Diversité génétique d'espèces structurantes en environnement marin : influence sur la réponse démographique des populations aux perturbations anthropiques

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Diversité génétique d'espèces structurantes en environnement marin : influence sur la réponse démographique des populations aux perturbations anthropiques

Thèse soutenue le 28 Novembre 2013
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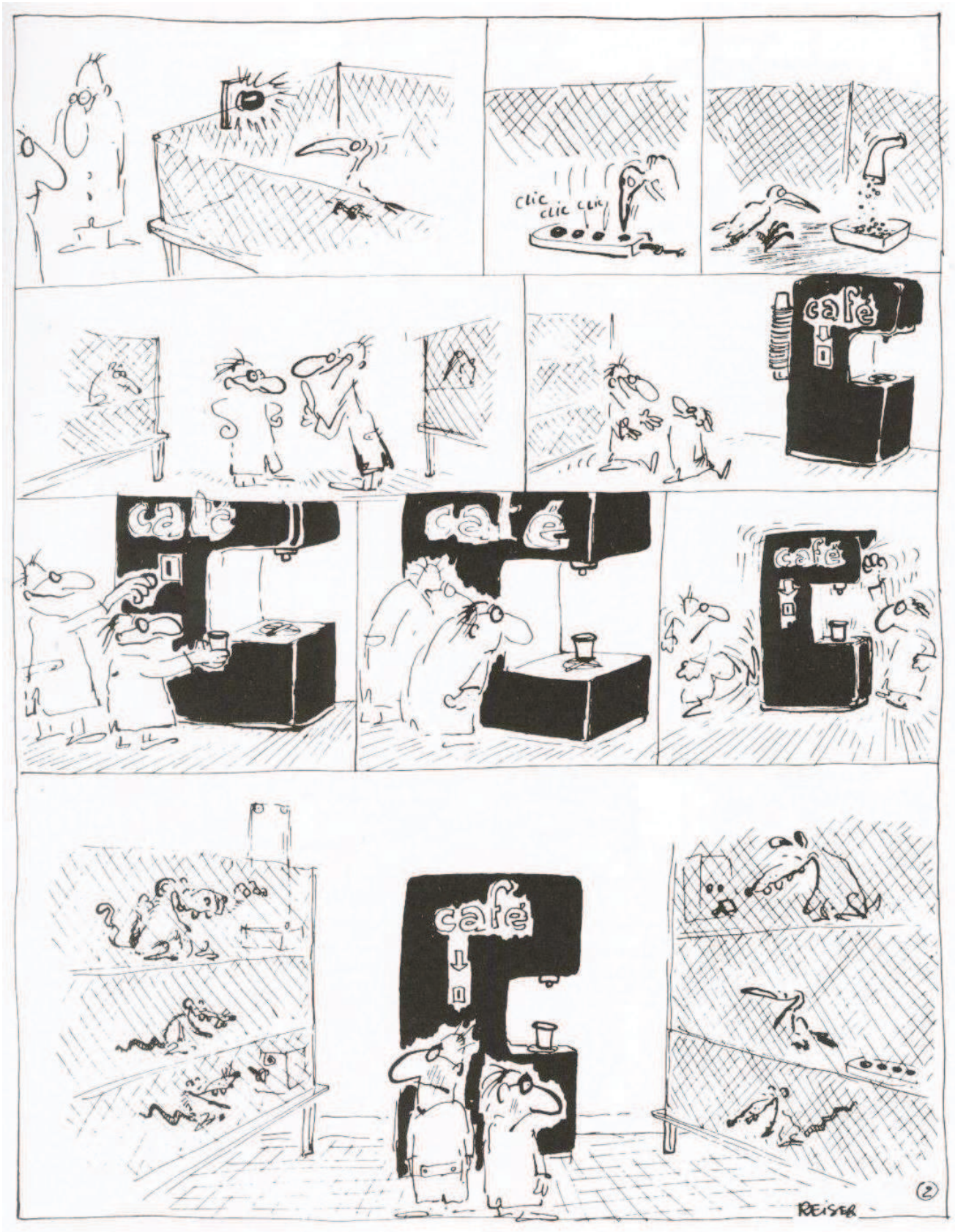
Un dernier petit mot à Khanou. Quelque part, elle est là, ta thèse…


TÜT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INTRODUCTION

LA DIVERSITE GENETIQUE, COMPARTIMENT BASAL DE LA BIODIVERSITE

La diversité génétique est un des trois compartiments de la biodiversité, à l'instar de la diversité des écosystèmes et des espèces (Fig.1; A). Elle est liée au polymorphisme allélique contenu au sein des populations et des espèces, et à la persistance ainsi le potentiel d'adaptation des espèces en dépendent. La diversité génétique est à l'origine, avec l'environnement, des variations phénotypiques, se traduisant notamment par la diversité de formes au sein et entre les espèces (Fig.1, B et C). Le niveau de diversité du patrimoine génétique d'une population varie sous l'effet de forces évolutives (migration, dérive, sélection et mutation. Pour exemple, Fig. 1; D). Ces forces sont généralement considérées indépendamment des forces qui influencent la diversité d'espèces, tels que la compétition, la dispersion ou encore la différentiation de niches écologiques (Neuhauser *et al*, 2003). Un nombre grandissant de travaux démontre l'existence d'interrelations entre ces deux niveaux de diversité biologique, au sein des communautés (par exemple, Lankau and Strauss, 2007), et suggère que cette dépendance joue un rôle majeur dans la dynamique et le maintien des populations et communautés.

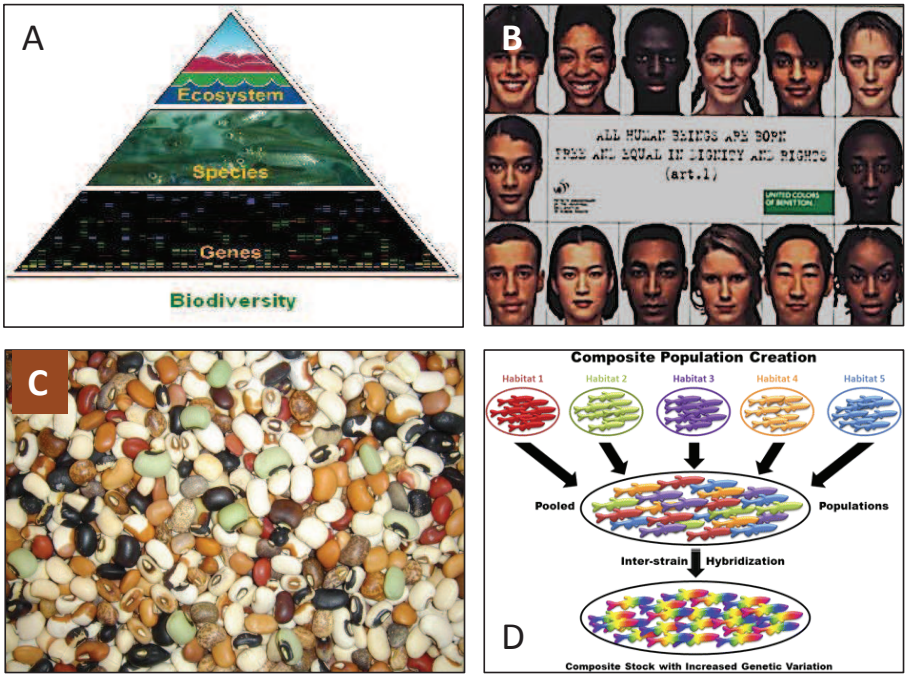

Figure 1 *La diversité génétique.*
Ces quatre images constituent un échantillon des images fournies par google.image, sur sa première page de résultats (la requête étant « diversité génétique »). L'image A (ahs-enviro.blogspot.com) illustre la hiérarchie entre les trois niveaux de biodiversité. Les images B (marketing-etudiant.fr) et C (topdefinitions.com) montrent le lien entre diversité génétique et variations phénotypiques. L'image D (pnas.org) provient d'un article de Brown et al. (2011), visant à illustrer le fait que l'hybridation entre populations localement adaptées est un mécanisme efficace pour générer de la diversité génétique.

Insert 1 Une illustration de l'effet de la diversité génétique sur le potentiel d'adaptation

Il s'agit d'un rapide exercice mené pendant le stage de Master 2, utilisant de façon simpliste la métaphore des pics adaptatifs. Elle fut initialement proposée par Wright, en 1932 (voir également Gavrilets, 1997).

Brièvement, un paysage adaptatif est un espace de N + 1 dimensions (N gènes et la fitness). La surface dite adaptative est la représentation de la fonction qui associe à chaque génotype (une combinaison des N gènes) une valeur de fitness (Fig.2). Les extrema locaux dessinent une surface en creux et bosses, respectivement appelé pics et vallées adaptives. Gavrilets estime que, pour un contexte environnemental donné, une population se situe sur la portion supérieure d'un pic.

