very large (e.g. in Brenne, France; Owen-Jones et al., unpublished data). However microsatellites diversity for such population is unknown. To our knowledge effective population size for microsatellite has never been estimated for European pond turtles and have barely estimated for freshwater turtles in general (Spradling et al., 2010, Pittman et al 2011). Ours simulations show the importance of initial population size in promoting genetic differentiation after isolation leading to population differentiation. Assuming a total rupture of gene flow, the smallest demographic population around the lake (L1) will be differentiated from other populations 3 times more rapidly on 100 generations. On the other hand, tens of generations are necessary to obtain a start of differentiation with larges populations (L2 and L3).

Even under a large historical population hypothesis, most of the results indicate that the upstream river (L5) is a different genetic unit than the southern and western populations of the lake and since many generations. Only clustering analyzes incorporate L5 site in the same genetic unit than other sites. However, Rodriguez-Ramilo and Wang (2012), indicate that this approach is inefficient in the case of low differentiation and when populations are in Hardy-

Weinberg equilibrium (as our study). Rivers are unfavorable habitats for *Emys orbicularis* (Ficetola et al., 2004): in our study, low summer water level due to dam located more north in Bulgaria, and water extraction in Greece create a suboptimal habitat. Upstream area is also more anthropized: dam, road, highway and villages, greatly limiting actual dispersal. However, this recent fragmentation of the habitat has not yet been able to impact genetic structure and presumably genetic variations has been influenced by past landscape features (Richarson, 2012). Indeed, the upstream area is very different from other sites: just the river corridor a narrow riparian forest and absence of ponds. Over generations, isolation by landscape structures could be a factor of genetic differentiation.

Conclusion

The history of the species in the region, with a probable large and continuous population could have masked the impact of the dam and anthropogenic pressures on populations of European pond turtle. It will take many generations to have a genetic footprint of the consequences of such construction. Our study is not unique. The same lack of differentiation was observed for *Graptemys geographica* at dam in Canada, built there a hundred years ago (Bennett et al., 2010). Larges generations time in chelonians slow down the genetic erosion by genetic drift. Despite their low dispersal abilities, enhanced by the fragmentation of the landscape, terrapins seem to show a delayed response to global change compared to most species. However, their fate is inevitable by the constant increase in human constructions. It is therefore essential to maintain corridors between populations to avoid the isolation of populations and threaten their long-term survival.

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Table 1. Sample sites of *Emys orbicularis* in the Kerkini area in Greece. Acr.: Site Acronym. N = sampling size.

Acr.	Habitat	*Coordinates*	N	Male/Female
L1	Strymon River dowstream the dam	41°08N, 23°13E	38	18/20
L2	Kerkinitis River and ponds	41°14N, 23°01E	50	26/24
L3	Ponds and Strymon river upstream Vironia bridge	41°14N, 23°17E	51	27/24
L4	Ponds below Sidirokastro bridge	41°15N, 23°19E	41	21/20
L5	River upstream Sidirokastro bridge	41°19N, 23°20E	32	25/6

Table 2. Distribution of nucleotidic polymorphism and haplotypes (number and relative frequency) based on 1428 pb mtDNA Cytb sequences of 187 *Emys orbicularis* from 5 locations (L1-5) in the Kerkini area, Northern Greece. H: haplotype; Sites*: numbers refer only to polymorphic sites relative to the haplotype Ia published sequence (accession number: AJ131407) arbitrarily chosen as a reference sequence.

H	Sites*							Location					
	1	1	5	6	7	8	9	L1	L2	L3	L4	L5	Tot
	5	7	7	2	2	0	9						
	3	4	5	7	3	9	6						
Ia	C	C	C	A	C	T	A	0.43	0.43	0.59	0.57	0.75	100
Ib	T	-	-	-	-	-	C	0.03	0.17	0.18	0.16	0.25	29
IIa	-	T	T	G	T	C	C	0.54	0.40	0.23	0.27	0	58
Tot								35	47	44	37	24	187

-: identical nucleotide relative to the reference sequence.

Table 3. Diversity at 9 microsatellites within 5 *Emys orbicularis* sites in the Kerkini area, Northern Greece. N: sample size; A: mean number of allele; Ar and PAr: mean number allele and private allelic richness corrected by rarefaction the method for a common size of 32 individuals, respectively; RA: mean number of rare allele (<0.05 in all dataset); Ho and He: observed and expected heterozygosity, respectively; F_{IS}: Hardy-Weinberg Equilibrium measure.

Pop	N	A	Ar	PAr	RA	Ho	He	F_{IS}
L1	38	12.4	9.8	0.47	5.2	0.81	0.86	0.062 ^{ns}
L2	50	12.8	9.8	0.60	5.6	0.80	0.85	0.075 ^{ns}
L3	51	12.3	9.5	0.37	5.1	0.79	0.85	0.071 ^{ns}
L4	41	12.2	9.7	0.64	5.1	0.80	0.86	0.077 ^{ns}
L5	32	10.7	9.7	0.46	3.6	0.84	0.87	0.031 ^{ns}

ns: non significant deviation

Table 4. Population size variations of 5 *Emys orbicularis* sites in the Kerkini area, Northern Greece. N: sample size; TPM and SMM: p value of bottleneck detection (one tailed Wilcoxon test) for Two Phase Model and Stepwise Mutation Model respectively. N_{demo} and N_e: Demographic and effective population size estimated using CMR and Linkage Disequilibrium method respectively. In parenthesis 95% confidence interval (CI).

Pop	TPM	SMM	N_{demo}	N_e (95% CI)
L1	0.97 ^{ns}	0.85 ^{ns}	119 (09-137)	145 (52 - ∞)
L2	0.63 ^{ns}	0.63 ^{ns}	543 (500-622)	390 (103 - ∞)
L3	0.82 ^{ns}	0.82 ^{ns}	1471 (1355-1684)	657 (1068 - ∞)
L4	0.46 ^{ns}	0.82 ^{ns}	na	∞ (115 - ∞)
L5	0.21 ^{ns}	0.25 ^{ns}	na	239 (48 - ∞)

ns: non significant deviation. Na : not applied

Table 5. Connectivity as estimated through the number of adult individual, among the 1224 European pond turtle, initially marked at L3 site along the 14 years of CMR, monitoring at this site and caught during the 2011 blood sampling campaign. D: Number of days of sampling. N1: number of individuals caught in 2011 and N2 the number among N1 that were initially marker in L3.

Site	D	N1	N2
L1	11	71	0
L2	4	137	0
L3	7	99	99
L4	4	230	17
L5	9	40	0

Table 6. Differenciation between 5 sites of *Emys oricularis* from the Kerkini area, Northern Greece. Pairwise F_{ST} values for 9 microsatellites loci; D_{est}: Estimator of Jost's D measure of differentiation and θ_{ST} for Cytb mtDNA sequences

	Microsatellites		**mtDNA**
	F_{ST}	D_{est}	θ_{ST}
L1-L2	0.0041 ns	0.025 ns	0.0080 ns
L1-L3	0.0042 ns	0.035 ns	0.0972 ns
L1-L4	**0.0070 ***	**0.070***	0.0660 ns
L1-L5	-0.0353 ns	**0.087***	**0.3021 *****
L2-L3	-0.0008 ns	0.017 ns	0.0255 ns
L2-L4	0.0031 ns	0.027 ns	0.0066 ns
L2-L5	-0.0404 ns	0.032 ns	**0.1772 *****
L3-L4	-0.0104 ns	-0.009 ns	-0.0230 ns
L3-L5	-0.0484 ns	0.012 ns	0.0451 ns
L4-L5	-0.0333 ns	0.024 ns	0.0709 ns

Values in bold were significant after Benjamini – Yekutieli correction (α = 0.017) and at the nominal threshold (α = 0.05).

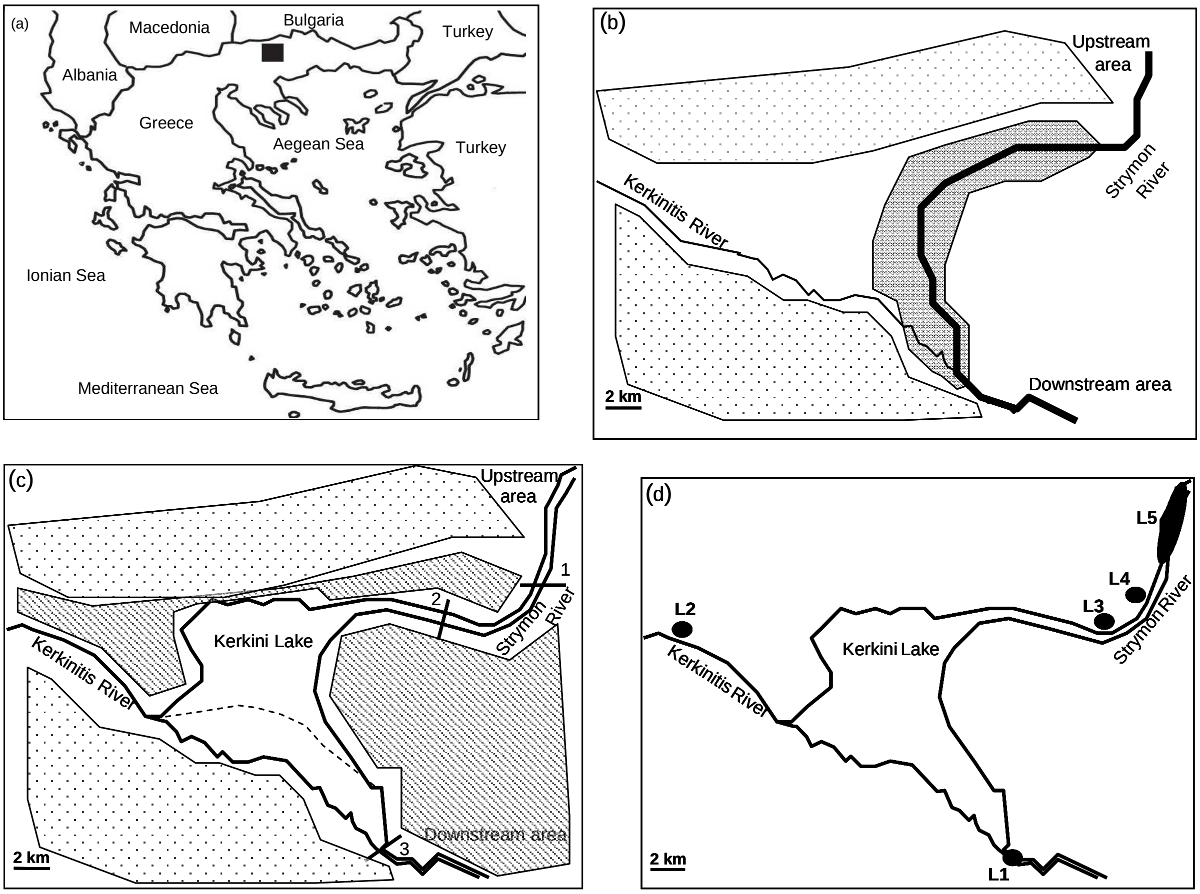

Figure 1. (a) Map of Greece with location (black square) of the Kerkini area (from Chelazzi *et al.*, 2006). (b) Map of Kerkini area before dam constructions. Dotted area: mountains. Light grey: floodplain). (c) Map of Kerkni area after dam constructions formation (1932 and 1982). Dashed line shows the initial delimitation of the lake after first dam building. Shaded area: agricultural patches (Crivelli et al., 1995a,b). 1: Sidirokastro Bridge; 2: Vironia Bridge; 3: Dam. (d) Location of sampled sites (black circles and ellipses). See Table 1 for details.

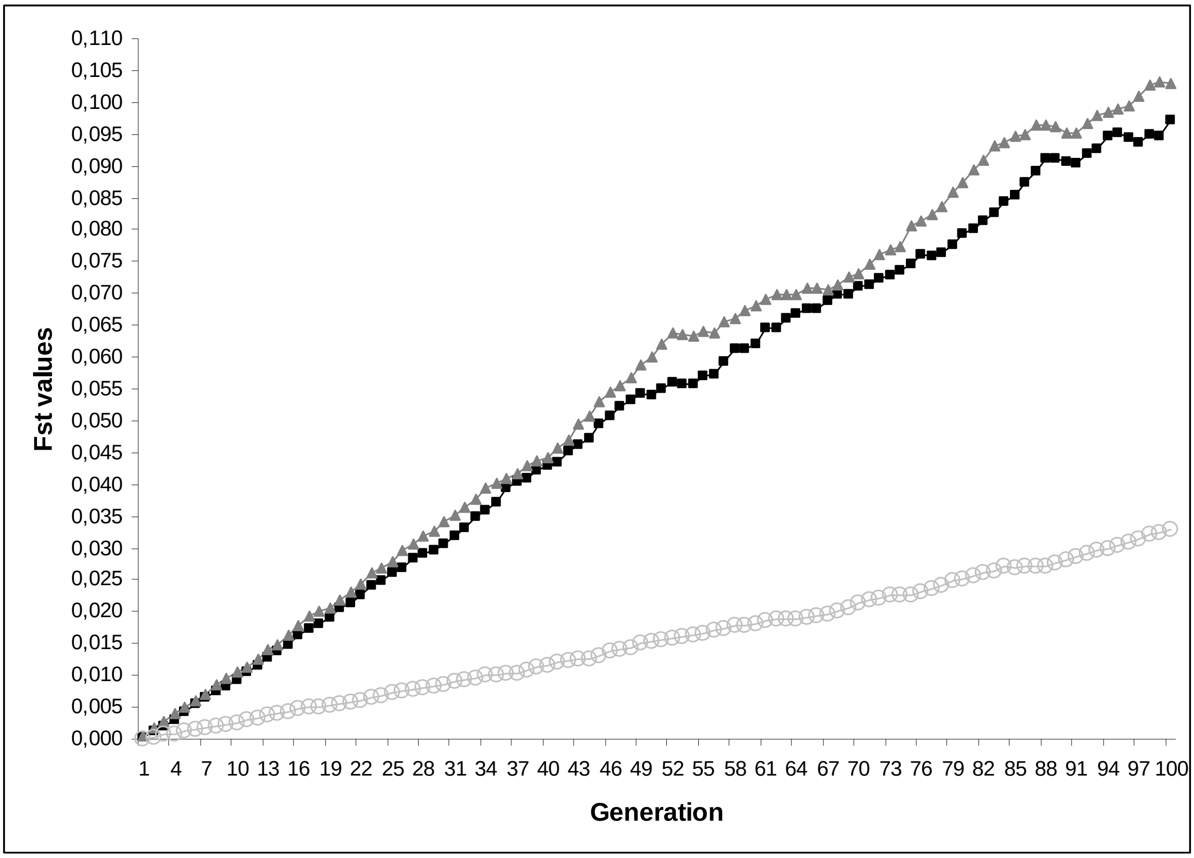

Figure 2. The simulation of future pairwise population differentiation (square for L1-L2, triangle for L1-L3 and ring for L2-L3) via F_{ST} values for different patch capacity (2000, 700 and 200 for L3, L2, L1; respectively) and sex-ratio of 0.5 to 0 from 100 generations without dispersion between sites in *Emys orbicularis*.