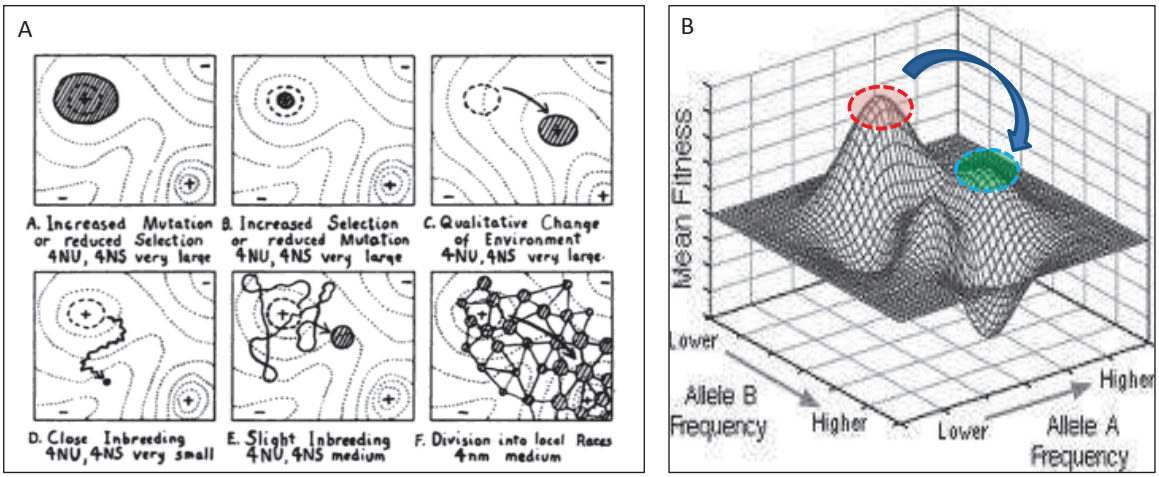

Figure 2 *Représentation des paysages adaptatifs*
Selon Wright (1932) à gauche (A). A droite (B), une illustration dAdam Goldstein (sur shiftingbalance.org), où la fitness moyenne dépend des fréquences alléliques. La population originale est présente sur le sommet du pic rouge. Suite à une fluctuation environnementale, cette population doit atteindre le pic vert.

Suite à une fluctuation environnementale, une population doit être en mesure de « sauter » d'un pic à un autre, sans passer par une vallée adaptative. La dérive et les effets fondateurs sont des mécanismes qui ont été évoqués pour expliquer ces sauts, entre autres dans le cadre de la théorie de « shifting balance », proposée par Wright et les travaux de Whitlock (1997) et Gavrilets & Hastings (1996).

L'objectif de cet exercice est de caractériser l'effet de la diversité génétique sur la probabilité de saut de pics adaptatifs.

Hypothèses de départ :

1. La population est réunie autour d'un pic adaptatif unique (en rouge sur la Fig.2, B).

2. La population est diploïde et suit les conditions d'Hardy-Weinberg.

3. Les allèles sont présents en équi-fréquence.

4. Tous les génotypes ont donc la même probabilité d'apparition.

On travaille avec un seul gène, présentant k allèles. Le nombre d'arrangements possibles est k^2, auquel il faut retrancher, pour obtenir le nombre de génotypes possibles, les répétitions (en effet, le

génotype (Aa) est identique à (aA)). Les répétitions sont tous les couples d'allèles du même locus. C'est donc le nombre de répétitions de 2 éléments parmi k, que l'on notera C(k ;2). Le nombre de génotypes possibles N, après un événement reproductif, est donc :

$N = k^2 - C(k ; 2) = k^2 - (k!) / [2!(k-2)!]$

soit $N = [k (k + 1)] / 2$

On suppose que chaque nouveau génotype se répartit aléatoirement sur la surface adaptative de surface « S ». Un nouveau génotype sera adapté s'il appartient à la portion supérieure du nouveau pic, de surface « s » (en vert sur la Fig.2, B). Pour un génotype pris au hasard, la probabilité de « tomber » dans cette aire donnée vaut $p = s / S$. La probabilité que ce génotype tombe à côté est donc $1 – p$.

Nous sommes alors dans un schéma de Bernouilli, de paramètres (N ; p).

Il est nécessaire pour la population d'avoir au moins un nouveau génotype, au niveau de la portion supérieure du nouveau pic. Nous devons alors estimer $P(X \geq 1)$, qui correspond alors à la probabilité de changer de pic :

$P (X \geq 1) = 1 – P(X < 1)$, soit $P (X \geq 1) = 1 – P(X = 0)$

Après développement, nous obtenons une expression simple :

$P(X \geq 1) = 1 – (1-p)^N$

Il s'agit d'une fonction croissante de N, tendant vers 1 quand N tend vers l'infini. Or, N est fonction croissante de k, le nombre d'allèles par locus. La diversité génétique augmente donc la probabilité de sauter de pics adaptatifs. La figure 3 illustre la forme de cette relation. La forme sigmoïde de cette relation pourrait également faire le lien avec les travaux de Saccheri *et al* (1998), qui concluent que la probabilité d'extinction des populations est d'autant plus forte que le niveau de diversité génétique est faible.

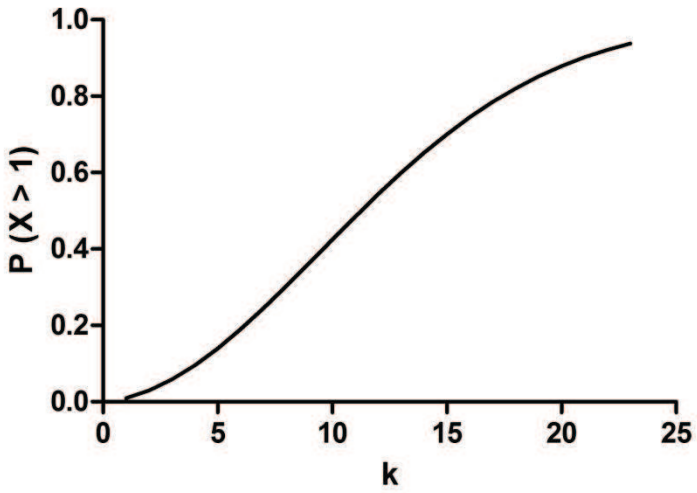

Figure 3 *Courbe représentant la fonction reliant la richesse allélique k et la probabilité de saut de pics adaptatifs P (X ≥ 1).*

DIVERSITE GENETIQUE ET POTENTIEL D'ADAPTATION DES POPULATIONS

En biologie de la conservation, la diversité génétique est proposée comme le reflet du potentiel adaptatif des populations, permettant de faire face à des changements environnementaux majeurs, du type de ceux classiquement imposés par les activités humaines, ou attendus dans le cadre du changement global. La pérennité d'une population dépend, en particulier, de sa capacité à engendrer un ensemble varié de nouveaux génotypes, dont une partie sera porteuse de combinaisons d'allèles permettant de vivre dans de nouvelles conditions environnementales. L'insert 1 fournit une illustration de ce lien entre diversité génétique et adaptabilité, en s'appuyant sur la théorie des pics adaptatifs.

L'influence de la diversité sur la persistance et le potentiel évolutif, constitue la raison essentielle pour laquelle l'IUCN, ainsi que la Convention pour la Diversité Biologique (CBD, sommet de Rio en 1992), ont inscrit ce niveau de biodiversité comme l'un des trois axes de conservation prioritaire (Laikre *et al*, 2010; Reed and Frankham, 2003). Elle relève de la capacité d'adaptation de la population dans sa globalité. L'adaptabilité individuelle est également un critère pris en compte par la CBD, puisque celle-ci est potentiellement influencée par la diversité génétique de l'individu, c'est-à-dire son niveau d'hétérozygotie.

L'hétérozygotie d'un individu peut être corrélée à sa performance écologique ou sa fitness. Par exemple, il a été empiriquement montré que, dans les cas d'extrêmes réductions démographiques, les populations sont simultanément affectées par une dérive génétique (Insert 2, modèle de Wright-Fisher) intense, réduisant substantiellement leur variabilité génétique globale, et par l'*inbreeding depression* liée aux diminutions de diversité individuelle. Il s'agit du phénomène qui, suite à des évènements de reproduction entre apparentés, provoque une diminution de la fitness de la population (conséquence de la fixation d'allèles délétères dans la population, et/ou de l'apparition d'homozygotes pour des allèles délétères). Ainsi, en deçà d'un certain seuil de réduction démographique, la population est affectée par une perte de diversité génétique jusqu'au niveau individuel (diminution de l'hétérozygotie globale et individuelle) et par une diminution de la fitness globale. Il s'agit d'une explication des corrélations entre hétérozygotie et fitness (Insert 3). Saccheri *et al.,* (1998) ont montré notamment que les populations contenant une faible variabilité génétique, présentent une plus forte probabilité d'extinction (Insert3).

Insert 2 Les concepts de base en génétique des populations

Les prémices de la génétique remontent à 1865, lorsque Mendel découvre les principes régissant la transmission du matériel héréditaire. La génétique des populations, raisonnant en termes de fréquences des allèles dans les populations, est apparue dans les années 1920, lorsque l'on a cherché à concilier la théorie darwinienne et les lois de Mendel. Les pères de cette conciliation sont Fisher, Haldane et Wright.

Les populations évoluent sous l'effet des forces évolutives, qui modifient dans le temps et l'espace les fréquences alléliques. Ces forces, phénomènes naturels ou statistiques, sont au nombre de quatre ○ **la sélection ;** ○ **la mutation ;** ○ **la dérive génétique ;** ○ **la migration.**

Pour comprendre l'effet de ces forces évolutives, des modèles théoriques d'évolution des fréquences alléliques entre générations ont été élaborés. Ce sera donc la comparaison des signatures génétiques observées, par rapport aux prédictions de ces modèles, qui permettra au généticien des populations d'inférer sur les mécanismes évolutifs agissant sur les populations d'étude. Ci-dessous sont présentés les deux principaux modèles

1. **Le modèle de Hardy-Weinberg (1908) ; cas idéal**

Ce modèle a été développé simultanément et indépendamment par Hardy (mathématicien anglais) et Weinberg (médecin allemand). Les hypothèses, sur lesquelles il repose sont :

○ taille infinie de populations. La fréquence d'un évènement est égale à sa probabilité. Il s'agit de la loi des grands nombres, la stochasticité n'a pas d'effet (pas de dérive génétique).

○ panmixie (les gamètes s'associent au hasard, par rapport aux gènes considérés)

○ Il n'y a ni sélection, ni mutation, ni migration

Dans une population diploïde, le gène considéré est représenté par deux allèles, A et a, de fréquence respective p et q, avec $p + q = 1$. Le génotype [AA] possède une fréquence génotype valant p^2, [Aa] de $2pq$, et [aa] de q^2. Etant donné qu'aucune force n'agit sur cette population, les gamètes qu'elle produit contiennent :

-l'allèle A en fréquence $p' = p^2 + ½ (2pq) = p$

-l'allèle a en fréquence $q' = q^2 + ½ (2pq) = q$

Ainsi, à la génération suivante, les fréquences des génotypes sont invariantes, de même que les fréquences alléliques.

NB : s'il n'y a pas panmixie, les fréquences génotypiques évoluent. En revanche, les fréquences alléliques restent constantes. Il s'agit simplement d'une redistribution, non liée au hasard, des allèles entre individus.

2. **Le modèle de Wright-Fisher**

Ce modèle a été élaboré afin de caractériser l'effet du hasard dans l'évolution des fréquences alléliques (dérive génétique). Comme pour le modèle de Hardy-Weinberg, il n'y a pas de mutation, de migration et de sélection. Trois hypothèses supplémentaires sont apportées :

○ taille finie de populations N

○ pas de chevauchement de générations

○ autofécondation possible avec un nombre infini de gamètes

Suite Insert 2

Les allèles A et a ont des fréquences respectives de p et q (avec p + q = 1). La dérive génétique correspond aux variations de fréquences de ces allèles dans le temps. La proportion d'hétérozygotes à la génération t + 1 est :

$H_{t+1} = (1 – 1/(2N))H_t$ et $H_1 = (1 – 1/(2N))H_0$

Par récurrence, on obtient :

$H_t = (1 – 1/(2N))^t H_0$

Cette suite géométrique prédit une disparition des hétérozygotes, conséquence de la fixation d'un des deux allèles (p ou q tendent vers 0). Ce résultat est repris dans l'Insert 3, et ses conséquences en génétique de la conservation, discutées.

Ce modèle introduit également la notion de taille efficace de population, notée **Ne**, qui correspond au nombre d'individus reproducteurs, prenant effectivement part à la constitution du pool de gènes de génération suivante. Elle est déterminante de l'effet de la dérive, c'est-à-dire de la diminution du polymorphisme au fil des générations, un phénomène qui ne peut être contré que par la mutation (et la migration dans les systèmes ouverts) qui en revanche introduit de nouveaux allèles.

Les validations empiriques du bénéfice de la diversité génétique, pour les populations et espèces, ont à ce jour principalement été menées sur des cas particuliers de populations extrêmement réduites et appauvries au niveau génétique(pour revue, voir Spielman *et al*, 2004b). Pourtant, l'influence de la diversité génétique sur les capacités de résistance et résilience des populations est un attendu plus global, devenu un paradigme de l'écologie évolutive. Un second paradoxe persiste en biologie de la conservation : malgré le paradigme énoncé ci-dessus, la variabilité génétique reste largement négligée par les mesures de protection de la biodiversité (Laikre *et al*, 2010). Cette lacune peut avoir plusieurs causes, parmi lesquelles le manque de démonstration formelle concernant les conséquences écologiques des variations de diversité génétique. L'objectif fondamental de cette thèse est de poursuivre autant que possible l'exploration de ces relations, y compris dans les populations dont la taille (et la diversité génétique) n'ont pas été réduites au point de les considérer comme étant au bord de l'extinction.

Les réponses des individus aux changements environnementaux doivent être également être prises en compte. Ceci repose sur le concept de plasticité phénotypique, qui correspond à la capacité d'un individu à ajuster son phénotype aux conditions environnementales rencontrées. Ainsi, les capacités de réponses d'une population aux fluctuations de son environnement dépendent de (1) la plasticité phénotypique, se définissant à l'échelle individuelle et (2) la diversité génétique contenue par les populations.

Insert 3 Lien entre démographie, variabilité génétique, et fitness.

Les liens entre démographie et génétique sont variés. Ici est présenté un exemple, repris dans l'introduction du papier de Reed & Frankham (2003). Sous le modèle de Wright-Fisher, l'équation régissant l'évolution de l'hétérozygotie dans le temps (en nombre de génération) est la suivante :

$Ht/H_0 = (1 – (1/(2Ne))^t$ ou $H(t) = (1 -1/(2Ne))^t * H_0$ (1)

Il s'agit d'une suite géométrique de raison (1 – (1/2Ne)). La raison étant inférieure à 1, cette suite est décroissante, sauf si Ne tend vers l'infini, auquel cas, l'hétérozygotie est constante. Cette équation n'a pu être posée que sous certaines hypothèses, mais elle permet de prédire qu'en cas de forte réduction démographique (i.e quand la dérive génétique n'est pas compensée par d'autres forces évolutives), la perte de variabilité génétique est inversement proportionnelle à la taille efficace Ne. La figure 4 illustre la réduction de l'hétérozygotie en fonction du nombre de génération.

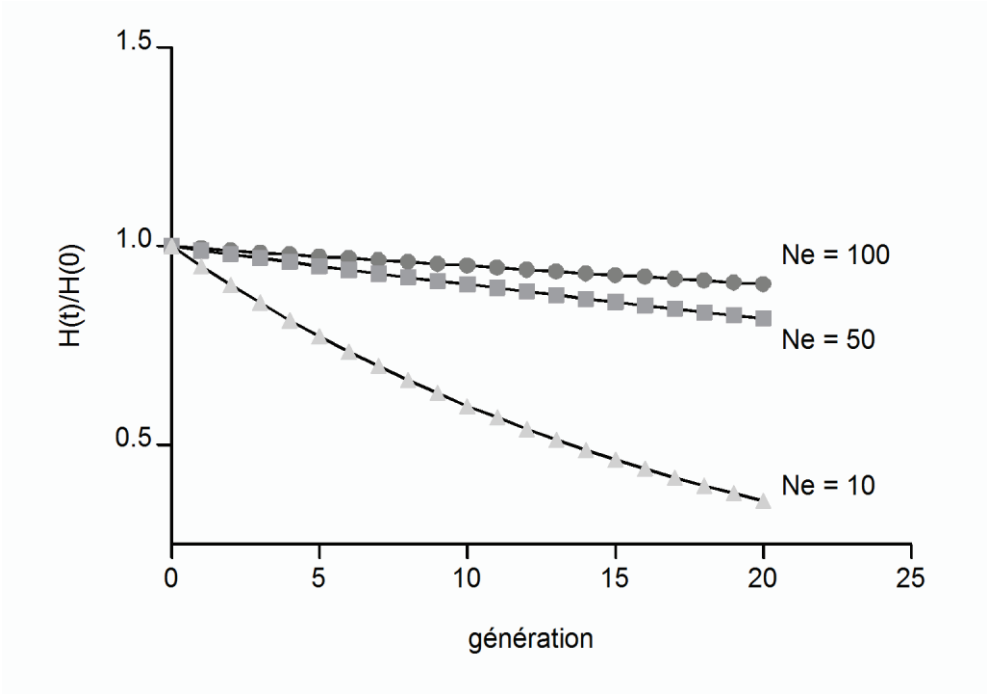

Figure 4 *Réduction du taux d'hétérozygotie dans le temps pour une population sexuée isolée, seulement soumise à la dérive génétique. Il s'agit de l'illustration de l'équation* $H(t)/H(0) = (1- (1/(2Ne))^t$ *en prenant : Ne = 100 ; Ne = 50 ; Ne = 10.*

Ceci illustre une des différentes relations qui peuvent exister entre démographie et génétique. Ici, le mécanisme sur lequel reposent ces réductions attendues de variations génétiques est la **dérive génétique** (sous l'hypothèse de l'absence de mutation, migration et sélection)**.** La figure 5, quant à elle, présente l'imbrication entre réduction démographique, dérive et *inbreeding depression*, une des origines de l'idée d'une corrélation entre niveau d'hétérozygotie et fitness (avec le phénomène d'hétérosis ; Reed & Frankham, 2003).

Suite Insert 3

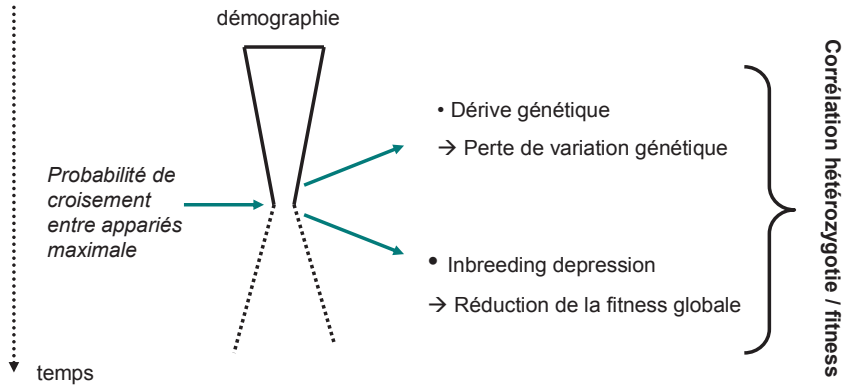

Figure 5 *Une des origines de l'idée des corrélations hétérozygotie-fitness*

La notion de fitness est centrale en écologie évolutive, et correspond au produit de la viabilité (probabilité d'un individu de vivre jusqu'à l'âge reproducteur) et la fertilité (nombre moyen de descendant à la génération suivante). La fitness d'un individu correspond donc à la probabilité qu'il transmette ses gènes à la génération suivante, donc à sa participation à la constitution du « pool » génétique de la génération suivante.