Chapitre 2

Study of demographic and genetic dispersal in a metapopulation context, example of the European pond turtle (*Emys orbicularis*) in Camargue, France.

Study of demographic and genetic dispersal in a metapopulation context, example of the European pond turtle (*Emys orbicularis*) in Camargue, France.

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Abstract

Dispersal is an essential ingredient of metapopulation dynamics. In the last decades, habitat loss and fragmentation pose the greatest threats to biodiversity and may precipitate a rapid decline in the probability of metapopulation persistence. In this context, understanding the dispersal in species with low movement capacities has become a prime importance to preserve their population survival. We studied the demographic and genetic dispersal in three populations only 2 km distant of European pond turtle in the Camargue by using capture-mark-recapture, set up on two of the three kernels over a 15 years period, and nuclear (microsatellites) and mitochondrial (cytochrome b) markers. Results show an absence of genetic differentiation and sex-biaised dispersal by a strong female philopatry and few male genes flow. We supposed that the carrying capacity is not reached in ours studied sites causing a relaxation of intra-specific competition not promoting dispersal. The stronger philopatry of female could be explained by the low availability of nesting site, increasing the cost of dispersal. In male, the avoidance of inbreeding depression could increase the benefit to dispersal. However, we showed that the disappearance of favourable habitat for *Emys orbicularis* and the rarefaction of nesting site, can be favour a strong site fidelity and separate populations even if they are closed geographically. This study show the importance to perverse the natural heterogeneity of wetland and conserve permeable corridors allowing to European pond turtle to move between sub-population and promoting the homogenisation of the genetic diversity by a metapopulation functioning.

Keyword

capture-mark-recapture, sex-biased dispersal, *Emys orbicularis*, metapopulation, microsatellite DNA, mitochondrial DNA.

Introduction

Habitat loss and fragmentation can have a very detrimental effect on metapopulation dynamics (Carlson, 2000) and are considered as being among the greatest threats to biodiversity (Wilcove et al., 1998). The decline of movements between patches and loss of suitable habitat may have very negative consequences on the dynamics and structure of populations and endangering species persistence (Gibbs, 1993). Species with limited dispersal capacities are particularly sensitive to fragmentation and habitat loss because the recolonization dynamics may be seriously altered preventing the metapopulation functioning (Cushman, 2006).

Wetlands are particularly the target of destruction or degradation. In particular, multiple sources of degradation such as fragmentation, invasive species or pollution have detrimental impacts on wetland species metapopulation dynamics (Marsh and Trenham, 2001) and are responsible of the decline of many species in herpetofauna (Rizkalla and Swihart, 2006). The consequence of habitat loss and degradation has been well documented in amphibians (Marsh and Trenham, 2001) but is still poorly known in freshwater turtles. This lack of knowledge results partly from the scarcity of studies on the metapopulation dynamics of freshwater turtle. Hence, the dispersal abilities of turtles in a fragmented wetland landscape were the connectivity are largely undetermined needs to be investigated (Rizkalla and Swihart, 2006). Indeed, Gibbs (1993) revealed that local populations of freshwater turtles faced a significant risk of extinction after loss of small wetlands. Freshwater turtles have limited dispersal capacities but several studies show a metapopulation functioning. For example, 33% of the individuals of *Chelodina longicollis* moved among the landscape patch (Roe et al., 2009). In a theoretical approach is was shown that the probability of extirpation was reduced by 42% under a metapopulation model suggesting that dispersal between breeding ponds is important for population persistence (Enneson and Litzgus, 2009). However, very few studies have documented freshwater turtle dispersal in a landscape with a potential metapopulational functioning.

The Camargue, the geographical zone that corresponds to the Rhone delta, is one of the most extensive European wetland, but it is subjected to multiple pressures that generate habitat degradation (Picon, 2008). This area consist of a floodplain of 145 300 ha, is recognized worldwide for its diversity of habitats (salt steppes, lagoons, marshes) and its biological richness (Mathevet, 2004). The European pond turtle (*Emys orbicularis*), one of three French native turtle species is one of the emblematic species of this complex ecosystems. However, the species is largely impacted by diverse anthropogenic factors such as habitat loss (drainage; Bodie, 2001), construction of canals, regulation of rivers and shoreline development that generate a strong homogenization of unfavourable habitat and fragmentation of populations (Rogner, 2009). The strong impact of these human modifications on the *Emys orbicularis* populations are partly explained by the limited dispersal abilities of this species that are severely reduced by impenetrable habitats (e.g. Duguy and Baron, 1998; Olivier, 2002;Cadi et al., 2008). Generally, female European pond turtles showed higher site fidelity (Bona et al., 2012) and it seems to be dependent of nesting site availability. Males had more important movements assumed to be related to mating search and therefore favouring gene flow (Olivier et al., 2010). Such a pattern is generally observed in the majority of freshwater turtles (Sheridan et al., 2010)

In this study, we investigated the dispersal of three kernels (i.e. area of spatial aggregation of individuals or at high density seen feeding and basking) of *E. orbicularis* on the Natural Reserve of the Tour du Valat in the Camargue, supposed to be less impacted by human activities. These kernels, distant by 2 km at maximum, are connected by ecological corridors such as marshes and canals of variable structural connectivity. This landscape structure suggests that the three populations are part of a metapopulation and are connected by dispersal processes. We used two complementary approaches to quantify functional connectivity between the kernels. The first approach was based on capture-mark-recapture (CMR) to estimate population dynamics whereas the second approach was based on molecular markers analysis. Since 1997 a CMR method has been implemented on two of the three kernels allowing the estimation of the probabilities of individual movements between these two units. The CMR method allows estimating precisely the movements of transitions, but cannot determine if this movements leads to gene flow. To estimate the effective dispersal, we used mitochondrial (Cytb) and nuclear (microsatellites) genetic markers. These markers were used to determine the population genetic structures and to identify recent migrants between the three kernels. The phylogeography and post-glacial colonization of European pond turtle are extensively studied in Europe (Fritz et al., 2005; Pedall et al., 2011). In Camargue, we suspected the presence of three haplotypic lineages originating from three different glacial refuges and associated with Holocene recolonization: the lineage II (*E. orbicularis orbicularis*) from the Balkans, V (*E. orbicularis galloitalica*) from South Italy and VI (*E. orbicularis fritzjuergenobsti*) from Spain (Pedall et al., 2011).

Materials and Methods

Study area

Our study sites were located at the Tour du Valat in Camargue (southern France, ~ 43°30’ N, 4°40’ E; Fig. 1). The three kernels studied, are ~2 km away from each other, and two of them (Esquineau and Faïsses, ~1.5 km distant) have been monitored by CMR since 1997 (see below). This annual monitoring feeds a database with a cumulative total of 736 individuals identified in 2011 for Esquineau and Faïsses combined. Esquineau Faïsses and the third kernel (Draille) have a total area of 250, 100 and 275 ha, respectively. At all sites *Emys orbicularis* inhabit two kinds of habitats: permanent and semi-permanent marshes managed by man-made irrigation and drainage canals (Olivier et al., 2010). Each site is composed by a unique meadow used as a nesting site. Both sites are interconnected by a network of canals and marshes representing structural connectivity for *E. orbicularis* (Fig. 1).

Capture-mark-recapture protocol

From 1997 to 2011, turtles of the Faïsses and the Esquineau were captured from April to August with fishtraps and by hand. Traps were placed in canals and marshes (22 in Faïsses and 39 in Esquineau on average, Fig.1) and were deployed in the field, alternating weekly between kernels, during three to five consecutive days and were visited daily in the morning. We also made hand captures during these daily visits. We recorded for each capture, the age, sex and morphological measure. Sex was identified by observing male secondary sexual characteristics proposed by Zuffi and Gariboldi (1995): concave plastron, orange eyes (yellow in females), basic wide tail and cloacae away the plastron. Turtles were classified as adults if they had no visible growth rings (Castanet, 1988; Olivier, 2002). We marked each individual with permanent notches cut into the carapace marginal scutes with a small hacksaw (Cagle, 1939). Turtles were returned within 24 h to the place where they had been collected. The dataset consisted of capture–recapture histories from 528 different adult animals (274 females and 254 males). Juvenile data were excluded from analyses.

General framework and consideration of heterogeneity.

We used multi-state capture-recapture models to analyze the CMR database (Nichols and Kendall, 1995). In these models, the status of individuals is noted at each occasion. In our case study of a spatially structured population, "states" represent different localities (the two kernels studied). The advantage of this multi-state approach is to estimate the probabilities of transitions between states, in addition to the more conventional estimate capture and survival probabilities (Lebreton et al., 2003.; Choquet et al., 2009). We estimated the transition probability outside our studied site by creating factice “ghost site” for each kernel. The outside transition probability allows quantifying the probability that an individual that lived a given site return to the site or move to the other site. With this method, we estimated the indirect transitions between our kernels. To take into account heterogeneity of captures observed in our data set, we defined two categories of individuals: Fidelity (only present in real sites) and No fidelity (present in real and factice sites). To quantified the percentage of individuals that lived definitively the two kernel system, we created a supplementary “ghost site” witch estimated the movement from the kernels to this ghost site, without possible returns. Finally, we defined 8 elementary states for our dataset: Fidelity to Esquineau (F1), Fidelity to Faïsses (F2), No fidelity to Esquineau (NF1), No fidelity to Faïsses (NF2), Out from Esquineau (O1), Out from Faïsses (O2), Out definitely (OD), and Dead (D).

The observation of an individual in the field is an event that provides information regarding the underlying state of an individual which most of the time, remain uncertain (Pradel, 2005). However, multi-event models allow modeling the probability of an individual being in a given state depending on the events associated with this individual. In the field, *Emys orbicularis* observations is coded into 3 events, Not seen/not caught (S0), Seen/caught at Esquineau site (S1), and Seen/caught at Faïsses site (S2). We denoted Π the initial state, φ the apparent survival probability from one occasion (one year) to the next, ψ the conditional transition probability from the locality state *r* at time *t* to locality state *s* at time *t*+1, given that the individual survived to *t*+1, α the probability to change of strategy (No fidelity to Fidelity). Capture probability and event probability for an animal reencountered in a given state are defined by β. Designs matrices are given in Table 1.

Contraints

The survival probabilities per site were difficult to estimate. To improve survival estimation and modelling quality, we fixed the same survival for both sites. In the same way, the survival estimations were less precisely when we estimated the transitions to OD. The models were better when the probabilities to OD were fixed to 0. Therefore, no estimation of definitely transition outside Esquineau and Faïsses was determined.

Model building

Analyses were realised with E-SURGE (Choquet, 2007) and U-CARE software (Choquet et al., 2005). Model building is done gradually by the analysis of parameters on the transition, survival, strategy and capture probabilities estimations. Parameters tested are sex, time and sites. All models were compared on the AIC (Akaike's Information Criterion, Burnham and Anderson, 2002) basis as suggest by Lebreton et al. (1992) corrected for small sample size (QAICc). The best model to explain our data is the one with the smallest value. Models with a QAICc difference less than 2 are considered as equivalent to describe the data.

Blood sampling for the genetic analyses

We collected 196 blood samples from *E. orbicularis* at the Tour du Valat, 64 from the Faïsses (29 males and 35 females), 71 from the Esquineau (31 males and 40 females) and 61 from the Draille (33 males and 28 females; Fig.1). Blood were sampled from dorsal tail vein (or dorsal coccygeal vein) and stored in preservation buffer (Seutin et al., 1991). We collected blood samples of up to 0.2 ml from terrapins aged above 3-4 years old. Genomic DNA was extracted from 15µl of blood, using a standard phenol-chloroform protocol (Hillis et al., 1996). The resulting DNA pellets were resuspended in 100 µl TE buffer, pH 8.

Mitochondrial and microsatellites DNA amplification and sequencing

We amplified by Polymerase Chain Reaction (PCR) 1428 pb of cytochrome b (Cytb) from mitochondrial genome and fourteen microsatellites loci from nuclear genome. We used newly designed forward EM-GluCytb-F (5'-CCACCGTTGTATTCAACTAC-3') and reverse EM-ThrCytb-R (5'-AGTCTTCAGTCTTTGGTTTAC-3') Cytb primers. For microsatellites, we used primers proposed in the literature following a M13 tailing strategy (Schuelke, 2000): msEo21, msEo41 (Pedall et al., 2009), Emys 1, Emys 8, Emys 11 (Ciofi et al., 2009), GmuD16, GmuD51, GmuD55, GmuD62, GmuD87, GmuD88, GmuD93, GmuD107, GmuD114 (King and Julian, 2004). All PCR reactions were performed in a total volume of

20µl, including 2µl of 1/10 diluted DNA, 1X of HotMaster^{TM} Taq buffer (5PRIME®), 200µM of dNTPs, 200nM of each primers and 0.25 (cytb) or 0.45 (microsatellites) unit of Taq polymerase HotMaster^{TM} (5PRIME®). For microsatellites, 100nM of M13 fluorochrom (700 or 800) labelled primer were added. According to the DNA marker, PCR conditions were, for cytb/microsatellites respectively, 1 min 30 sec/5 min at 94°C followed by 34/35 cycles of 30/40 sec denaturing at 94°C, 45/30 sec annealing at 55/56°C, and 45 sec/1 min extension at 65°C. Final elongation step was at 65°C for 10/5 min. For mtDNA, PCR yield and specificity was checked by running a 2 µL aliquot on a 2% agarose gel, and PCR products were purified with Exonuclease 1 (2 units) and Shrimp Alkaline Phosphatase (1 unit) enzymes for 1 hour at 37°C. Sequencing was performed by MACROGEN® society in The Netherlands following a Big Dye sequencing protocol. We edited and aligned the sequencing chromatograms by manually with MEGA 5 software (Tamura et al., 2011). We included as references the haplotypes IIa (accession number: AJ131411), IVa (accession number: AJ131417) and Va (accession number: AJ131420).