Dans les cas les plus extrêmes de réduction de taille de populations, un phénomène d'auto-emballement, appelé vortex d'extinction, peut se produire (Fig. 6).

GENETIQUE DES POPULATIONS ET GENETIQUE DE LA CONSERVATION

La génétique des populations est une discipline focalisée sur la diversité génétique (liée au polymorphisme des allèles), et l'étude de sa distribution au sein des individus (niveau d'hétérozygotie), des populations (richesse en allèles contenue par la population, équivalent à la diversité α d'une communauté) et entre les populations (différences de nature et fréquence des allèles entre populations, équivalent à la diversité β pour les communautés), sous l'influence de forces évolutives.

Cette discipline repose à la fois sur un formalisme mathématique, qui fournit les outils d'étude de la distribution de la variabilité génétique, et sur des interprétations biologiques centrées sur l'intensité des différentes forces évolutives la démographie et la dynamique des populations considérées.

Une définition de la biologie de la conservation

Une nouvelle discipline qui s'adresse aux dynamiques et problèmes d'espèces, communautés et écosystèmes perturbés. […] Son objectif est de fournir des principes et outils pour la préservation de la diversité biologique (Soulé, dans le livre Science of scarcity and diversity)

La **génétique de la conservation** est une branche de la biologie de la conservation en général. Frankel a été un des principaux scientifiques ayant fait reconnaître l'importance des facteurs génétiques en conservation,

avec Soulé qui fut déterminant dans le développement interdisciplinaire de cette science (dans Frankham, 1995a).

Les 5 phénomènes majeurs susceptibles d'impacter la pérennité des populations sont (1) l'*inbreeding depression* (voir Insert 3), (2) l'accumulation et la perte de mutations délétères, (3) la perte de variabilité génétique dans les petites populations (voir Inserts 2 et 3) (4) l'*outbreeding depression*, (5) la fragmentation des populations et la réduction des taux de migration.

Les trois premiers peuvent agir de concert, pour déboucher sur un vortex d'extinction (Fig. 6). L'*outbreeding depression* correspond à la réduction de la valeur adaptive après hybridation de populations divergentes. Le mécanisme sous-jacent est la destruction des complexes de gènes co-adaptés, et les créations de génotypes hybrides dont les combinaisons alléliques ne sont pas optimales (ces génotypes hybrides se trouvent au niveau d'une vallée adaptative). Enfin, la fragmentation des habitats des populations augmente leur isolement spatial, ayant pour conséquences possibles une augmentation de la dérive et de l'identité par ascendance, ainsi qu'une dispersion moindre et de plus grandes difficultés de recolonisations en cas d'extinction locale. La fragmentation peut également conduire à un vortex d'extinction.

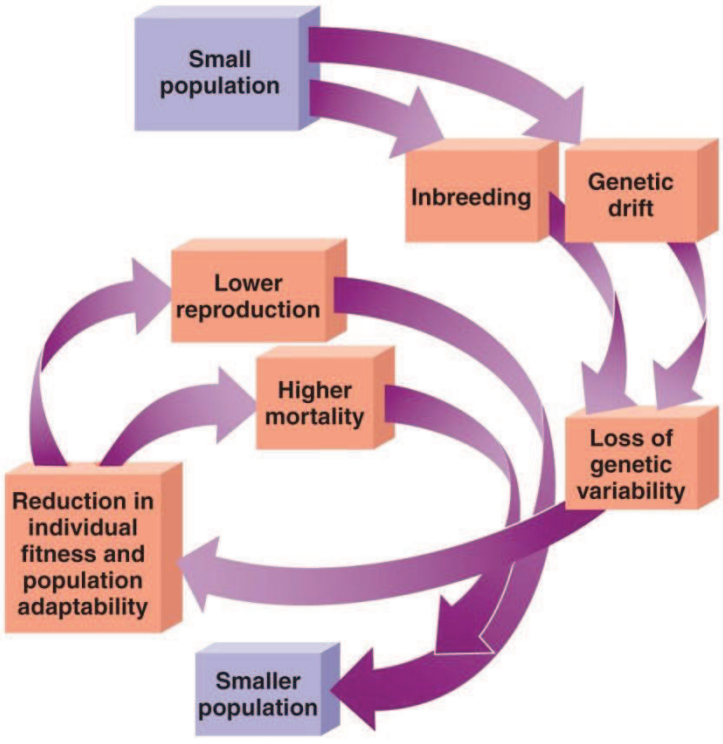

Figure 6 *Mécanismes à l'origine du phénomène du vortex d'extinction (apbiosemonefinalreview.pbworks.com)*

L'INTERFACE ENTRE GENETIQUE DES POPULATIONS ET DEMOGRAPHIE : POURQUOI EST-ELLE CRUCIALE ?

Les réductions démographiques peuvent mener, au-delà d'un certain seuil, à une intensification des phénomènes stochastiques. Cette stochasticité affecte aussi bien la génétique (via la dérive) que le fonctionnement dynamique des populations.

Considérant les conséquences génétiques et démographiques distinctes, Lande (1988) a publié un article largement controversé par la suite, suggérant que les réponses démographiques sont plus immédiates que les réponses génétiques. On peut résumer son point de vue de la façon suivante : les problèmes démographiques se manifestant de façon plus rapide et drastique que les conséquences génétiques, les plans de conservation doivent se concentrer sur la démographie.

Une méta-analyse plus récente (Spielman *et al*, 2004b) démontre que cette conclusion est erronée, mettant en évidence qu'une majorité de taxons menacés arbore une diversité génétique plus faible que les taxons proches non menacés. Entre autres, cette analyse met en évidence que Lande (1988) a sous-estimé les seuils démographiques en deçà desquels dérive et *inbreeding* se manifestent. De même, leurs effets sur la viabilité des populations ont été également sous-estimés, par manque d'information. En particulier, Lande n'a pas considéré les interactions génétique/démographie. Ces auteurs (Spielman *et al*, 2004b) concluent donc que la plupart des espèces ne sont pas conduites à l'extinction avant d'être affectées par les conséquences génétiques des aléas démographiques.

Les interactions potentielles entre génétique et démographie sont d'ailleurs implicitement suggérées par certaines définitions du concept de population. Une définition, intégrant au mieux les différentes composantes des définitions précédentes, a été proposée par Camus & Lima (2002) ainsi que par Berryman (2002) (Insert 4). Les concepts d'unité panmictique ainsi que de forces évolutives sont implicitement présents dans cette définition « écologique ». La notion de panmixie est suggérée par les termes « vivant ensemble », même si ce critère n'est qu'un pré-requis de la panmixie et ne la garantit pas pour autant. Le terme « changement numérique » évoque la démographie de la population. On peut ainsi considérer qu'il englobe la notion de taille efficace, et donc de la dérive génétique. Les termes de dispersion et migration évoquent quant à eux la connectivité au sens démographique (un flux d'individus) mais également la connectivité génétique, puisque les individus migrants sont les transporteurs des allèles qui, si ces individus produisent des descendants, s'intègreront à la génération suivante. Enfin, derrière les termes de naissance et mort, qui sont les déterminants essentiels de la démographie, se cachent les notions de chevauchement de générations (une hypothèse lourde qui sous-tends les modèles de génétique des populations étant l'absence de chevauchement), de fitness (capacité des individus à produire des descendants viables et donc de transmettre leurs gènes à la génération suivante), de sélection (force évolutive s'appliquant sur des génotypes, ou individus, modulant les fréquences d'allèles sous sélection).

Enfin, parce que les allèles de la population panmictique sont contenus dans les individus de la population écologique, diversité génétique et démographie sont en constante interaction, et tout phénomène affectant l'un de ces compartiments (forces évolutives et mécanismes de dynamique des populations) a, *de facto,* une répercussion possible sur le second. Une revue intitulée « Why evolutionary biologists should be demographers »(Metcalf and Pavard, 2007) insiste sur le fait que les phénomènes évolutifs sont indissociables des trajectoires démographiques des populations (incluant notamment la survie et la fertilité des individus, en fonction du stade dans le cycle de vie).

L'objectif de cet article est de mettre en évidence la nécessité d'une vision intégrative de la biologie évolutive et de la démographie.

Si l'existence de ces relations parait évidente, leur caractérisation demeure un thème de recherche à part entière. La notion même de population est problématique. La population théorique se conçoit très bien sur le plan conceptuel. En pratique, ni la population au sens écologique, ni l'unité panmictique ne sont facilement identifiables. La difficulté majeure pour appréhender les contours des populations et leur connectivité réside dans notre capacité à estimer les distances de dispersion, et leur intensité, comparer leur effets sur la démographie et les allèles par rapport aux phénomènes internes (naissance et mort). Ceci est indispensable pour fixer une limite entre les propositions « ce mouvement d'individus est un simple déplacement au sein de la population » et « ce mouvement d'individus est une migration de la population A vers la population B », et ainsi contraindre les échelles de temps et d'espace qui délimitent la population.