Hardy Weinberg Equilibrium (HWE) and disequilibrium linkage

The Weir and Cockerham (1984) estimator of F_{IS} were calculated for each kernel from multiloci microsatellite data, and significant difference of F_{IS} from zero (indicating a deviation from Hardy Weinberg Equilibrium) was tested using 1100 random permutations. When HWE departure was detected, the program MICRO-CHECKER (van Oosterhout et al., 2004) was used to test for genotyping errors origin. Three loci (msEo21, GmuD93 and GmuD51) showed significant heterozygote deficit for all sites and were excluded from all subsequent analyses. We estimated also potential linkage disequilibrium for each pair of loci and for each kernel. Both analyses were implemented with FSTAT 2.9.3. (Goudet, 2002a).

We adjusted the nominal level of significance (5%) correcting for multiple comparisons using Benjamini–Yekutiely corrections (BY; Benjamini and Yekutieli 2001). BY corrections provides a better compromise between type I and II errors (Narum, 2006) comparatively to Bonferroni correction that is very conservative.

Diversity measures

We estimated the genetic diversity at microsatellites loci by calculating the mean number of allele (A), and the mean number of allele (Ar) and private allelic richness (PAr) both corrected by rarefaction method, for a common size of 61 individuals (Kalinowski, 2004; Leberg, 2002). All these analyses were realised with HP-RARE 1.0 software (Kalinowski, 2005).

Phylogeography and population structure

To demonstrate that enough time ago to allow mixing for the nuclear genome between haplotypic lineages or complete introgression (as observed in other *E. orbicularis* contact zones Pedall et al 2011) and that phylogeographic process is not a driving force of the actually pattern of microsatellites differentiation, we first tested that no nucleo-cytoplasmic disequilibrium wad persisting by checking F_{ST} according to mtDNA lineage. Consequently, we supposed that the differentiation for microsatellite not would occur according to mtDNA haplotype lineage but rather than another factor (e.g. geography). Then, to test than enough ancient mixing have erased any anatomic differences between haploptypic lineages at the Tour du Valat, we attempted to explain morphometric differentiation using length of the back according to haplotypic lineage, sex and site (Esquineau and Faïsses, no data for Draille) by using Generalized Linear Models (GLM) with a Gaussian distribution for the error term. We evaluated the fit of models by checking normality, independence and homoscedasticity of residuals. Model selection was achieved using the Akaike Information Criterion corrected for small sample size (AICc; Burnham and Anderson, 2002). Model with the lower AICc value is considered as the best model for the data at hand. Models with an AICc difference less than 2 are considered as equivalent to describe the data. GLM were implemented in R version 2.7.2 (Development Core Team, 2008)

Genetic dispersal analysis

To compare with demographic dispersal estimated by CMR, we determined the genetic dispersal among kernels by three different approaches.

The first approach is based on comparisons of sample units (the kernels). Differentiation for nuclear markers was assessed for both sex combined and for male and female separately. If connectivity for one sex is higher than the other, one would expect lower or even non-significant differentiation when measured for this sex relative to the other measure for combined sexes (Goudet et al., 2002b). Similarly, differences in the level of differentiation for bi-parentally inherited markers (microsatellite) compared to maternally inherited markers (mtDNA) could be associated with differential dispersal according to sex (e.g. lower differentiation of mtDNA *vs* microsatellite is expected if females are less dispersing; Prugnolle and De Meeûs, 2002). For this, we quantified differentiation level using multi-loci F_{ST} index for microsatellites (Slatkin, 1995) and by θ_{ST} an analogue index for mitochondrial data (Excoffier et al., 2005). Indirectly, this method allowed measuring the global dispersal level between the kernels. For microsatellites, F_{ST} was also calculated for males and females separately to quantify gender-specific dispersal. Statistical significance was tested with 10 000 permutations of individuals between sites. All calculations were realised using ARLEQUIN version 3.5 software (Excoffier et al., 2005) and the nominal level of significance (5%) was adjusted with BY corrections.

The second approach is to infer genetic structure at the same time by clustering individuals into genetic units (called *K*) and estimating the probability an individual have to belong to this cluster *i*. We used the Bayesian clustering method implemented in the software STRUCTURE (Pritchard et al., 2000) to identify the most probable number of genetic units *K*. We performed five runs for each value of simulated K (between 1 and 5) to assess consistency of estimations of log-likelihood of the posterior probability of the data for a given *K* (ln Pr(X/K)). Our calculation were based on 500 000 MCMC iterations with an initial burn-in of 100 000 steps. Admixture model with non-independent allele frequencies were chosen Falush et al., 2003). The posterior value of *K* (Pr(K/X)) we computed following Eq. 3 in Pritchard et al. (2000) using R ver. 2.7.2 (Development core Team, 2008). We used the statistic proposed by Waples and Gagiotti (2006), recommended especially when the observed level of differentiation was low (see results), rather than Evanno's test (Evanno et al., 2005).

Third, we tried to identify first generation migrants (F0 migrant here after) characterised by the fact that they were produced genetically in one population but were sampled in another one. This approach is in part analogous to the previous one but can be based on other analytical tool and the rate at which type one error could occur can be controlled. We used GENCLASS2 2.0 software (Piry et al., 2004) to implement Likelihood L $=L_{origin/}$ L_{max}, using the frequencies-based method proposed by Paetkau et al. (1995) and the Monte Carlo re-sampling method (Paetkau et al., 2004), for a given allele frequency distribution [72]. This likelihood was computed according to Paetkau et al. (2004) [72] re-sampling algorithm for 100 000 simulations and an alpha level of 0.1%.

Results

Population dynamics

The survival is high for both sex (Table 2, M4 vs. M5, ΔQAICc=35) and reach the value of 0.920 ± 0.012 for male and 0.959 ± 0.007 for female.

The fidelity to site is lower in males 0.036 ± 0.046 than in females 0.136 ± 0.074. The probability to change from Fidelity to No fidelity categories is low 0.046 ± 0.024 and independent of any parameters (Table 2, M6 vs. M7, ΔQAIVc= 75).

The probability of capture is on average 0.78 ± 0.06. Taking account the effect of site and time on capturability reducing the QAICc (Table 2, M8 vs. M9, ΔQAICc=21).

Movements from Faïsses and Esquineau to their respective ghost sites, and vice versa, are important for both sexes (0.427 ± 0.071 and 0.363 ± 0.080 for females and male on average, respectively; Fig. 2). On the contrary, direct and indirect movements between Faïsses and Esquineau are low for males (0.027 ± 0.009 on average; Fig. 2) and null for females (Fig. 2). Values of QAICc are better when direct (from Faïsses to Esquineau, and vice versa) and indirect (from Faïsses to Esquineau via the ghost site, and vice versa) transitions are fixed equals for each sex respectively (Table 2, M1 vs. M2, ΔQAICc=53) and without time effect (Table 2, M2 vs. M3, ΔQAICc=180).

Genetic structure

Three haplotypes, IIa, IVa and Va from three haplotypic lineages (II, IV and V) were observed out of a total of 168 individuals, sequenced from our 3 studied sites, based on 1428 base pairs mtDNA cytochrome b sequences. We found 43%, 55% and 2% of haplotypes IIa, IVa and Va at Faïsses (for 63 individuals), respectively. Esquineau (for 61 individuals) is also including the three haplotypes: 64% of haplotype IIa, 5% of haplotype IVa and 31% of haplotype IVa. Finally, we found at Draille (for 44 individuals), 66 % and 34% of haplotype IIa and Va, respectively. Haplotype Va differed for 19 nucleotides with both haplotypes IIa and IVa (1.3% divergence) while a 20 nucleotides difference is observed between the haplotype IIa and the IVa (1.4% divergence). Haplotype IIa is the most frequent (59.7%) and haplotype IVa can be considered rare (frequency ≤ to 5%).

Microsatellite loci appeared to be inherited independently of each other, as no evidence for linkage disequilibrium was found for any pair of loci. Therefore, eleven loci were obtained for 196 individuals from the 3 sites (Table 3). The genetic diversity is high with an uncorrected (A) and a corrected for a common size of 61 individuals (Ar) average of 12.83 and 11.23 allele per locus, respectively. The private corrected allelic richness (PAr) was 1.23. All populations showed no deviation to Hardy-Weinberg Equilibrium. To finish, microsatellites showed no genetic structure by haplotypic lineage (F_{ST} = 0.0005, P=0.7204). The morphology (length of the back) of turtles was also not explained by haplotypic lineage but best explained by site and sex (Table 5, M1 *vs* M5, ΔAICc = 117.8).

All measures of differentiation site pairwise for microsatellites (F_{ST}) either for both sexes combined or for each sex alone were significant (Fig.4). Site pairwise estimates of θ_{ST} ranged from 0.003 (between Esquineau and Draille) to 0.092 (between Faïsses and Draille) and differentiation was revealed between Faïsses and Esquineau (Table 4). Bayesian clustering methods found also genetic structure and indicated both maximum likelihood and highest posterior probability for 3 genetic clusters corresponding to our 3 studied sites (K=3; P (K=3) =1; Fig. 3). We note one first generation migrant (F0), one male, genetically belonging to Esquineau but captured in the Faïsses cluster. GENECLASS analyses confirmed this result with the same male sampled in Faïsses genetically assigned to Esquineau. This male was captured in the adult stage for the first time in 1998 at the Esquineau sub-population and was recaptured 24 times only in the Faïsses sub-population since 1999.

Discussion

The objective of our study was to quantify the dispersal between populations kernels of the European pond turtle at the Tour du Valat, in the Camargue, connected by potentials aquatics corridors that would offer structural connectivity. We used data from a capture-mark-recapture (CMR) monitoring since 15 years between two kernels and genetic markers (mitochondrial DNA and microsatellites) to estimate the dispersal including a third kernel. The contribution of molecular markers is important because it provides information on the degree of differentiation among populations but is not sufficient to quantify dispersal behaviour because population genetic structure results mostly from a balance between genetic drift and gene flow (Bohonak, 1999). The CMR approach is a pertinent complement to quantify movements between sub-populations (Vandewoestijne and Baguette, 2004).

Our starting hypothesis was that several kernels of population were connected by corridors and form a unique genetic population with metapopulation functioning. Our results do not support this hypothesis. The CMR models and the genetic markers provide concordant information for no dispersal between Faïsses and Esquineau. Indeed, we found a significant global F_{ST}, three genetic clusters corresponding to the three studied sites and high level of private allele between populations (Kalinowski, 2004). We supposed very limited connectivity from males (with the detection of a single F0 male migrant) with a total demographic dispersal estimated by CMR at 2.7%, and with null movements estimated by CMR and genetic assignment for females.

This observed pattern could results from phylogeographic processes. Indeed, three mtDNA lineages have been found in Camargue (II, IV and V). These lineages results from glacial refugia and their co-occurrence in Camargue results from Holocene range expansion and secondary contact (Fritz et al., 2007; Pedall et al., 2011). Assortative mating for haplotype (i.e males with a haplotype lineage mating preferentially with females of the same haplotype lineage) could exist and each lineage could own a set of private alleles and since the relative frequency of lineage is unbalanced in each kernel, this could partially explain the F_{ST} and the private allele pattern found. However, no evidence of assortative mating for haplotype is known in *Emys orbicularis*. On the contrary, in this species, the mate choice is size dependent. Poschadel et al. (2006) showed through a simultaneous binary choice test that male prefers to mate with larger females. Female fecundity is positively correlated with female size in European pond turtles, which may account for male choosiness (Poschadel et al., 2006). However, females did not show a preference. In addition, we showed throught a GLM analysis that the individuals size is explained by sex and population. There is no significant difference of individual size according to haplotype. Mate choice, therefore, is probably not by assortative mating by haplotype. Moreover, this pattern of differentiation involved enough mixing or complete introgression occurring between lineages enough time ago as differentiation for microsatellite was not structure by haplotypic lineage.

The structuration pattern found among kernels could more likely the results from the behavioural ecology of pond turtle, where female are highly philopatric and where a male sex-biased dispersal occurs (Sheridan et al., 2010), althought it seems very limited here. In freshwater turtles, the female philopatry is explained by nesting site fidelity (Freedberg and Wade, 2001). At the Tour du Valat, the numbers of nesting site are limited (i.e one per kernel). For a female, the probability to find other favourable nesting sites is very low. Hence, reproductive success of dispersal behaviour in female is probably very low. More generally, female philopatry might be favoured by selection because female juvenile benefit from the territory of their mother and have good resources for laying (Greenwood, 1980). On the contrary, to avoid inbreeding depression, the dispersal is less costly in male (Ebert et al.,

2002) and they are advantaged by prospecting female (Andres and Chambers 2006).However, in the present study, the gene flow generated by males is insufficient to homogenize the genetic diversity. In addition, each kernel in our study is at HWE and the level diversity is in the upper range of what could be observed in European pond turtles (Velo-Anton 2007, 2008; Pedall et al., 2011; Molnar et al., 2011). Therefore, the need to avoid inbreeding depression might be questionable in each kernel, limiting dispersal decision taken by males. Even if the dispersal capacities are limited in the European pond turtle (Lenk et al., 1999), the movements of 1 or 2 km are quite feasible for both sexes (Kotenko, 2000; Cadi, 2003). This ability may be more important and has allowed the recolonization of Europe after the last glaciation via "long distance dispersal" of few individuals using rivers, creeks, lakes and swamps, including recolonization of South Scandinavia from the Balkans (Sommer et al., 2007). After northern Europe was reached, a minimum dispersal of 50 km per 100 years (500m per year) may be hypothesized to explain this recolonisation since ~10 000 ans (Sommer et al., 2007).