Insert 4 Définitions de population (d'après Camus & Lima, 2002) et de la stabilité (d'après McCann, 2000)

Définition de population : groupe d'individus appartenant à la même espèce, vivant dans un espace de taille suffisante pour permettre des comportements normaux de dispersion et/ou migration, et au sein duquel les changements numériques sont essentiellement déterminés par les processus de naissance et mort.

Définitions de la stabilité dynamique

Stabilité d'équilibre : une mesure discrète considérant qu'un système est stable s'il retourne à l'équilibre après une faible perturbation. Ainsi, un système stable ne connaît pas de variation en l'absence de perturbation.
Stabilité générale : une mesure qui suppose que la stabilité est d'autant plus forte que le niveau minimal atteint aux cours des fluctuations –démographiques, par exemple- est important. Pour une population, plus sa démographie s'approchera de 0 (variance forte) lors de ses minima locaux, moins elle sera stable.
Variabilité démographique : ceci tient compte de la variation de la densité de population dans le temps, habituellement mesurée par la variance

Définitions de la résilience et la résistance

Résilience: une mesure de la stabilité d'un système qui augmente quand le temps nécessaire pour retourner à l'équilibre après perturbation, diminue.

Résistance : mesure du niveau de variation d'une variable lors d'une perturbation, ou capacité à rester inchangé après perturbation. En considérant la démographie comme variable, la résistance de la population correspond à la capacité de maintien du niveau démographique (donc à la survie moyenne des individus).

LE ROLE POTENTIEL DE LA CONNECTIVITE

La dispersion des organismes marins, dont beaucoup sont caractérisés par un cycle de vie complexe comprenant une phase dispersante et une phase fixée, a longtemps été considérée comme forte. Le milieu marin étant un milieu très dispersif et les populations marines souvent de grande taille et de forte fécondité, une absence de structure génétique était attendue (l'intensité des flux de gènes homogénéisant les pools de gènes des sous-populations). Ceci dit, de nombreux exemples de limitation de flux de gènes en milieu marin ont été rapportés depuis (Cowen *et al*, 2000; Cowen *et al*, 2006; Jones *et al*, 1999; Palumbi and Warner, 2003; Swearer *et al*, 2002; Taylor and Hellberg, 2003). Etant donnée la difficulté, voire l'impossibilité de réaliser des suivis directs pour estimer le degré d'interconnexion des populations (Insert 5), les suivis indirects, via l'utilisation de marqueurs moléculaires, sont largement répandus.

La connectivité a-t-elle une influence sur la stabilité globale des populations (Fig. 7) ?
Sur une échelle de temps et d'espace écologique, la connectivité correspond au flux d'individus potentiellement impliqués dans le maintien de la démographie d'une population et les processus de recolonisation. A l'échelle évolutive, elle représente un flux de gènes influençant l'évolution de la diversité génétique (la migration est une force évolutive), contrebalançant l'effet de l'adaptation locale (Insert 5). Plusieurs ordres de grandeur séparent en général, ces deux types de connectivité. Pour que deux populations soient connectées au sens génétique, seul le nombre absolu de migrant compte théoriquement. Il suffit de quelques migrants efficaces par génération, un nombre d'individus devant se reproduire dans la population d'arrivée, mais dont l'effet démographique peut-être négligeable. En revanche, une connectivité au sens démographique dépend de la proportion d'individus migrants et requiert donc, en général, un nombre beaucoup plus important d'individus échangés, mais la transmission de gènes n'est pas requise.

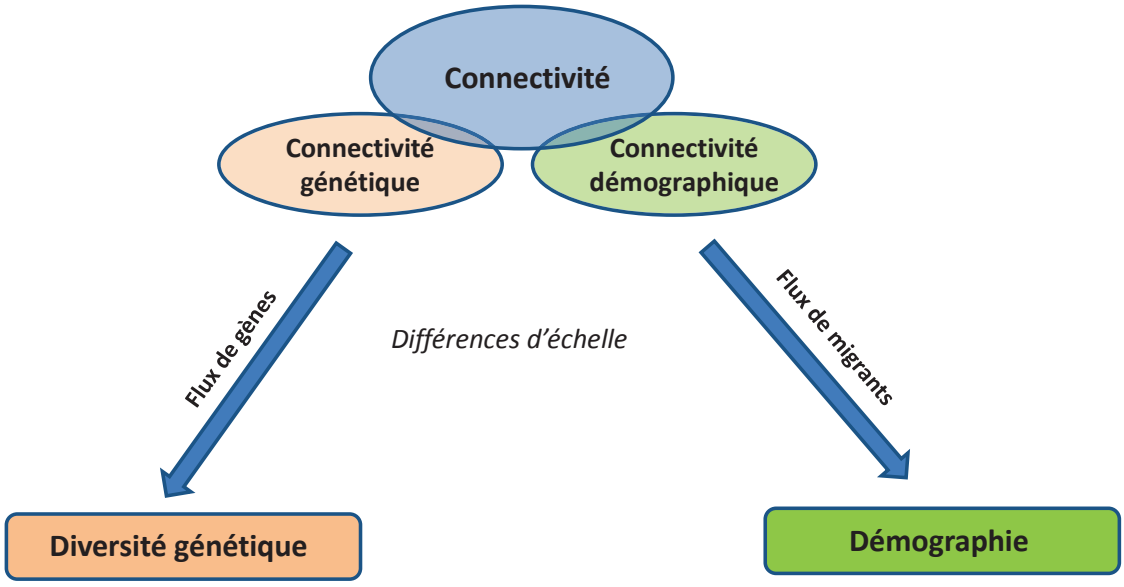

Figure 7 *Influence de la connectivité sur le niveau de diversité génétique et la démographie des populations*

Insert 5 La migration ; un phénomène difficile à suivre directement et pouvant contrer la sélection

Un exemple de la difficulté à réaliser un suivi direct

L'acquisition directe d'informations par la méthode de capture-marquage-recapture est, dans le cas d'espèces côtières, déjà particulièrement ambitieux. Jones *et al* (1999) ont marqué environs 10 millions d'œufs de poissons demoiselles à la tétracycline, un fluorochrome intégré à la matrice de l'otolithe, et n'ont pu ré-échantillonner que 15 larves en phase de pré-recrutement. Pourtant, il s'agit d'une espèce côtière, caractérisée par un fort taux d'autorecrutement (le cas de figure le plus favorable au ré-échantillonnage). Pour la plupart des organismes marins, appliquer une stratégie similaire est donc impossible.

Adaptation locale versus migration

L'adaptation locale représente la conséquence évolutive des variations spatiales de la sélection naturelle. La fitness dépend des interactions génotype * environnement. En l'absence d'autres forces, cette sélection divergente peut générer pour chaque population locale (dème) une évolution des traits représentant un avantage pour les conditions environnementales locales (ou habitat), sans tenir compte de la valeur adaptative de ces traits dans les autres habitats (Kawecki and Ebert, 2004). Le flux de gènes peut contrebalancer les forces sélectives, impliquées dans l'adaptation locale, en déstructurant les complexes de gènes co-adaptés.

LA STABILITE D'UNE POPULATION ; QUELQUES DEFINITIONS

Le sens commun de « stable » est « ce qui ne varie pas, ou très peu ». Le dictionnaire Larousse propose deux définitions de l'adjectif « stable » :

- Qui repose solidement sur sa base et dans une position d'équilibre bien assurée
- Qui se maintient durablement dans tel ou tel état

Il s'agit de définitions fixistes, qui n'ont qu'une faible validité en écologie, où l'intégralité des éléments de l'écosystème fluctue en permanence. La notion de stabilité dynamique est plus appropriée (voir Insert 6). En admettant une variabilité résiduelle permanente, tout l'enjeu réside dans la définition de ce que l'on considèrera comme des fluctuations « normales » pour une population jugée stable ou comme une variabilité anormale pour une population instable. Ceci implique de travailler soit :

- le long d'un gradient de variabilité, par exemple sur un ensemble de populations dont les variations de densité ou de biomasse sont plus ou moins fortes. Par comparaison, on peut alors affirmer que la population « A » est plus stable que la population « B » si ses variations de densité sont plus faibles.
- à la définition d'un état de référence qui constituerait le niveau « 0 » de l'instabilité écologique. Idéalement, cet état de référence doit être résumé dans un ensemble de paramètres décrivant un type donné d'écosystème hors perturbation. Ceci amène deux nouvelles questions. Existe-t-il des écosystèmes exempts de toute perturbation ? Qu'appelle-t-on perturbation ?

En français, nous utilisons essentiellement les mots « perturbation » et « impact ». Les anglophones, quant à eux, parlent dans la littérature de « disturbance » et « perturbation », qui sont strictement synonymes dans l'usage courant. Néanmoins il existe une nuance en écologie, explicitée dans une revue de Rykiel (1985). « disturbance » fait référence à la cause, qu'elle soit biotique ou abiotique, provoquant une « perturbation », décrivant un écart aux fluctuations « normales » ou « résiduelles » dans le système considéré. Là encore, cette terminologie est relative à un état de référence i.e un système supposé non perturbé (Bazzaz, 1983; Vitousek and White, 1981).
Pour en revenir au français, « impact » serait l'équivalent de la « disturbance », et perturbation serait également la conséquence.