Our pattern of differentiation is another example to demonstrating that aquatic connectivity of habitats does not necessarily equate to genetic connectivity (Sheridan, 2010; Molnar et al., 2011). Several studies showed that dispersal is triggered by density-dependence (Travis et al., 1999). Indeed, is the resources are abundant and the density of individuals is low, the intra-specific competition is relaxed. Consequently, the philopatry is less costly that dispersal because the probability to find another favourable site is low. At the Tour du Valat, we supposed that carrying capacity is not reached because the density is moderate and food abundant (Olivier, 2002) allowing increase of population sizes (Ficheux, unpublished data). For both sexes, it could be more advantageous to show high fidelity, and could allow allocating energetic resources in the survival and reproduction. The higher survival rate of female found in our results can be explained by sex-biaised survival frequently found in turtle (Litzgus, 2006; Martins and Souza, 2009). This mechanism allows maximizing the reproductive success (Litzgus, 2006). The life history theory provides a compromise between survival and reproduction. In chelonians, late sexual maturity and iteroparity maximize reproductive success of individuals and offset the high variability of eggs and juveniles survival (Cunnington and Brooks, 1996). This strategy, called bet-hedging, could partly explain the observed differences in survival between sexes. The survival is lower in males because the reproduction costs are more important with the partner research (Litzgus, 2006). On the other part, the economy of energy resources by female philopatric could favorite the reproductive success by multiple lay, two or three clutches in Camargue are frequent (Olivier, 2002). The sperm storage capacities, know in European pond turtle (Roques et al., 2006), allow a multiple paternity and promoting the genetic diversity of these clutches.

The pattern of dispersal between the Faïsses and the Esquineau is also observed with the third kernel, the Draille. The F_{ST} results show a significant differentiation between all sub-populations. We cannot rule out a connectivity problem at the Tour du Valat. Although potential corridors are abundant in Camargue, the Rhone delta have experienced major habitat degradation and loss associated with fragmentation (Picon, 2008). Fragmentation affects in priority species with low dispersal abilities (Gibbs, 1993). It is also quite possible that the quality of ecological corridors i.e. their permissiveness to turtle, is poor. In a recent study, Cosentino et al. (2010) found that the habitat patche surfaces, their connectivity and habitat quality influenced the spatial dynamics of the freshwater turtle *Chrysemys picta*.

Comparing F_{ST} per sex and both sexes combined as well as with θ_{ST} was inconclusive about differential dispersal according to sex. Such results could be explained by the over low level of differentiation, limiting the power of such approach (Goudet et al 2002, Prugnolle and De Meeûs, 2002). Moreover, the CMR data identity high transition with ghost kernel suggesting that the kernel are not the actual spatial limits of the demographic populations. In addition, some individuals overlapped areas of the studied sites whereas others seemed to be capture within the same area over several years. This capture heterogeneity has been taken into account by the determination of two states: Fidelity and No fidelity. The probability of changing from one state to another is very low. In other part, the rate of intra-population transition, as measured by the transitions between the sites and their ghost sites, are important. Generally, when a pond turtle leaves the Faïsses or Esquineau sites, the animal returns to their original site. We supposed that these intra-population transitions pattern suggest that in some years these individuals will be unavailable (i.e. not catchable, because at the periphery of kernel) and will be considered to have temporarily "transited out" of the study areas.

From a demographic and genetic population point of view, population kernels at the Tour du Valat are not functioning as a dynamic metapopulation (Hanski and Gaggiotti, 2004) with strong female philopatry and few gene flow provided by male. Through this study, we show that the disappearance of favourable habitat for *Emys orbicularis* and the rarefaction of nesting site, can be favour a strong site fidelity and separate populations even if they are closed geographically. Consequently, in this case, if an extinction of a local population occurs, the absence of connectivity would not allow the recolonization of the site (Cushman, 2006). On the other part, if the isolation continues, the genetic diversity will decrease producing a higher vulnerability to stochasticity (Reed and Frankham, 2003). It is crucial to perverse the natural heterogeneity of wetland and conserve permeable corridors allowing to European pond turtle to move from a familiar site to an alternative site and promoting the homogenisation of the genetic diversity.

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Table 1. Design matrices of capture-mark-recaptures models with initial state matrix (a) and transitions matrix decomposed in survival probabilities (b), transitions probabilities between locality state (c), probabilities to change of strategy (d) and events probabilities (e).

a)

F1	**F2**	**NF1**	**NF2**	**O1**	**O2**	**OD**
π	π	π	*	-	-	-

b)

	F1	**F2**	**NF1**	**NF2**	**O1**	**O2**	**OD**	**D**
F1	φ	-	-	-	-	-	-	*
F2	-	φ	-	-	-	-	-	*
NF1	-	-	φ	-	-	-	-	*
NF2	-	-	-	φ	-	-	-	*
O1	-	-	-	-	φ	-	-	*
O2	-	-	-	-	-	φ	-	*
OD	-	-	-	-	-	-	φ	*
D	-	-	-	-	-	-	-	*

c)

	F1	**F2**	**NF1**	**NF2**	**O1**	**O2**	**OD**	**D**
F1	*	-	-	-	-	-	-	-
F2	-	*	-	-	-	-	-	-
NF1	-	-	*	ψ	ψ	-	ψ	-
NF2	-	-	ψ	*	-	ψ	ψ	-
O1	-	-	ψ	ψ	*	-	-	-
O2	-	-	ψ	ψ	-	*	-	-
OD	-	-	-	-	-	-	*	-
D	-	-	-	-	-	-	-	*

d)

	F1	**F2**	**NF1**	**NF2**	**O1**	**O2**	**OD**	**D**
F1	*	-	-	-	-	-	-	-
F2	-	*	-	-	-	-	-	-
NF1	α	-	*	-	-	-	-	-
NF2	-	α	-	*	-	-	-	-
O1	-	-	-	-	*	-	-	-
O2	-	-	-	-	-	*	-	-
OD	-	-	-	-	-	-	*	-
D	-	-	-	-	-	-	-	*

e)

F1	**S0**	**S1**	**S2**
F1	*	β	-
F2	*	-	β
NF1	*	β	-
NF2	*	-	β
O1	*	-	-
O2	*	-	-
OD	*	-	-
D	*	-	-

Table 2. Modelling transition (*Ψ*) between localities (Faïsses and Esquineau and their respective ghost sites), for survival, strategy and capturability probabilities (φ, α, β respectively) in the European pond turtle (*Emys orbicularis*) at the Tour du valat, South France. Results of model selection include: number of mathematical parameters (k), deviance and Akaike Information Criterion value corrected for small sample size (QAICc) and difference of QAICc (ΔQAICc) between models and the model with the lowest QAICc for each modelling (M2, M4, M7 and M9, respectively).

No.	Models	Constraints and biological hypothesis	k	Deviance	QAICc	ΔQAICc
		A) Model selection for localities transition				
M2	Ψindirect&direct-site.sex	Equals indirect + direct transition for both sex respectively	21	5803	5845	0
M1	Ψsite.sex	Site and sex dependence	16	5966	5898	53
M3	Ψindirect&direct-site.sex.time	Equals indirect + direct transition for both sex respectively and time dependence	197	5585	6023	125
		B) Selection hypothesis, survival analysis				
M4	φsex	Survival function of sex	74	5549	5702	0
M5	φsex.time	Survival function of sex and time	99	5529	5737	37
		C) Selection hypothesis, strategy				
M7	α	Strategy independent	46	5622	5716	0
M6	αsite.sex.time	Strategy dependent of site, sex and time	98	5584	5791	75
		D) Selection hypothesis, capturability				
M9	βsite.time	Capturability dependent of time and site	50	5616	5718	0
M8	β*site.sex.time*	Capturability dependent of sex, site and time	76	5581	5739	21

Table 3. Diversity at 11 microsatellites within three sites of *Emys orbicularis* at the Tour du Valat, South France. N: sample; A: mean number of allele; Ar and PAr: mean corrected number allele and private allelic richness by rarefaction method for a common size of 61 individuals, respectively; RA: mean number of rare allele (<0.05 in all dataset); F_{IS}: Hardy-Weinberg Equilibrium measure.

Population	N	A	Ar	PAr	F_{IS}
Draille	61	12.9	11.4	1.39	0.059 ns
Esquineau	71	13.1	11.3	1.26	0.064 ns
Faïsses	64	12.5	11.0	1.00	0.055 ns

ns: non significant deviation

Table 4. Differentiation between three sites of *Emys oricularis* from the Tour du Valat, South France. F_{ST} , $F_{ST\text{-}Female}$ and $F_{ST\text{-}Male}$: pairwise F_{ST} values for 11 microsatellites loci for both sex combined, females only and males only, respectively. θ_{ST} for Cytb mtDNA sequences.

	Microsatellites			**mtDNA**
	F_{ST}	$F_{ST\text{-}Female}$	$F_{ST\text{-}Male}$:	θ_{ST}
Faïsses-Esquineau	0.01*	0.026*	0.011*	0.069*
Esquineau-Draille	0.013*	0.010*	0.030*	0.003
Faïsses-Draille	0.017*	0.021*	0.028*	0.092

* Significant values significant after Benjamini – Yekutieli correction (α = 0.027) of the nominal threshold (α = 0.05).

Table 5. Detection of first generation (F_0) migrants assessed by GENECLASS 2 of European Pond Turtle (*Emys orbicularis*) as a percentage of individuals (absolute number in parenthesis) assigned genetically to a putative kernel of origin given the kernel were individuals were sampled in.

		Assigned to (%)		
Sampled in	N	Draille	Esquineau	Faïsses
Draille	61	**1 (61)**	0.0	0.0
Esquineau	71	0.0	**0.9843 (70)**	**0.156 (1)**
Faïsses	64	0.0	0.0	**1 (64)**

N = sampling size.

Table 6. Generalized Linear Models to explain length of pond turtle *Emys orbicularis* back shell variations by different explanatory variables with selection by AICc at the Tour du Valat, South France. *Site*: Esquineau, Faïsses; Δ: difference of AICc between models and the model with the lowest AICc (M2); K: number of parameters; Ω: Weight of each AICc in the global analysis; * effect with interaction between qualitative variables; + effect without interaction.

No.	**Model**	**AICc**	**Δ**	**K**	**Ω**
M2	site + sex	1113.0	0	5	0.81
M3	sex	1116.8	3.8	3	0.12
M1	Site*sex*haplotype	1117.9	4.96	12	0.07
M4	sites	1223.5	110.55	4	0
M5	haplotype	1230.8	117.82	3	0

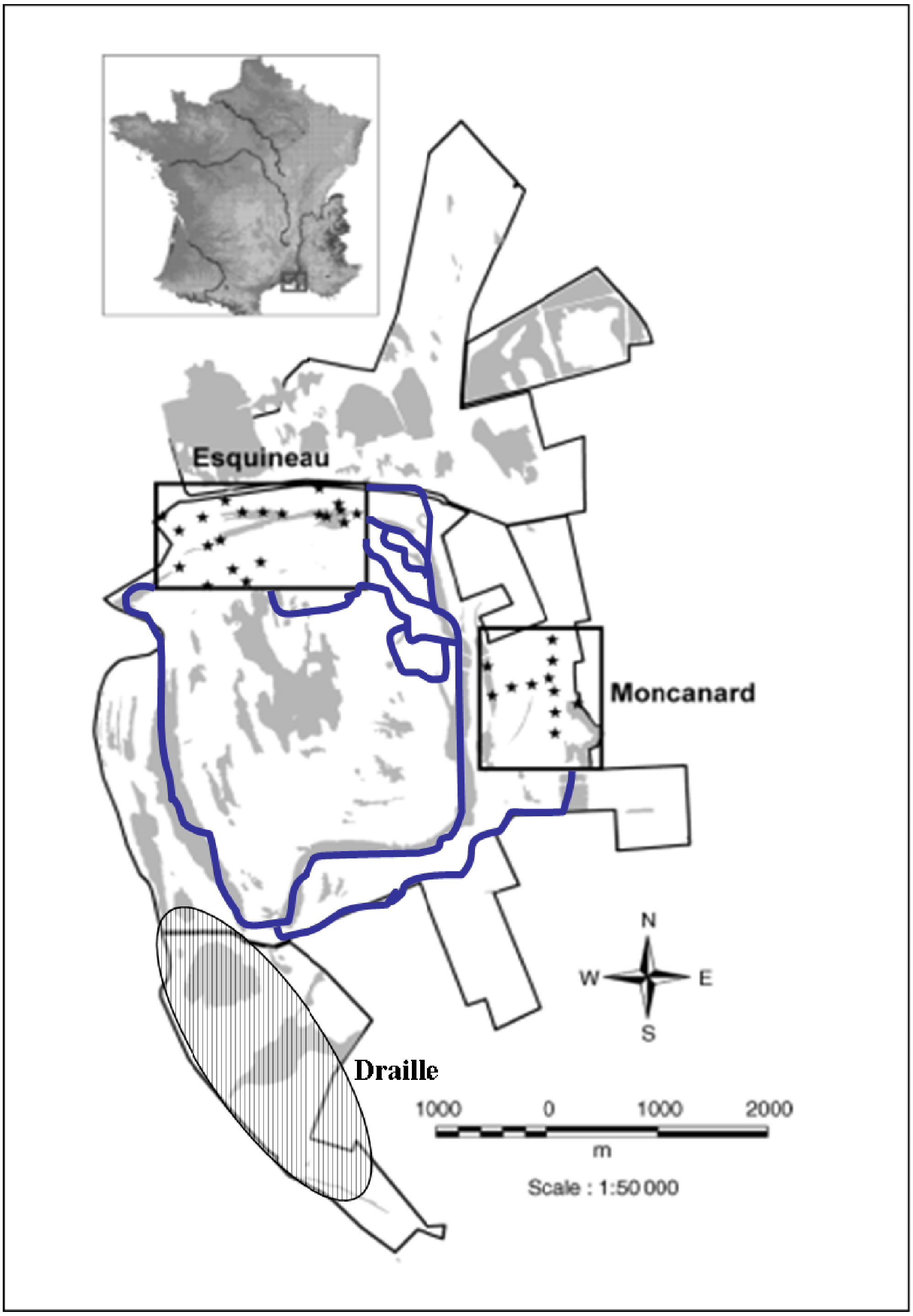

Figure 1. Location of the three study sites at the of *Emys orbicularis* at the Tour du Valat, in the Camargue, France. Esquineau and Faïsses (black rectangles) were both studied for CMR and genetics while Draille (shaded area) was only used for genetics. The stars are the location of the higher number of fixed two-trapping sites. The aquatic corridors between the two sites are highlighted in dark blue. Modified from Olivier et al. (2010).