En bref, l'instabilité « anormale » constitue une perturbation, en réponse à un impact s'appliquant au système considéré (ou disturbance pour les anglophones).

Si l'on ramène ces considérations au niveau de la population au sens écologique, la stabilité se traduit par une faible variance du nombre d'individus (ou des dérivés tels que densité de population, biomasse, pourcentage de recouvrement, etc.). Ceci revient à considérer dN/dt, N étant un proxy de la démographie, et t le temps, et ses variations dans le temps. La stabilité dynamique implique que ce rapport n'est jamais nul, à un temps « t », mais sa valeur moyenne sur le moyen et long terme tend vers 0.

L'ensemble des perturbations d'origine anthropique (pollution, changement global, fragmentation des habitats…) représente un défi pour les populations. La pérennité d'une population dépend de deux points :
- de ses capacités intrinsèques à se maintenir dans un environnement fluctuant ou impacté. Ceci inclut les capacités de résistance et résilience des individus présents, et les capacités d'adaptation sur le court terme (plasticité phénotypique) et les moyen et long terme (évolution du pool génétique de la population)
- de facteurs extrinsèques, dépendant du degré d'interconnexion avec les populations voisines. L'apport régulier d'individus externes favorise le maintien de la démographie, peut alimenter la population en diversité génétique, et dans le cas de populations fortement impactées (forte diminution démographique), la recolonisation constitue un facteur de résilience.

LES RELATIONS DIVERSITE-STABILITE

Les premières études rapportées sur ce thème ont été réalisées dans les années 50, par Elton, Odum et McArthur (dans McCann, 2000). Suite à des observations répétées de communautés terrestres, ils aboutissent à des conclusions similaires : les communautés terrestres grandement simplifiées subissent des plus fortes variations d'abondance que les communautés diversifiées. Par exemple, les îles, qui comportent généralement peu d'espèces, sont davantage sujettes aux invasions biologiques que les continents. Bien plus tôt, en guise d'anecdote, Darwin écrit dans son livre « L'origine des espèces » (1859) « Si l'on ensemence deux parcelles de terre identique, l'une avec une seule espèce d'herbe, l'autre avec une variété de genre, on récolte dans la seconde une plus grande et plus riche quantité de foin ».

Dans les années 1990, les approches se sont multipliées, de l'expérience à la modélisation, se focalisant sur les relations entre diversité spécifique et diversité fonctionnelle ainsi que sur leur influence sur la stabilité des communautés. Exceptés de rares travaux, la plupart des articles conclut que les systèmes les plus complexes, c'est-à-dire les plus diversifiés sur le plan fonctionnel, ont des capacités de résistance et de résilience accrues (Hector *et al*, 2002; Hector *et al*, 1999; Naeem *et al*, 1996; Tilman *et al*, 2001; Tilman *et al*, 1996). Ceci rejoint les observations empiriques d'Elton, Odum et McArthur, et l'interprétation de ces résultats repose sur deux grandes familles de théories (Insert 5). La première famille est statistique, et prédit que les fluctuations des différentes populations qui composent la communauté diminuent quand le nombre de populations (i.e la diversité spécifique) augmente (Tilman *et al*, 1998; Walker, 1992). L'autre famille est basée sur les traits fonctionnels, correspondant aux propriétés de l'espèce, et qui sont répartis entre espèces de façon redondante ou complémentaire. Suivant la façon dont ces traits sont répartis au sein de la communauté, les théories écologiques proposent des hypothèses décrivant l'effet de la diversité des communautés sur la stabilité globale du système (Insert 6).

Insert 6 Les théories diversité-stabilité

Les théories statistiques

L'effet porfolio prédit une diminution de la variance totale des communautés, quand le nombre d'espèces augmente.

L'effet de la covariance négative fait intervenir des interactions négatives entre espèces, qui ont pour conséquence une diminution de la variance totale (Var (A + B) = Var (A) + Var (B) + cov (A,B), avec cov A,B négative).

Les théories écologiques

La redondance fonctionnelle (Walker, 1992) : ce terme désigne la présence de plusieurs entités biologiques présentant des traits fonctionnels similaires (Tilman *et al*, 1997). Cette hypothèse peut être déclinée de la façon suivante :

- le fonctionnement d'un écosystème dépend plus de sa diversité fonctionnelle que de sa diversité biologique ;
- chaque groupe fonctionnel étant composé de une ou plusieurs espèces, la perte de 10 espèces réparties en 10 groupes fonctionnels différents est moins grave que la perte des 10 espèces composant la totalité d'un groupe.

La forme de la relation est une courbe hyperbolique croissante, avec une forte pente à l'origine et, donc, un seuil de saturation vite atteint. Cette théorie est bâtie à partir d'expériences de culture en champs. Ceci étant, Naeem (1998) met en garde contre cette vision, dans le cas d'un écosystème naturel complexe, précisant qu'il est nécessaire de considérer conjointement la redondance fonctionnelle et le nombre de groupes fonctionnels. En effet, il démontre analytiquement que la redondance augmente la fiabilité d'un tel système, mais que la probabilité de disfonctionnement de l'écosystème augmente avec le nombre de groupes fonctionnels.

Suite Insert 6 :

La complémentarité : cette théorie explique une utilisation optimale de la ressource, par son partage dans le temps et l'espace (Hector *et al*, 1999; Loreau and Hector, 2001; Tilman *et al*, 2001). Ce mécanisme repose sur deux phénomènes sous-jacents :

- la différenciation de niche, qui suppose que chaque unité biologique possède une niche écologique propre et originale ;
- la facilitation, désignant un type d'interactions positives entre espèces. Par exemple, les légumineuses produisent leur nitrate par fixation de diazote atmosphérique, et facilitent ainsi le pompage de nitrate pour les autres plantes (Loreau and Hector, 2001).

On peut également citer le fait que la diversité phénotypique des plantes d'un milieu permet une utilisation optimale de la lumière, par l'éventail de taille et de forme (Loreau and Behera, 1999; Naeem *et al*, 1994). La forme de la relation entre richesse spécifique et stabilité, sous l'hypothèse de la complémentarité, est similaire à celle de la redondance (hyperbole croissante), avec une pente à l'origine moins forte.

La synergie : cette théorie repose sur le rôle crucial des interactions au sein d'un réseau trophique, en particuliers les interactions faibles (McCann, 2000). Une interaction faible entre deux espèces (proie et prédateur) correspond à un flux d'énergie de faible intensité, entre celles-ci. Les interactions faibles tamponnent l'impact des interactions fortes, ayant par là un rôle stabilisant fondamental. Le nombre d'interactions augmente de façon exponentielle avec la richesse biologique. Si le nombre d'interactions faibles augmente en proportion, la stabilité est liée à la richesse suivant une relation exponentielle (Danovaro *et al*, 2008; Downing and Leibold, 2002).

Les rivets pop : dans le cas des écosystèmes à espèces structurantes (herbiers de phanérogames marines, forêt de kelp, moulières, mangroves, etc), la diversité au niveau des espèces structurantes est faible, et il n'y a pas ou peu de redondance fonctionnelle (Micheli and Halpern, 2005). La théorie, dite des rivets pop, prévoit des chutes brusques de fonctionnement, en cas de disparition de la ou des espèce(s) structurante(s). Ceci concerne également les écosystèmes à espèces clé-de-voute, qui peuvent présenter une faible abondance mais être néanmoins fondamentales pour la dynamique et le maintien de l'écosystème. Les caractéristiques de ces systèmes font qu'ils sont particulièrement sensibles à différentes perturbations, telles que la destruction des habitats, la pollution.

L'idiosyncracie : cette théorie prévoit l'absence de relation entre diversité et stabilité. Chaque communauté possède son propre fonctionnement, que l'on ne peut prévoir, et qui dépend moins du nombre d'espèces, que de la qualité de celles-ci (Downing and Leibold, 2002).

Dans les années 2000, des travaux similaires ont été conduits pour étudier l'influence de la diversité intra-spécifique chez des espèces partiellement clonales. C'est d'ailleurs essentiellement la Zostère marine, *Zostera marina* (l'une des espèces au cœur de cette thèse) qui a été utilisée par ces expériences. Il s'agit également d'une espèce structurante. Ce statut particulier de la population structurante permet potentiellement d'étudier les conséquences de la diversité intra-spécifique à l'échelle de l'écosystème que cette population structure (voir le paragraphe suivant). Le système reproducteur de ces espèces alterne reproduction sexuée et reproduction végétative (voir Annexe 1). Ainsi, le clone et la population sont les transposés de l'espèce et de la communauté. Ces montages expérimentaux, fixant le nombre de lignées clonales, ont montré que :

- la richesse clonale, désignée par le terme richesse génotypique, influence positivement la résistance au broutage des zostères par les oies bernaches (Hughes and Stachowicz, 2004). La variable d'étude est la densité (nombre de ramets/m²) des zostères,
- la richesse clonale influence positivement la résilience après un choc thermique, en l'occurrence la canicule de 2003 (Reusch *et al*, 2005). La variable d'étude pour laquelle une relation a pu être établie ici est toutefois le nombre de feuilles par pied de zostères, ce qui semble moins pertinent que la densité,
- La richesse clonale influence positivement la production primaire, la diversité et l'abondance des espèces associées à la plante structurante *Solidago altissima* (Crutsinger *et al*, 2006).