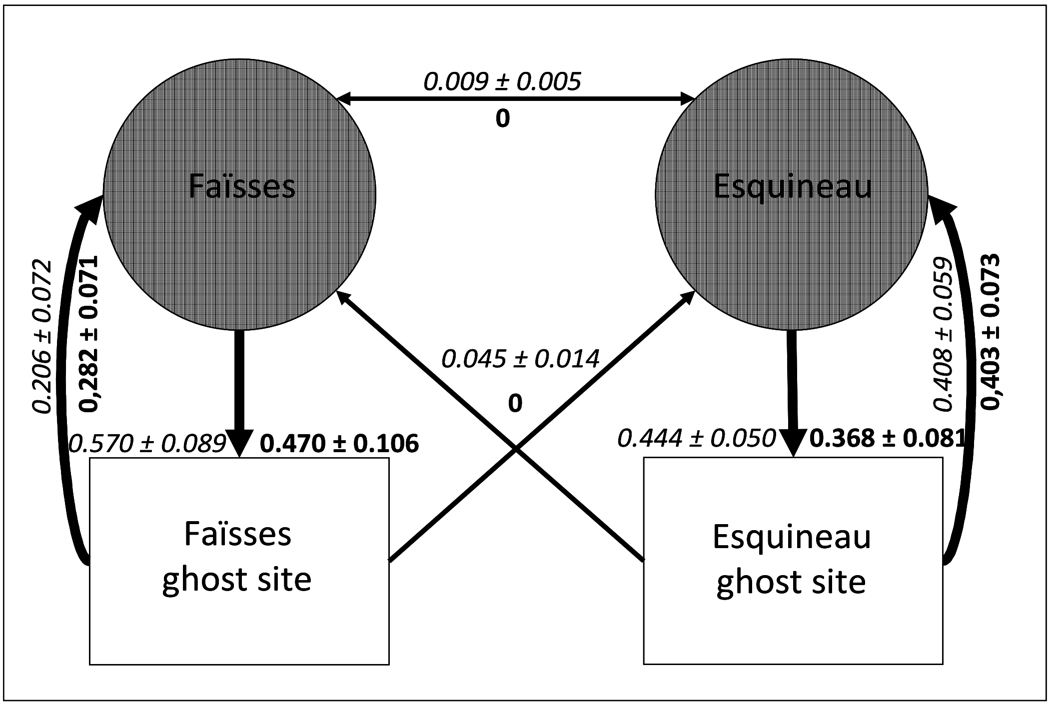

Figure 2. Schematization of the probabilities of transition (± standard errors) between Faïsses and Esquineau and their respective ghost site for the European pond turtles (*Emys orbicularis*) at the Tour du Valat, South France. Values in bold and in italic represents females and males transition, respectively.

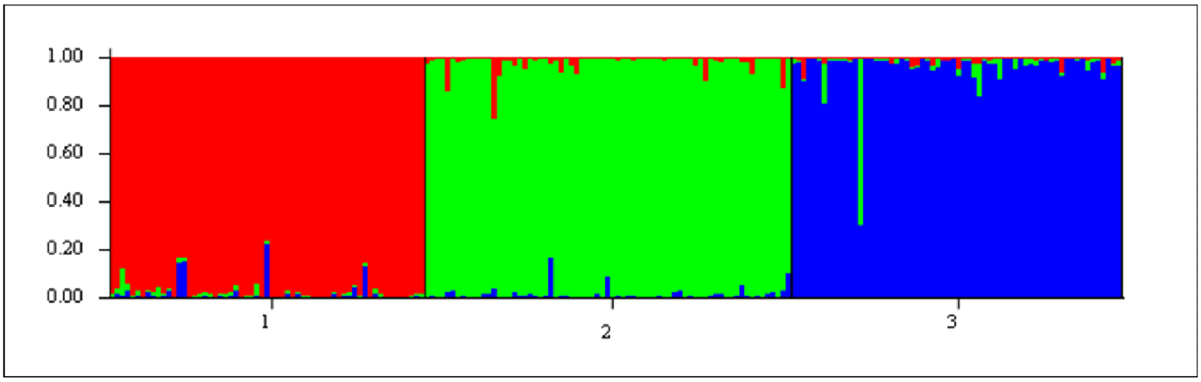

Figure 3. Summary barplot of estimates of Q: the estimated membership coefficients for each individual in each STRUCTURE cluster. Each individual is represented by a single vertical bar broken into K colored segments, with lengths proportional to each of the K inferred clusters. The numbers (1, 2 and 3) correspond to the sampled sites (1=Esquineau, 2= Faïsse and 3=Draïlle).

Chapitre 3

Rapid response of a long-lived species to improved water and grazing management: the case of the European pond turtle (*Emys orbicularis*) in the Camargue, France.

Rapid response of a long-lived species to improved water and grazing management: the case of the European pond turtle (*Emys orbicularis*) in the Camargue, France.

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Abstract

Among human activities, the effect of habitat management by grazing on population viability is ambiguous. Indeed, beneficial effects of grazing are expected by maintaining open meadows, but overgrazing is supposed to increase mortality by trampling. Grazing has been shown to negatively impact the survival of European pond turtle (*Emys orbicularis*) in the Camargue. Consequently, a new management plan was defined. We investigated the consequences of this management using capture-recapture methods to estimate variations of population sizes in this managed site and a control site over a 17 years period. Results show an increase of the number of adults and juveniles on the managed site after the management change. Our results suggest that improved water management with flooding in autumn provided better hibernation conditions, and that reduced grazing intensity in autumn/winter likely decreased the risk of trampling. Population was restored in less than 4 years following the management change, probably by the relaxation of density-dependence. It is an original result for a long lived-species supposed to have an important time of resilience to perturbations.

Keywords

capture-mark-recapture, density-dependence, *Emys orbicularis*, population size, hydrologic management, pastoral management.

Introduction

One third of the reptiles inhabiting marine and freshwater environments are threatened of extinction (Böhm et al., 2013). Higher level of threats to freshwater and marine habitats compared with terrestrial ones are likely responsible for this figure. In particular, 46-57% of the freshwater turtle species are threatened by habitat degradation (Böhm et al., 2013; World Commission on Dams, 2000). Many of these species are particularly affected by home range reduction due to the loss of favorable habitats, competition with invasive species and landscape fragmentation (Williams and Osentoski, 2007).

The European pond turtle (*Emys orbicularis*) is the reptile which has shown the most important range reduction in Europe (>2%) between 1970 and 1990 with increasing fragmentation of populations and the extinction of several relict populations in Eastern Europe (Servan, 1999). Poaching (especially capture with fishtraps), habitat loss and transformation (Rogner, 2009) are the main causes of this negative trend. In particular, drainage, channel constructions, water regulation and dike management generally cause strong habitat homogenization and population fragmentation (Rogner, 2009). Furthermore, the conversion of wetlands to croplands generates additional threats for European pond turtles by exposing them to water pollution.

European pond turtles require both good quality freshwater habitat for foraging and terrestrial habitat with open areas and low plant cover for successful nesting (Ficetola et al., 2004). In this context, cattle grazing may provide open meadows offering favorable nesting sites, essential for the breeding dynamics of the species as was shown in the bog turtle *Glyptemys muhlenbergii* (Tesauro and Ehrenfeld, 2007). However, the benefits of grazing remain controversial: some authors argue that intensive grazing negatively affects the survival of herpeto-fauna by changing the macrohabitat (vegetation structure) or microhabitat (ground temperature), decreasing prey abundances (Wilgers et al., 2006), or increasing the numbers of injured animals by trampling (Olivier et al., 2010).

Olivier et al. (2010) compared the dynamics of two European pond turtle populations of the Camargue (south of France) facing different managements, the Esquineau population face intense grazing and a variable water management while the Faïsses site has constant conditions of management characterised by moderate grazing intensity and stable water levels. The Esquineau population was found to be declining and individuals were found to present shell damaged by cattle trampling. It was thus hypothesized that trampling increased turtle mortality and consequently reduced the numbers of individuals (Fig. 1). In parallel, we suspected that natural winter flooding was too late to offer optimal hibernation conditions since overwintering starts as soon as autumn (Rogner, 2009). Therefore, sites offering better conditions for hibernation due to the dense vegetation (that allows stable conditions to be maintained during the winter; Thienpont et al., 2004) were unavailable, forcing European pond turtles to take refuge in areas less favorable, which may have increased mortality during the hibernation period. Based on these hypotheses, in 2007, a new management plan was implemented at the Esquineau so that flooding started in autumn and the timing and stocking rates of cattle were changed with moderate grazing intensity outside the active period of turtles (Fig. 1). In an adaptive management perspective, monitoring the change of turtle population size following this management change should allow evaluating the effect of grazing and water management on this species. Adaptive management is a conservation strategy that aims to "learn by experimenting" (McLain and Lee, 1996; Walters, 1986). The principle is to establish a management policy based on assumptions and models of the functioning of the system, and to assess their effectiveness *a posteriori* (Berkes et al., 2000; Maris and Béchet, 2010). Hence, 7 years after management change we evaluated the effectiveness of the conservation measure taken to prevent the decrease of the *E. orbicularis* population to validate the hypotheses made on the functioning of the system. In particular, we expected that mitigating the grazing pressure and changing water management would allow population size to return to its initial state.

Materials and Methods

Study area and sampling

The two populations of *E. orbicularis* studied are located in the natural reserve of the Tour du Valat in the Camargue (southern France, ~ 43°30' N, 4°40' E). The first site, the Esquineau, has a total area of 250 ha. The second site, the Faïsses (Moncanard in Olivier et al., 2010) covers a surface area of 100 ha. The studied populations are ~ 1.5 km apart. At both sites European pond turtles inhabit two kinds of habitats: permanent and semi-permanent marshes managed by man-made irrigation and drainage canals (Olivier et al., 2010).

From 1997 to 2013, turtles of both populations were captured from April to August with fishtraps and by hand. Traps were placed in canals and marshes (22 in the the Faïsses, 39 in the Esquineau) and were deployed in the field alternating weekly between the Esquineau and the Faïsses during three to five consecutive days and were visited daily in the morning. Hand captures were also made during these daily visits. For each capture, the age, sex and shell condition (damaged or not) were recorded. Sex was identified by observing male secondary sexual characteristics: concave breastplate, orange eyes (yellow in females), basic wide tail and cloacae away from the plastron (Zuffi and Gariboldi, 1995). Turtles were classified as adults if they had no visible growth rings (Castanet, 1988). In juveniles, reading these growth rings allows to determine the individual birth year (Olivier, 2002). Each individual was marked with permanent notches cut into the carapace marginal scutes with a small hacksaw (Cagle, 1939). Turtles were returned within 24 h to the place where they had been collected. A total of 7059 captures corresponding to 963 different individuals was obtained over the 17-years study period. More precisely, the dataset includes 364 adults and 276 juveniles from the Esquineau and 207 adults and 116 juveniles from the Faïsses.

Both horses and cows graze on land and in the marshes. Grazing intensity was calculated as P = (*number of adult livestock * number of months of grazing*) */* (*grazed area (ha)*). We arbitrarily defined 3 thresholds of grazing intensity: low under a value of 2, high above of 5 and moderate between these two values. . The duration of grazing periods varies between years: i) all year long between 1997 and 2001; ii) in spring and summer between 2002 and 2006; iii) in autumn and winter after 2007 (Fig. 1).

Water levels were measured 2 to 3 times per month and dry period were recorded. Before 2002, water was present in marshes and canals all year long. Since 2002, water levels were modified with a dry period at the end of summer and a natural flooding by rainfall in winter. After 2006, flooding was artificially supplemented by pumping in autumn. Both management changes only affected the Esquineau site so that the Faïsses site can be considered as a control site.

Statistical analysis

We estimated annual variations of the European pond turtle population size at each site separately for each age-class (adult and juvenile) by using close population models. Each year from two to five sampling periods were performed. These sampling periods were used to build the capture histories. Each sampling period length between three to five days was then pooled in our analysis. We first tested for each year whether the population was closed or not by using CLOSE-TEST (Stanley and Burnham, 1999). Then we estimated population size at each of the 17 years with the CAPTURE module (Rexstad and Burnham, 1991) of MARK software (White and Burnham, 1999). CAPTURE allows varying assumptions regarding capture probabilities including: i) inter-individual heterogeneity of capture (M*h*); ii) time variation in capture probability (M*t*); iii) a behavioural response to the first capture (M*b)* and the different possible combinations of effects on capture probabilities. The best model is selected based on the maximum value (between 0 and 1) of a discriminate model selection criteria specific to CAPTURE. We calculated the size of the whole population by adding the number of adults and juveniles estimated each year for each population.