Il s'agit des trois travaux pionniers. D'autres plus récents ont conduit à des conclusions similaires (Crutsinger *et al*, 2008; Ehlers *et al*, 2008; Hughes and Stachowicz, 2009). Mais les approches mises en œuvre présentent des limites évidentes, appelant à une certaine vigilance vis-à-vis des conclusions proposées par les auteurs. Notamment, la simplification inhérente aux approches expérimentales, incluant un faible nombre de clones et une fenêtre spatio-temporelle très petite, empêche l'extrapolation aveugle de ces conclusions à des populations naturelles, dans lesquelles des observations contradictoires ont parfois été rapportées (Arnaud-Haond *et al*, 2010). En effet, ces dernières sont bien plus complexes, résultant d'années de croissance clonale, d'impacts répétés, abritant une biodiversité jouant potentiellement un rôle dans leur stabilité, et connectées aux populations voisines qui peuvent les approvisionner en migrants.

Ces différents travaux expérimentaux suggèrent que les théories diversité-stabilité sont extrapolables à la diversité clonale. Par exemple, des différences fonctionnelles entre lignées clonales ont été mises en évidence (2011; Hughes *et al*, 2009) confortant les auteurs dans l'idée que la complémentarité fonctionnelle est un facteur clé de la stabilité des populations clonales. Si l'on conclue à de la complémentarité en travaillant sur moins de 10 clones dans un mètre carré, que passe-t-il en population naturelle ?

La confusion induite par ces travaux :

Dans ces travaux le terme « richesse clonale » est synonyme de « richesse génotypique » dans le cas d'espèces partiellement clonales. Or la richesse génotypique est une variable qui était utilisée en génétique des populations avant l'avènement de marqueurs hyper-variables, tels que les microsatellites dans les années 1990. A cette époque, le pouvoir résolutif des marqueurs ne permettait pas d'obtenir systématiquement des codes barre uniques à l'échelle individuelle. Un même génotype multilocus pouvait être partagé par plusieurs individus génétiques, y compris chez des espèces non clonales. Ainsi, la richesse génotypique a longtemps été un proxy de la diversité génétique. Or, les travaux exposés ci-dessus, dédiés à des espèces clonales, ont continué à utiliser ce terme de « diversité génétique » comme un synonyme, dans ces études, de « richesse clonale »(Ehlers et al, 2008; Frankham, 2005a; Hughes and Stachowicz, 2004). Globalement, les conclusions indiquent qu'une augmentation du nombre individus génétiques différents augmente la capacité de réponse d'un petit assemblage d'individus à une variation environnementale, soit « la diversité clonale ou génotypique est un facteur de résistance et/ou de résilience ». Cette conclusion a été reprise, de façon erronée, remplaçant diversité génotypique par diversité génétique, et l'idée que la démonstration de l'influence de la diversité génétique sur la stabilité des populations était acquise, a perfusé. Or, ces deux niveaux de diversité sont distincts, tant dans leur définition qu'en terme de signification écologique et évolutive. Une diversité génétique est effectivement nécessaire pour générer une diversité de clones, et ces deux niveaux de diversité sont indissociables pour les faibles valeurs de

clones et allèles (Massa et al, accepted). Dès lors qu'un seuil de faible diversité est dépassé, ces deux variables deviennent indépendantes.

LES CAS PARTICULIERS DES ESPECES STRUCTURANTES : UN PREMIER PAS VERS L'ETUDE DES CONSEQUENCES DE LA DIVERSITE GENETIQUE A L'ECHELLE DE L'ECOSYSTEME ?

Dans certains écosystèmes, des espèces ont un rôle prépondérant, et contraignent les interactions interspécifiques. On parle d'espèces ingénieures. Parmi les espèces ingénieures, des types sont à distinguer. Les ingénieurs allogéniques, tels que les castors, qui modifient l'écosystème en transformant du matériel biotique ou abiotique, par un mécanisme physique. En revanche, les espèces ingénieurs autogéniques modifient l'environnement via leur propre structure physique, i.e. leur tissu vivant ou mort (Jones *et al*, 1994). C'est ce dernier type d'espèces que l'on appellera ***« espèces structurantes »,*** et qui seront considérées dans ce travail.

Voici deux brefs extraits d'articles parus dans le même numéro de Oikos, en 1994 :

Jones, C. G., Lawton, J. H. and Shachak, M. (1994). Organisms as ecosystem engineers.

Ecosystem engineers are organisms that directly or indirectly modulate the availability of resources to other species, by causing physical state changes in biotic or abiotic materials. In so doing they modify, maintain and create habitat.

Lawton, J. H. (1994) What do species do in ecosystems?

Eliminating beavers has a dramatic, cascading effect on many other taxa and ecosystem processes; there is no redundancy built into the role beavers play in ecosystem

Ce dernier extrait illustre le rôle crucial des espèces ingénieures, sur lesquelles repose l'ensemble du fonctionnement du biotope auquel elles contribuent et de la biocénose qu'elles abritent. Un lien avec les théories diversité-stabilité est possible ici. Si la communauté repose sur une seule espèce structurante, sans redondance (voir la redondance fonctionnelle, Insert 6), la disparition de cette espèce provoque l'effondrement de son écosystème (voir la théorie des rivets pop, Insert 6).

Dans cette thèse, nous avons travaillé sur le concept de population, le recrutement et la connectivité, ainsi que la diversité génétique et la stabilité chez deux types d'espèces structurantes en milieu marin :

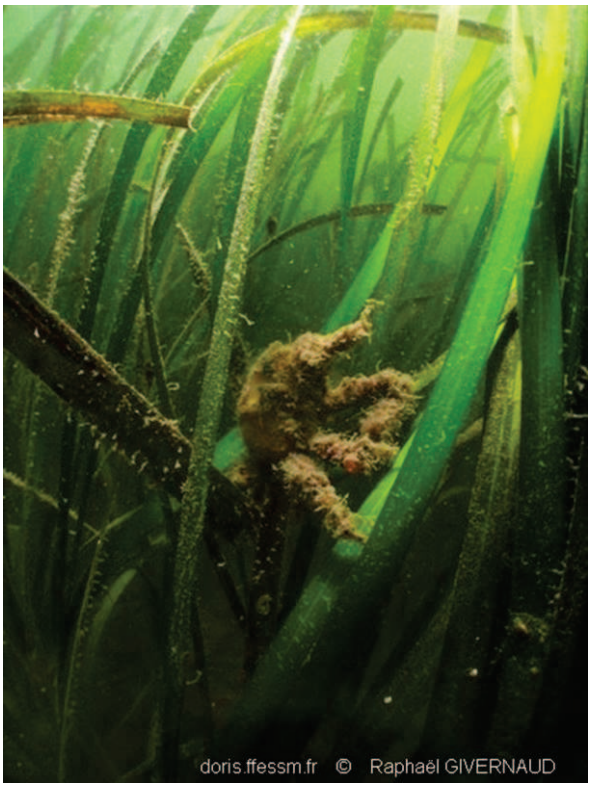

Figure 8 *Un herbier de Zostères marines, dans le Bassin d'Arcachon*

Les phanérogames marines. Ces plantes constituent les bases structurelles des herbiers marins qui sont des écosystèmes marins clés (Fig. 8) structurant les écosystèmes côtiers sur l'ensemble de la planète, aussi bien dans les tropiques qu'en zone tempérée. Elles sont d'importants producteurs primaires du milieu côtier qui influencent l'hydrodynamisme, les cycles des éléments, et la structure du réseau trophique (Hemminga and Duarte, 2000a). De plus, elles supportent une forte diversité spécifique et une forte biomasse (Orth *et al*, 2006), constituant une source de nourriture pour une multitude d'invertébrés et de poissons ainsi que pour les grands herbivores tels que les dugongs et les tortues marines, et un habitat pour certaines espèces de poissons d'intérêt commercial ou récréatif (Beck *et al*, 2001), ainsi qu'un puits de carbone essentiel pour l'ensemble de la planète (Fourqurean *et al*, 2012). Répartis à l'échelle planétaire, excepté aux pôles, les herbiers marins sont en déclin généralisé (Orth *et al*, 2006; Waycott *et al*, 2009). L'espèce sur laquelle ce travail s'appuie en grande partie, *Zostera marina*, largement distribuée dans l'hémisphère Nord, est également concernée par ce déclin (pour exemple, voir Plus *et al*, 2010). Les causes principales sont les activités anthropiques : impacts physiques (dragage, mouillage des plaisanciers), espèces invasives, activités de pêche, développement de l'aquaculture, blooms d'algues…

Les coraux profonds. Les deux principales espèces constructrices de récifs sont *Lophelia pertusa* et *Madrepora oculata*. Elles sont réparties sur l'ensemble des océans, excepté les pôles, là encore (Cairns, 1994; Zibrowius, 1980). Ces coraux sont des scléractiniaires ahermatypiques, (i.e. des coraux durs) produisant un squelette calcaire et dépourvus de micro-algues symbiotiques. Ils se trouvent à des profondeurs très variables (de 39 à plus de 2000 mètres), mais typiquement entre 500 et 1000m. Les récifs de coraux profonds, également appelés coraux d'eau froide se développent dans des zones de reliefs sous-marins marqués, tels que les monts, falaises et flans de canyons. Ces régions sont caractérisées par un fort hydrodynamisme, limitant le taux de sédimentation et constituant un vecteur d'apport de nourriture. Les récifs profonds constituent un habitat hétérogène complexe (Buhl-Mortensen *et al*, 2010) abritant une forte biodiversité (Fig. 9) et influencent fortement le réseau trophique qui leur est associé. De même, ce sont des écosystèmes caractérisés par de forts recrutements de juvéniles de nombreuses espèces de différents groupes taxonomiques (poissons, gastéropodes, spongiaires, cnidaires, crustacés…) et qui remplissent la fonction de nourricerie (Baillon *et al*, 2012) pour de nombreuses espèces de poissons, incluant des espèces d'intérêt commercial (Freiwald *et al*, 2004; Freiwald and Roberts, 2005). On ne connaît pas encore le nombre d'espèces de poissons qui leur sont strictement inféodés. Contrairement aux phanérogames marines, plus faciles d'accès, et donc plus faciles à