We attempted to explain annual population size variations at the two sites by using Generalized Linear Models (GLM) with a Gaussian distribution for the error term. We included the standard error of the population size as weight to take into account inter-annual variations in the precision of the estimate. We evaluated the fit of models by checking normality, independence and homoscedasticity of residuals. We first tested for a difference of population size between sites. Then we tested for each population the effect of age (juvenile or adult), and of the three periods corresponding to the three management plans: a single period 1997-2013, two periods 1997-2001/2002-2013 and 1997-2006/2007-2013, and three periods 1997-2001/2002-2006/2007-2013. Model selection was achieved using the Akaike Information Criterion corrected for small sample size (AICc; Burnham and Anderson, 2002). Model with the lower AICc value is considered as the best model for the data at hand. Models with an AICc difference less than 2 are considered as equivalent to describe the data. GLM were implemented in R version 2.7.2 (Development Core Team, 2008)

Results

At the Esquineau, grazing intensity was stable and low between 1997 and 2001. In 2002, the livestock density was multiplied by 2.5 and the grazing intensity was high. Since 2007, the grazing intensity has been more fluctuating and can be considered as moderate (Fig. 1). At the Faïsses, grazing intensity has remained moderate from 1997 to 2013.

At the Faïsses, we noted 3 phases of recruitment (Fig. 2) determined from the number of newly marked individuals in 2000, 2003 and 2007 with 7, 8 and 10 individuals, respectively. At the Esquineau, we observed 2 phases (Fig. 2) in 1998 with 15 individuals and between 2004 and 2007 with a maximum of 26 individuals in 2007.

Damaged shells were noted on 32 individuals mostly on adult females and male juveniles at the Esquineau (9 and 7, respectively; Table 1). Trampling seems to be more important between 1997 and 2001 and between 2007 and 2012 than between 2002 and 2006 (15, 12 and 5, respectively; Table 1).

Close tests indicated that the closure assumption within primary periods was supported in ~70% of years. Among the top six models receiving the highest scores based on the CAPTURE algorithm for model selection, model with time-variation in capture probability (M*t*) was selected for adult data sets. For juvenile data sets, models without effects (M_0) and with heterogeneity at the capture (M*h*) were selected for the Esquineau and the Faïsses, respectively.

The Faïsses and the Esquineau populations appear to fluctuate independently of one another over the study period (Table 2, M1 *vs* M2, ΔAICc= 22.4) supporting localized effects of management actions.

The Faïsses population does not show any consistent size variations across the three management plans (Table 2, M10 *vs* M11, ΔAICc= 4.1) so that this population appears relatively stable with 73 ± 21 individuals (Fig.3c). Results suggest that the Faïsses show a strong age effect but independent of any of the management plans (Table 2, M9 *vs* M14, M15 ΔAICc≥ 4.4).

Contrary to the Faïsses, the Esquineau population size varies depending on the three management plans (Table 2, M5 *vs* M6, M7 and M8, ΔAICc≥ 7.7; Fig. 3c). Model selection supports the hypothesis that variations of population size are age-dependent (Table 2, M3 *vs* M4, ΔAICc= 2.4). Adult population size was 206 ± 19 individuals during the first period, decreased to 145 ± 30 between 2002 and 2006 and increased to 300 ± 90 during the last period (Fig. 3a). The juvenile population follows similar variations with a greater magnitude (Fig. 3b). Population size dropped from 43 ± 30 to 15 ± 9 between the first and the second period, a decline of 65% compared to adult population which decreased of only 30% over the same period. After 2006, the average population size of juveniles increased by 700% reaching 120 ± 50 individuals, while this rise was only 107% for adults. The increase of the juvenile population size seems to start in 2006 (Fig. 3b), two years before the increase observed in the adult population (Fig. 3a).

Discussion

In an adaptive management approach, our study aimed at evaluating the effect of two management measures taken to restore European Pond turtle populations in the Camargue. In particular, we hypothesised that high grazing intensity and late flooding management could be two factors that negatively impact European pond turtles.

Our results suggest distinct situations between sites in the variations of population size. We showed a local effect at the Esquineau site where population sizes vary following the changes of management plans. This pattern is not observed at the Faïsses site where management remained the same throughout the study period. At this site, the whole population slightly fluctuates between 1997 and 2013. At the Esquineau, the number of adults and juveniles increased steadily after the second management plan in 2007 and remained high up until 2013. Even if the respective effects of grazing and water management can not be disentangled, our results suggest beneficial effects of these two factors.

The availability of water is essential for the survival of European pond turtle (Rogner, 2009). *Emys orbicularis* uses wetlands for feeding, mating and hibernating. In the Camargue, marshes are dry by the end of summer. The natural flooding of marshes may occur at the end of autumn or in the beginning of winter. But flooded marshes constitute favourable sites to hibernate that European pond turtles seek as early as October (Olivier, 2002). Therefore, in natural conditions, marshes would most often been dry when turtles start hibernation, so that they are constrained to hibernate in channels where thermal conditions are supposed less stable due to the lack of dense vegetation and more variable water levels (Thienpont et al., 2004). By artificially flooding marshes in autumn we expected that turtles would get better hibernation conditions, thus promoting higher winter survival. Increased population sizes after this change suggest that this measure positively affected population size.

The number of trampled turtles by management plan and per site is insufficient to detect an evolution of damaged shells and the individual mortality, here not detected, caused by the changes of management. In addition, the low overlap between grazing periods and the European pond turtle availability to trampling inevitably leads to a decreased risk of being crushed. However, data suggest that the trampling of turtles by livestock remain significant on our study sites and seems to be more important at the Esquineau than at the Faïsses for both age categories (Olivier, 2002). In the short term, high grazing intensity can therefore negatively affect turtles. In *Mauremys rivulata*, overgrazing causes heavy disturbance by trampling, suggested to be a factor of juvenile and adult mortality. It can also have negative consequences on the reproductive biology, with a possible disruption of nesting areas *(*Chelazzi et al., 2007). In European pond turtles, trampling, which occurs during the movements of individuals (dispersal for males, and terrestrial nesting for females), likely increased mortality on adults and therefore contributed to decrease the numbers of individuals between 2002 and 2006 (Olivier et al., 2010). Nevertheless, European pond turtle at the Tour du Valat do not seem to be severely affected by the relatively high grazing intensity implemented in autumn and in winter during the last management plan since even if the grazing intensity was high, the impact on turtles remained low because individuals are inactive during hibernation (Rogner, 2009) and protected from trampling by mud (i.e. the earth soil layer).. In the case of *Emys orbicularis*, grazing prevents the closure of terrestrial habitat and maintains favourable nesting sites. .

Our results also emphasize that the rapidity of the response of this long-lived species to the change of management. Indeed, European pond turtles are long-lived animals with a late age of first reproduction (Rogner, 2009). Consequently, the generation time is very high and the time of resilience to perturbations is also expected to be high (Couturier et al., 2011; Pitt and Nickerson, 2013). Our results contrast with this prediction. Yet they are similar to the observations made by Cheylan and Poitevin (1998) who studied the impact of fire on population of *Emys orbicularis* in the Massif des Maures (Southern France). They showed that despite the decrease of abundance and mortality of individuals after a fire, the population could be restored in only 5 years by high recruitment.

The relaxation of density-dependence may explain the low time of resilience to perturbation observed in our populations (Vincenzi et al., 2010). Indeed, as population size dropped during the second plan, the decrease of intraspecific competition may have facilitated an important and rapid recruitment of the newly born individuals from 2004 (Fordham et al., 2009; Fig. 2) despite a low and stable fecundity across years (5 per 1000 on average; Olivier, 2002). This recruitment can result from an increase of the numbers of juveniles newly marked from 2006 (we observed at the Tour du Valat that the juveniles are caught for the first time around 2-3 years). The sudden increase of the number of adults captured from 2008 onward marks the transition from the marked juveniles to the adult state (around 5-6 and 7-8 years for males and females, respectively) in the dataset. The recruitment of both age categories intensifies with the third management plan. In contrast the decrease of the number of newly marked individuals after 2008 (<5 individuals in recent years) may be explained by the increasing intraspecific competition following population restoration. As the majority of individuals born from this last recruitment have been captured and marked, we predict that in a few years the numbers of juveniles in the whole population will progressively decrease. On the contrary, the population of adults should increase in the next 3 to 5 years.

Our study shows the importance on one hand of the adaptive management of aquatic habitats in an anthropic system, and on the other hand of the long-term monitoring of populations for the conservation of freshwater turtles species.

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Table 1. Number of *Emys orbicularis* individuals found with severely damaged shell by management plan, site, sex and age at the two study populations Esquineau and Faïsse in the Camargue.

	Adult				
	Female Esquineau	**Male Esquineau**	**Female Faïsses**	**Male Faïsses**	**Total Esquineau/Faïsses**
1997-2001	6	3	0	1	9/1
2002-2006	2	0	0	1	2/1
2007-2013	1	3	2	0	4/2
	Juvenile				
	Female Esquineau	**Male Esquineau**	**Female Faïsses**	**Male Faïsses**	
1997-2001	2	2	1	0	4/1
2002-2006	1	1	0	0	2/0
2007-2013	2	4	0	0	6/0

Table 2. Generalized Linear Models to evaluate a) the effect of site on population size; b) the effect of age and of the different management plans on annual population sizes at the Esquineau (b) and the Faïsses (c). *pop*: Esquineau, Faïsses; *A*: 1997-2001; *B*: 2002-2006; *C*: 2007-2013; Δ: difference of AICc between models and the first model (M1, M3 and M9, respectively). K: number of parameters; Ω: Weight of each AICc in the global analysis; * effect with interaction between qualitative variables; + effect without interaction.

a)

No.	**Model**	**Deviance**	**AICc**	**Δ**	**K**	**Ω**
M1	(A / B / C)**Pop*	1093.6	737.6	0	7	1.00
M2	(A / B / C)+*Pop*	1643.2	760..0	22.4	5	0.00

b)

No.	**Model**	**Deviance**	**AICc**	**Δ**	**K**	**Ω**
M3	(A / B / C)*Age	50.25	322.9	0	7	0.60
M4	(A / B / C)+Age	64.52	325..3	2.4	5	0.40
M5	(A / B / C)	604.3	398.6	75.7	4	0.00
M6	A-B / C	817.89	406.3	83.4	3	0.00
M7	A / B-C	1054.9	414.9	92	3	0.00
M8	1997-2013	1249.6	418.3	95.4	2	0.00

c)

No.	**Model**	**Deviance**	**AICc**	**Δ**	**K**	**Ω**
M9	Age	130.33	302.2	0	3	0.489
M14	(A / B / C)+Age	155.33	306.6	4.4	5	0.260
M15	(A / B / C)*Age	130.33	307.1	4.9	7	0.242
M10	1997-2013	504.06	337.6	35.4	2	0.003
M13	A / B-C	492.90	339.3	37.1	3	0.002
M12	A-B / C	503.15	340.0	37.8	3	0.002
M11	(A / B / C)	489.27	341.7	39.5	4	0.002

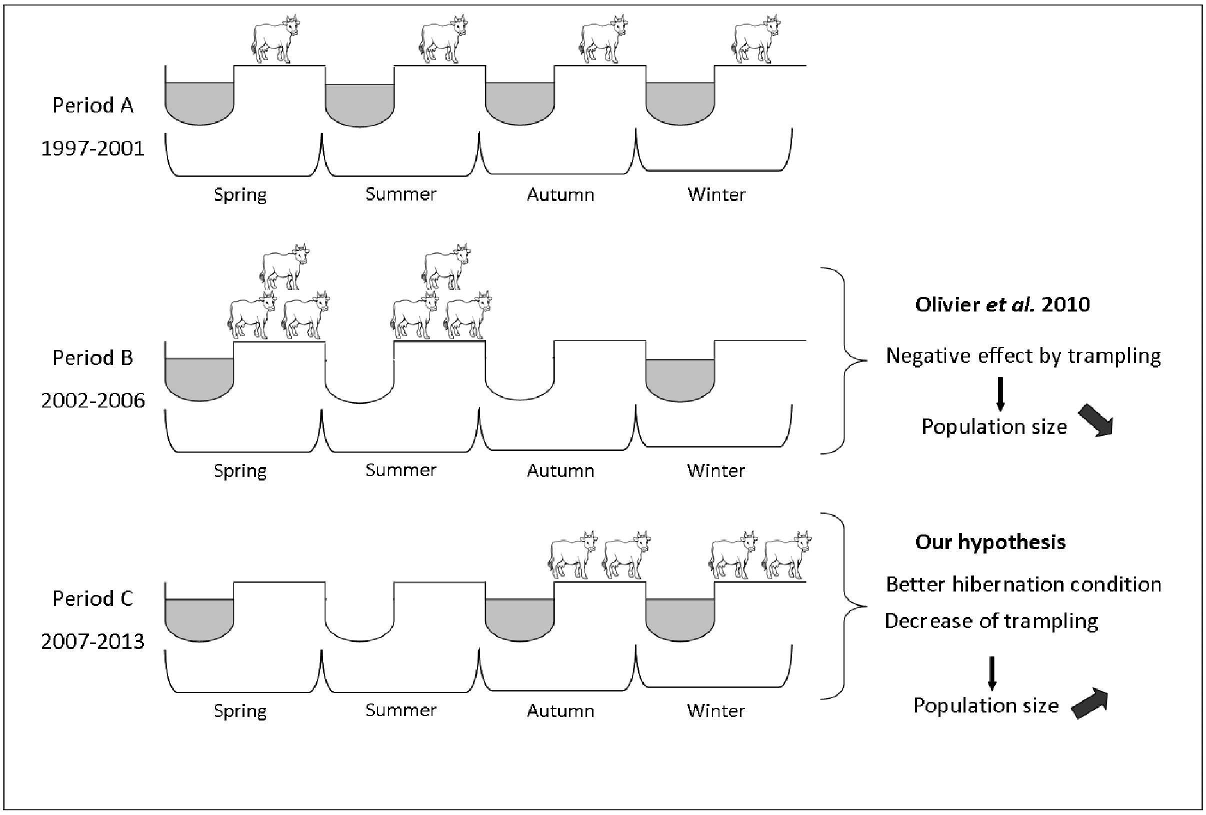

Figure 1. Characteristics of the different management plans at the Esquineau European pond turtle population (Tour du Valat) from 1997 to 2013. The number of cows reflects cattle grazing intensity: low (one), moderate (two) and high (three). Areas in grey show the presence of water and blank areas its absence. Flooding of marshes in autumn was artificial and in winter was natural.

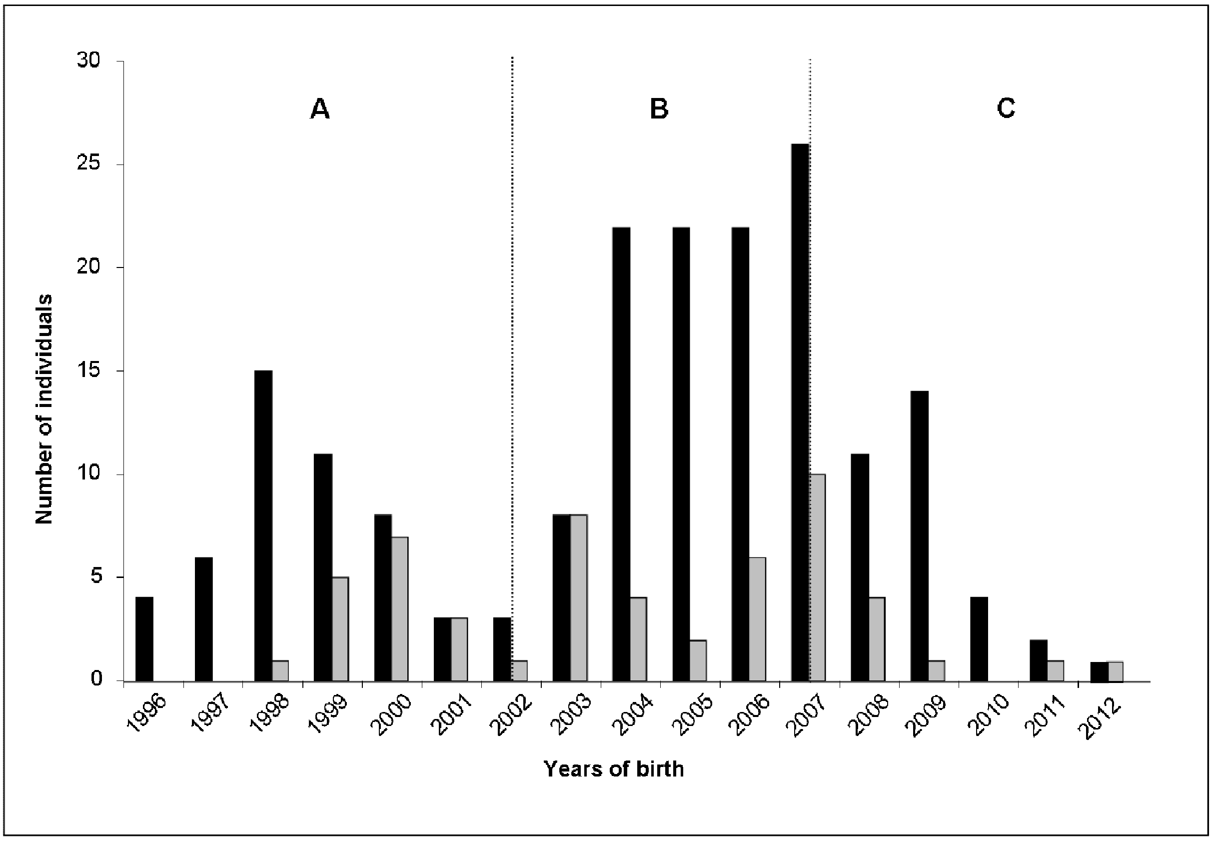

Figure 2. Number of European pond turtle births per year as estimated from the age of the newly marked individuals of *Emys orbicularis* captured at the Tour du Valat for each study site (the Esquineau in dark and the Faïsses in grey) for the three different management plans.

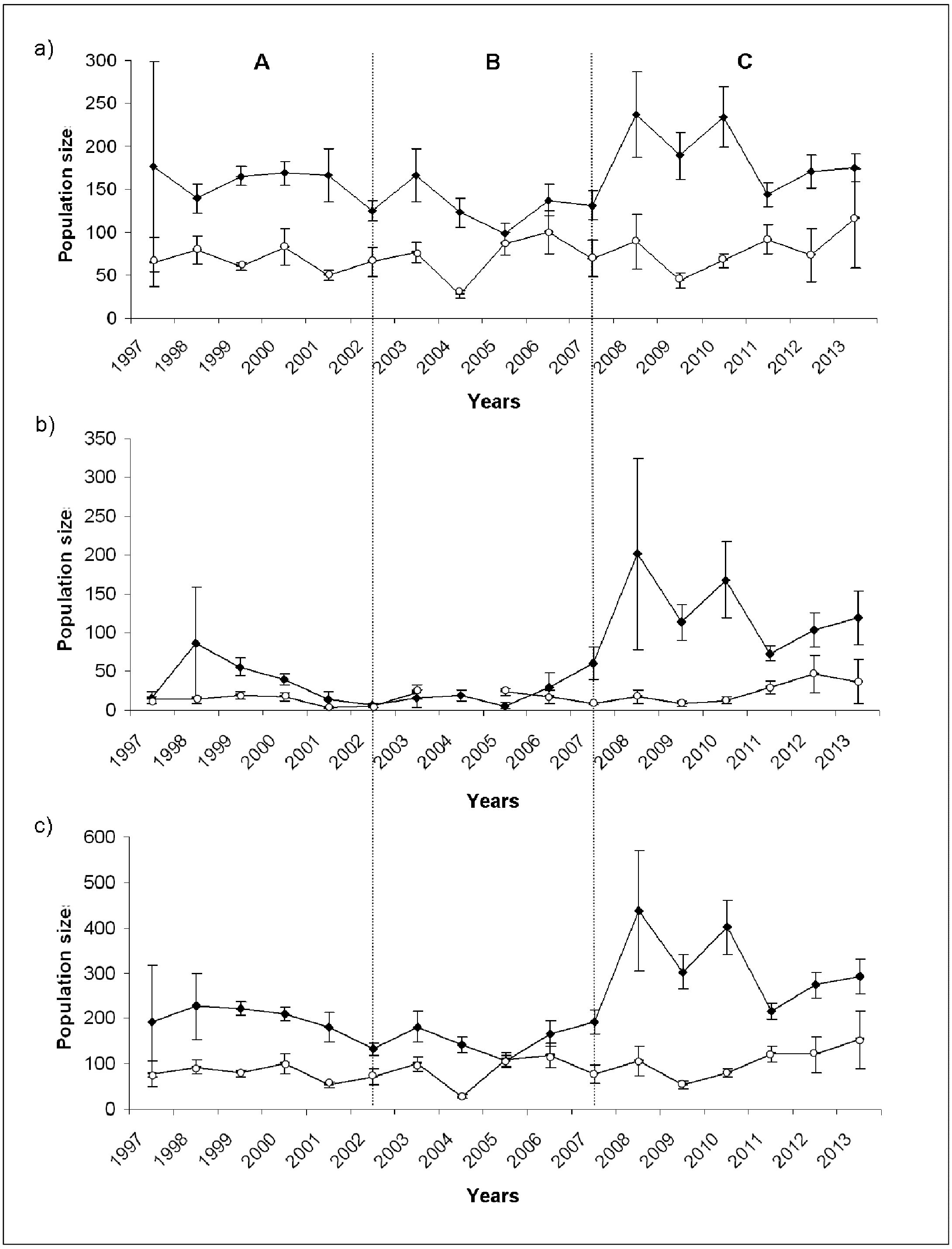

Figure 3. Population size (± SE) of *Emys orbicularis* at Tour du Valat, for the three different management plans. (a) For adults. (b) For juveniles (c) For the whole populations. Black square curve are for the Esquineau and white circle one for the Faïsses site.

Conclusion

Conclusion générale

Nous préconisons, lorsque c'est possible, de combiner les modèles de dynamique et de génétique des populations afin d'étudier la dispersion chez la Cistude d'Europe. En effet, chez cette espèce, il est impossible de dissocier d'un point de vue démographique les mouvements de transition des mouvements de dispersion. En ce sens, l'apport des outils moléculaires est essentiel pour les déduire. Cette approche offre également l'avantage d'étudier, en plus des flux de gènes contemporains, les mouvements d'individus passés. En revanche, la génétique des populations ne peut être que qualitative dans l'estimation des flux entre les populations. En effet, l'estimation des taux de dispersion entre populations à partir de la différenciation génétique et notamment à partir des F_{ST} est extrêmement imprécise (Whitlock and McCauley, 1999). De même, les méthodes d'assignement sont restreintes à l'identification des migrants F0 lorsque la différentiation entre populations est faible (ce qui est le cas dans notre étude). L'intégration génétique de ces individus dans les populations receveuses reste donc à déterminer. La dynamique des populations, et dans notre cas l'utilisation de modèles CMR, est quant à elle beaucoup plus adaptée à la quantification des mouvements entre populations. L'étude de la dispersion est délicate, et la caractérisation de ce comportement chez les tortues d'eau douce n'échappe pas à cette généralité. Il est difficile de dégager un schéma global de l'impact de la fragmentation sur la connectivité et par conséquent sur la structure et la dynamique des populations de cistude. Ces 3 ans de recherche auront permis d'explorer de nouvelles pistes d'investigation.

La première a consisté en l'étude de populations non protégées et de grands effectifs. Nous avons souhaité dans ce cas sortir du contexte de l'étude plus répandue de petites populations en milieu protégé. Si les connaissances de l'impact de la fragmentation sur les populations de petite taille ne manquent pas (Davy, 2013) et ce qui est compréhensible car sujettes à un risque accru d'extinction, celles sur les populations de grande taille manquent. Pourtant les cistudes, et les tortues d'eau douce en général, sont des animaux aux capacités de dispersion limitées (Mitro, 2003) et l'impact de la fragmentation peut par conséquent également menacer les populations de grande taille. À travers notre étude sur les cistudes à Kerkini, développée dans le chapitre 1, nous avons tout d'abord confirmé et pointé du doigt ce paramètre très important à prendre en compte en écologie et en biologie de conservation qu'est la taille des populations (Van Treuren et al., 1991). D'autre part, nous avons mis en évidence que les grandes populations peuvent être menacées à plus long terme et que leur situation actuelle comprenant une forte diversité génétique masque un danger futur potentiel. Chez les chéloniens, cette taille d'effectif joue un rôle clé dans la vitesse de différenciation. Malgré les capacités de dispersion limitées de ces tortues ne favorisant pas l'homogénéisation génétique des unités démographiques, le processus de différentiation des populations sera beaucoup plus long lorsque leurs tailles sont importantes, comme c'est le cas à Kerkini. Dans ce cas, le processus d'érosion génétique, déjà ralenti par des temps de génération long (environ 12 ans chez la cistude), sera renforcé. Par conséquent, aujourd'hui, les populations de Kerkini ne semblent pas être impactées par la forte fragmentation anthropique liée à la création des deux barrages. Une étude similaire, au Canada (Bennett et al., 2010) suggérait que l'absence de différenciation était probablement due à d'importants flux de gènes. Nous avons avancé ici une hypothèse alternative par l'existence d'une grande population historique présente avant la construction du barrage, dont seraient originaires les populations échantillonnées autour du lac. Ce schéma expliquerait l'absence de différenciations entre ces populations malgré l'absence de flux de gènes. Par ailleurs, aucune des 1240 cistudes marquées depuis 1999 sur un des trois sites autour du lac n'a été échantillonnée dans les deux autres sites. Bien que ponctuel, cet élément renforce l'idée d'une absence de connectivité récente.

Le second point que nous souhaitions développer était de combiner les méthodes de dynamique et de génétique des populations pour les raisons dont nous avons parlé au début de ce chapitre. Peu d'études (Sheridan et al., 2010 ; Freedberg et al., 2005) ont combiné ces deux méthodologies pourtant complémentaires dans l'analyse de la dispersion chez les tortues d'eaux douces. Dans ces deux études, les auteurs ont utilisé des données de CMR de façon qualitative et ponctuelle pour estimer la transition. Nous avons développé une étude quantitative d'estimation de la transition par CMR par l'utilisation de modèles multi-états et multi-événements. L'association de ces modèles avec l'utilisation de marqueurs génétiques nous a permis dans un premier temps d'étudier la concordance de ces deux méthodes et dans un second temps de déterminer si les mouvements de dispersion observés conduisaient à un apport de flux de gènes. Les deux outils d'analyse de la dispersion nous montrent ainsi une fidélité totale des femelles caractérisée par un flux de transition nul et une forte structuration génétique à la fois par les microsatellites et l'ADN mitochondrial. À l'inverse, les mâles semblent assurer un faible flux de gènes avec un taux de transition inférieur à 3 % en 17 années de suivi. Notre étude confirme donc les faibles capacités de dispersion chez cette espèce, déjà montré par Ayres and Cordero (2007). Nous mettons également en évidence l'absence de connectivité globale à la Tour du Valat montrant que la dynamique de cette métapopulation est affaiblie, rendant les sous-populations plus vulnérables à la stochasticité environnementale et démographique.

Le déclenchement de la dispersion chez les cistudes semble dépendant de la qualité du milieu et notamment des corridors écologiques. Initialement, à la Tour du Valat, la forte proximité des noyaux, très connectées en corridors, supposait que la dispersion serait facilitée. Au vu des résultats révélant une structuration génétique significative entre ces populations, nous supposons désormais que les corridors écologiques soient très peu empruntés car peu favorables à l’espèce. Ainsi, il serait intéressant d’évaluer la qualité des corridors, en termes de disponibilité en eau, de ressources, de sites d’ensoleillement, de pollution, etc., permettant d’expliquer l’absence de connectivité réalisée en présence de connectivité aquatique à priori favorable aux mouvements. D’autre part, l’espèce semble sensible à la compétition intra-spécifique entre adultes (Poschadel et al., 2006). En Camargue, la compétition entre adultes semble limitée du fait de la faible densité des individus et des populations (Olivier, 2002). Par conséquent, les individus possèdent des ressources favorables (nourriture, site de ponte,…) ce qui rend trop couteuse la dispersion vers d’autres sites. En Grèce, où les densités sont bien plus importantes, les mouvements sont plus nombreux à une échelle spatiale comparable, notamment pour les femelles (Ficheux et al. résultats non publiés, voir perspectives). Le coût à la dispersion serait moins important en Grèce puisque la probabilité de trouver un site de meilleure qualité est plus importante. D’autre part, l’ubiquité des sites de ponte rend la dispersion moins coûteuse favorisant la sélection de ce comportement chez les femelles. A l’inverse, en Camargue, la faible disponibilité des sites de ponte a pu favoriser la sélection de la philopatrie. Les femelles qui sont territoriales (Olivier, 2002) engendrent des jeunes qui bénéficieront de la qualité de leur habitat. Les jeunes femelles n’auront pas besoin ni de quitter leur habitat natal, ni de rentrer en compétition pour disposer d’un territoire et d’un site de ponte favorables. Il est donc plus avantageux pour une femelle en Camargue de rester fidèle à son site de naissance. Les mâles bénéficient aussi de la qualité d’habitat de leur lieu de naissance. Cependant, ils peuvent être avantagés à disperser pour éviter la compétition entre apparentés et surtout la consanguinité. On peut donc comprendre la mise en place du biais de la dispersion pour les mâles en Camargue, et plus généralement dans les habitats où les sites de ponte sont une ressource rare. Par ailleurs, les ressources énergétiques économisées par l’absence de déplacement peuvent être allouées dans la reproduction, permettant aux femelles de pondre plusieurs fois par an (2 à 3 pontes en Camargue ; Olivier, 2002), et dans la survie (Olivier et al., 2010). D'ailleurs, notre étude de dynamique de population à la Tour du Valat révèle une survie plus importante chez les femelles que chez les mâles, et concordante avec d'autres étude chez cette espèce (Keller, 1997)

A la Tour du Valat, le taux de fécondité est peu élevé, nous considérons que 5 œufs sur 1000 pondus donneront un jour des adultes (Olivier, 2002) malgré les pontes annuelles multiples en Camargue et une faible densité d'individus. Nous pouvons donc supposer que le recrutement de juvéniles soit plus important en Camargue. Cependant, le nombre de jeune nouvellement marqués reste plus faible qu'en Grèce. La localisation précise des sites de ponte, et par conséquent la concentration des œufs pondus facilitent sans doute une prédation accrue, bien recensée chez cette espèce (Zuffi, 2000). Par ailleurs, au vu du caractère fluctuant des juvéniles nouvellement marqués, nous supposons que les jeunes cistudes soient également sensibles à la compétition avec les adultes. En effet, dans le chapitre 3, nous avons démontré que la relaxation de la densité-dépendance des adultes en Camargue permettrait un recrutement important des juvéniles. Cet aspect est une force de la dynamique des populations de cistude, car elle permet une réponse très rapide à une perturbation du milieu. C'est un résultat crucial pour une espèce longévive ayant une reproduction tardive et des générations longues suggérant un temps de résilience important (Pitt and Nickerson, 2013). Cette démarche met également en avant l'intérêt de l'application d'une science théorique qu'est la dynamique des populations au service de la gestion. Elle montre l'importance des suivis à long terme chez les chéloniens pour permettre une gestion adaptative. La gestion adaptative est une stratégie de conservation qui vise à « apprendre en expérimentant » (McLain and Lee 1996); le principe est de mettre en place une politique de gestion en s'appuyant sur des hypothèses et des modèles, pour en évaluer l'efficacité a posteriori (Maris and Béchet, 2010). Elle va dans le sens d'une nécessité de collaboration de plus en plus étroite en biologie de la conservation entre le monde de la recherche et celui de la gestion, de façon à ce que la biologie de la conservation sorte des concepts académiques sans prise sur le monde réel (Besnard 2013).

Perspectives

Comme tout travail de recherche, cette thèse ouvre la voie à de nouveaux questionnements pouvant offrir des pistes d'investigation pour les chercheurs et étudiants s'intéressant à ces sujets. En Camargue, les échantillons de sang récoltés depuis 2010 vont nous permettre d'établir les relations de parenté dans les deux noyaux de populations suivis par CMR. Ainsi, il sera possible de déterminer le régime d'appariement des cistudes de la Tour du Valat. Par conséquent, nous pourrons savoir si les mâles disperseurs fécondent plus de femelles que les mâles locaux, dans quelle proportion, etc. De même, un individu disperseur engendrera-t-il des individus qui ont une probabilité plus grande de disperser ? De récentes études (Korsten et al., 2010 ; Tschirren and Bensch, 2010) montrent que le polymorphisme du récepteur D4 de la dopamine (DRD4) peut être lié aux variations des comportements exploratoires, et donc en lien avec la dispersion. Peut-on déterminer si la dispersion des cistudes suit les mêmes modalités, est-elle héritable?

Par ailleurs, nous souhaitons dans un futur proche étudier la transition dans un contexte de compétition interspécifique. À Kerkini, la cistude vit en sympatrie avec l'Emyde des Balkans (*Mauremys rivulata*). Depuis 1999, les cistudes et les emydes sont sujettes à un programme de suivi par CMR. Toutes les deux semaines, d'avril à septembre, pendant deux jours consécutifs, des individus des deux espèces, regroupés sur environ 100 hectares, sont capturés, marqués et relâchés. Quelques résultats préliminaires sur les cistudes montrent que les individus ont une survie plus faible (0,89) qu'en Camargue (0,94, chapitre 2) et qui ne semble pas dépendante du sexe. D'autre part, les taux de transition intra-sites ne différent pas entre les mâles et les femelles. Ces résultats vont dans le sens de ce que nous avons expliqué en conclusion. La forte disponibilité des sites de ponte et la forte densité n'ont probablement pas favorisé la sélection de la dispersion. Néanmoins, il est délicat de comparer les situations entre la Tour du Valat et Kerkini, car ils s'opposent sur de nombreux points : qualité du milieu, densité des populations très hétérogène, lignées halotypiques différentes, absence de sympatrie en Camargue, zone non protégée en Grèce… Il est donc impossible de déterminer quels facteurs influencent la survie et la transition. En revanche, la comparaison de la dynamique des populations de cistude et de *Mauremys* à partir de 15 années de CMR sur un site identique se révèle particulièrement intéressante. Est-ce que la survie est différente entre les deux espèces ? Est-ce que la forte densité des émydes favorise l'émigration des cistudes ? Est-ce que les deux espèces sont impactées de la même manière par la dynamique de la durée d'inondation ? La configuration du site offre l'avantage de suivre les tortues dans les marais et dans une portion de rivière. Ainsi, peut-on observer une disparité dans la répartition spatiale des espèces ? Y a-t-il compétition inter-spécifique pour l'habitat ? Nous essayerons également de déterminer si les phénomènes de densité-dépendance interspécifique ont un impact sur les taux de transitions définitives (avec non retour des individus). De plus, pendant notre campagne de terrain en Grèce, 209 prises de sang ont été faites sur les emydes appartenant aux 5 mêmes populations étudiées autour du lac Kerkini dans le chapitre 1. Ainsi, nous allons pouvoir comparer la structure génétique des cistudes et des emydes des Balkans. En effet, l'emyde, qui semble plus adaptée aux milieux à courant vif (Chelazzi et al., 2007), sera-t-elle impactée différemment par la construction du barrage et du lac de rétention d'eau ? Quelles sont les modalités de la dispersion chez les emydes à Kerkini ?

Pour finir, au cours de ces années de thèse, nous avons eu l'occasion de participer à des campagnes ponctuelles de CMR dans 4 populations situées à la limite de répartition septentrionale de cette espèce en France. Deux de ces populations sont localisées en Bourgogne, dont l'une dans l'étang de Pierre Poulain sur la commune de Pouilloux en Saône et Loire, et l'autre dans l'étang des Grands Georgeats au niveau de la commune de Lucenaylès-Aix dans la Nièvre. Les deux autres populations se trouvent en Auvergne, au nord du département de l'Allier dans des étangs se trouvant sur les communes de Dompierre-sur-Bresne et Varenne-sur-Allier. Ces campagnes furent l'occasion de prélever des échantillons sanguins. Ces populations, classiquement caractérisées de relictuelles de par leur position à la limite septentrionale de l'aire naturelle de distribution, semble caractérisées par de très petits effectifs (quelques dizaines d'individus). De plus, plusieurs dizaines de km séparent ces populations (entre 25 pour les plus proches et 80 km pour les plus éloignées) dans un milieu totalement fragmenté : présence de routes, de zones commerciales, d'habitations et une absence générale de corridors biologiques. Il est inconcevable qu'actuellement des individus puissent disperser entre ces populations. À travers une étude de génétique des populations, actuellement en cours, nous allons essayer de déterminer l'impact de la fragmentation sur ces populations relictuelles. Ainsi, nous allons mesurer la dispersion historique et déterminer si les populations étaient autrefois connectées. Pour cela, nous allons évaluer le niveau de diversité génétique pour des marqueurs mitochondriaux (cyt b) et nucléaires (microsatellites). Par ailleurs, comme pour nos autres études, le marqueur mitochondrial va nous permettre de déterminer les haplotypes présents en Bourgogne. Les premières recherches montrent la présence de la lignée *Emys orbicularis orbicularis* (haplotype IIa). Ce résultat est peu surprenant car cet haplotype est le plus fréquent et cette lignée est celle qui a recolonisé la France par le nord depuis les Balkans (Sommer et al., 2009). En revanche, nos données montrent aussi la présence de la lignée IV *Emys orbicularis hellenica* (haplotype IVa) dans trois sites (absent de Pierre Poulain). Cette lignée est originaire d'Italie (nord et est, Sommer et al., 2009) et est également présente en Camargue. Du point de vue des marqueurs nucléaires, les prémisses de résultats montrent que la diversité génétique de ces trois populations est importante, bien que plus faible qu'en Camargue et à Kerkini. En revanche, la population de Pierre Poulain semble montrer beaucoup moins de diversité que les trois autres sites.

Cependant, toutes les populations semblent être à l'équilibre de Hardy-Weinberg, suggérant l'idée d'absence de consanguinité. Les premières mesures de F_{ST} indiquent que les populations sont très fortement différenciées génétiquement.

La génétique des populations, bien que coûteuse, est dans ce sens un outil précieux pour évaluer rapidement la connectivité entre les populations. Les résultats obtenus seront intégrés dans les actions menées par les gestionnaires (ici avec ceux de l'Allier et de la Bourgogne) pour mettre en place des actions concrètes de conservation et de gestion dans le cadre du ''Plan national d'action 2011-2015 pour la Cistude d'Europe (Emys orbicularis)'' mis en place par le Ministère de l'Ecologie, du Developpement Durable et de L'Energie (Thienpont, 2011). La restauration de corridor biologique semble illusoire. Dans les populations très fragmentées et espacées de plusieurs dizaines de km comme c'est le cas en Bourgogne/Allier, il faut au contraire favoriser la philopatrie pour éviter les déplacements et donc inéluctablement la hausse de la mortalité. Il est donc crucial de veiller à la qualité des habitats en termes de ressources : nourriture, sites d'ensoleillement, végétation pour l'hivernation, sites de ponte, refuges… Il est tout aussi important de surveiller les changements d'effectif des populations et éviter la charge complète de la capacité d'accueil, bien que peu probable en limite d'aire de présence d'espèce (conditions environnementales moins favorables pour l'espèce), pour éviter les phénomènes de dispersion liés à la densité dépendance ou encore une compétition trop intense entre juvéniles et adultes. Il faut donc diminuer le coût à la philopatrie, à défaut de favoriser la dispersion. L'élément clef est, à notre sens, la localisation des sites de pontes, leur préservation et leur restauration si nécessaire. La première raison est qu'une forte disponibilité des sites de pontes favorisera des déplacements limités et donc moins coûteux (écrasement par les voitures, prélèvement d'individus pour la captivité, prédation des nouveaux nés lors du retour au milieu aquatique…). La seconde est qu'un site de ponte de mauvaise qualité peut provoquer des phénomènes de dystocie. Le manque d'habitats propices à la nidification provoque souvent chez les femelles le maintien des œufs dans les trompes de Fallope jusqu'à ce que l'environnement soit favorable à la ponte (Buhlmann et al., 1995 ; Galbraith et al., 1988). Dans certains cas, la rétention des œufs entraîne un stockage dans la cavité abdominale par des trous érodés dans les parois de l'oviducte (Cagle and Tihen, 1948) ou un péristaltisme inverse (Jackson et al., 1971). Les œufs se trouvant dans la cavité abdominale sont souvent infectés par des bactéries, provoquant par conséquent des réactions inflammatoires (Jackson et al., 1971) et pouvant conduire à la mort. La dernière raison, et probablement la plus importante, est que la mauvaise qualité d’un site de ponte dans des habitats ayant des climats à tendance continentaux, comme en Bourgogne ou en Auvergne, peut entrainer un très faible succès d’éclosion ou des phénomènes de sur-prédations par absence d’effet de dilution. Dans l'ensemble, l'altération ou la perte des sites de ponte pourrait conduire à une réduction du succès de reproduction, à des changements de sexe-ratios dans les populations, et donc menacer leur viabilité à long terme. Pour finir, à défaut de pouvoir réduire les coûts de dispersion des mâles, il faut également favoriser leur philopatrie. Cependant, le maintien du flux de gènes via les mâles est particulièrement important pour la conservation de cette espèce, d’autant plus que les cistudes présentent une paternité multiple (Roques et al., 2006). L’apport de flux de gènes pourrait se faire de manière artificielle en amenant quelques mâles compatibles génétiquement qui proviennent de populations ayant des dynamiques actives et des niveaux de diversité génétique élevés. Ainsi, peut-on déplacer quelques mâles des populations de Brenne vers la population de Pierre Poulain ?

D’une manière générale, en biologie de la conservation, la mise en place de plans de gestion efficaces passe au préalable par la connaissance du fonctionnement des populations : structure, démographie, connectivité, histoire... En ce sens, les études en dynamique et génétique des populations apportent des éléments clefs dans la conservation de la nature et le renforcement des populations naturelles et c’est dans cet esprit que s’insère cette thèse, mieux connaître pour mieux protéger.

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