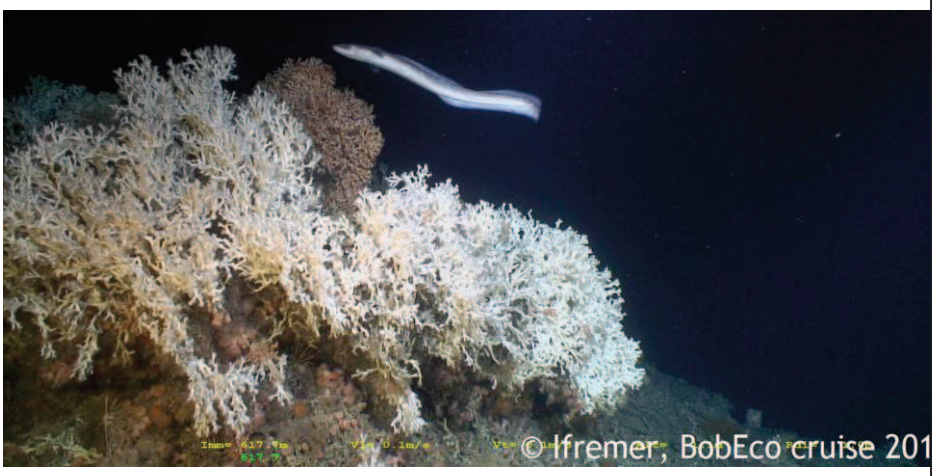

Figure 9 *Un récif profond dans le Golfe de Gascogne, survolé par un congre*

étudier, on ne peut pas clairement affirmer si les récifs profonds sont en déclin ou non. Pour autant, de nombreuses menaces pèsent sur ces écosystèmes, parmi lesquelles le chalutage de fond, l'exploration et la production de gaz et pétrole, la pose de câbles sous-marins et de pipelines, de même que les activités de recherche qui se concentrent sur les rares récifs à proximité des côtes. Par exemple, 30 à 50% des récifs norvégiens sont impactés par le chalutage de fond (Fossa *et al*, 2002), une activité particulièrement destructrice, pouvant remonter des blocs de $1m^3$, représentant des décennies de croissance récifale (Hall-Spencer *et al*, 2002). Le changement global représente également une menace par l'intermédiaire de deux mécanismes (Guinotte *et al*, 2006). D'une part, l'acidification des océans et la remontée de la surface de saturation de l'aragonite constituent une source potentielle d'arrêt de la croissance des colonies (Fabry *et al*, 2009). Expérimentalement, il a été montré qu'une diminution du pH de 0.15 à 0.30 unités amenait à une diminution de 30 à 56% de la calcification de *Lophelia pertusa* (Maier, 2008; Maier *et al*, 2012). Ces auteurs ont tout de même observé la capacité des coraux à contraindre la précipitation des carbonates, malgré des conditions sous-saturantes. D'autre part, le changement global pourrait se traduire par une modification des régimes de courants à grande échelle, induisant de fait, des modifications des patrons de sédimentation et des apports de nutriments. Ainsi, Kano *et al,* (2007) relient la présence des coraux dans le Golfe de Gascogne à l'existence de la veine d'eau méditerranéenne qui, comme la formation des récifs (Frank *et al*, 2009) elle-même, est sensiblement affectée par les variations climatiques.

Points communs entre phanérogames marines et coraux profonds

Phanérogames marines et coraux profonds présentent plusieurs similitudes :

- ce sont des espèces structurantes
- ils forment des habitats caractérisés par une forte diversité et une importante biomasse
- les écosystèmes que ces espèces structurent sont des zones de nourricerie pour des espèces d'intérêt commercial
- ces écosystèmes sont impactés par les activités humaines
- le changement global représente une menace

Par ailleurs, il s'agit d'espèces partiellement clonales, capables de se reproduire par la voie sexuée ainsi que par la voie végétative. Les conséquences écologiques et évolutives de la clonalité sont profondes, influençant le fonctionnement dynamique des populations ainsi que les trajectoires évolutives. La clonalité partielle des espèces structurantes considérées constituera la trame de fond de ce travail.

Parce que ces espèces structurantes contraignent les interactions dans leur environnement, et influencent l'ensemble d'une communauté, les conséquences écologiques de la diversité génétique de cette espèce, si elles sont significatives, devraient être plus facilement identifiables (Fig. 10).

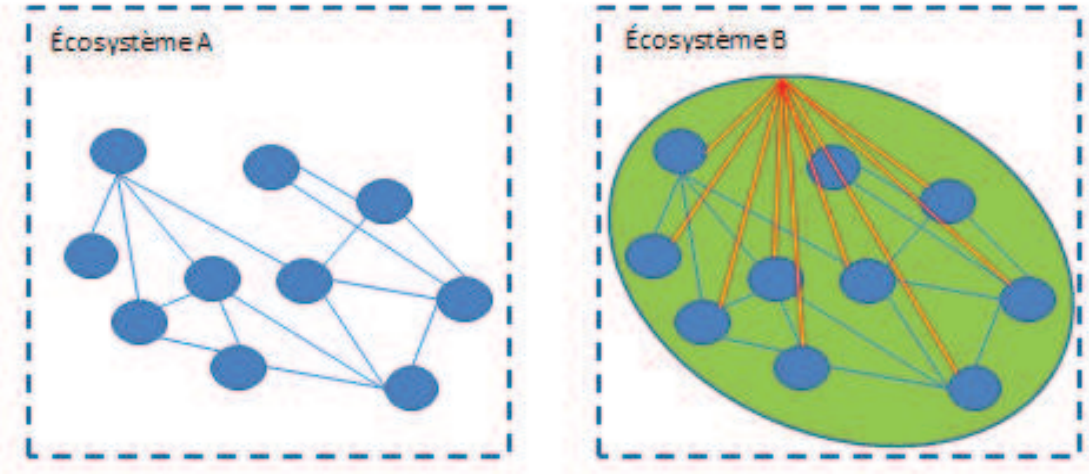

Figure 10 *Différence de distribution des interactions interspécifiques*
Les espèces sont représentées par les disques bleus, et leurs interactions par les liens bleus. L'écosystème A n'est pas structuré par une seule espèce. L'écosystème B est structuré par un espèce (disque vert), qui interagit avec l'ensemble de la communauté (liens rouges).

OBJECTIFS DE LA THESE

Diversité génétique, démographie et connectivité sont les trois compartiments en interaction à considérer dans l'étude des relations diversité/stabilité. La figure 11 illustre le schéma théorique global de ce travail, et constitue le fil directeur du manuscrit.

L'objectif central est de parcourir ce schéma, en s'appuyant sur des populations naturelles structurantes. Les implications évolutives et écologiques de la clonalité constitueront en trame de fond de ces travaux.

Le premier chapitre sera dédié à l'étude de la connectivité passée des coraux profonds.

La connectivité ancienne entre populations de coraux profonds (*Lophelia pertusa* et *Madrepora oculata*) de Méditerranée et Atlantique sera considérée dans ce volet. De Mol *et al*. (2005) ont émis l'hypothèse suivante : à la suite du dernier évènement glaciaire (10-12 000 ans), l'Atlantique Nord-est a été recolonisé par les coraux d'eau froide depuis la Méditerranée. Cette hypothèse repose sur des données géologiques et paléogéographiques. L'objectif de ce paragraphe est de tester ce scénario par le biais d'analyses phylogéographiques.

Le second chapitre considerera les interactions entre dispersion et clonalité de la zostere marine.

- ✓ L'influence de la croissance clonale sur le patron de distribution spatiale de la variabilité génétique sera étudiée dans un premier temps, au sein des herbiers de *Zostera marina*. L'hypothèse « la clonalité influence la structure génétique à fine échelle spatiale » sera testée. Le concept de population pour les espèces partiellement clonales sera discuté à cette occasion.
- ✓ En considérant ces mêmes herbiers de zostères, l'effet de la clonalité sur la variation temporelle de la diversité génétique constituera le second volet de ce chapitre. En considérant conjointement dispersion et clonalité, nous testerons l'hypothèse « la diversité génétique et l'architecture clonale sont stables à l'échelle annuelle ».

Le troisième chapitre considèrera l'effet *in situ* de la diversité génétique sur la démographie

L'objectif central de ce second chapitre est de tester cette théorie clé de la génétique des populations, en considérant volontairement des populations non dramatiquement impactées.

- ✓ Le premier paragraphe s'appuiera sur les herbiers de zostères considérés dans le premier chapitre, et mettra en relation les niveaux de diversités génétique et clonale de ces herbiers avec leur démographie (bénéficiant d'un suivi bisannuel depuis 2004, voir le site www.rebent.org). L'hypothèse testée ici est la suivante : la diversité génétique d'une population influence sa démographie.
- ✓ Sous réserve d'une validation de l'hypothèse précédente, une brève exploration des relations entre le niveau d'hétérozygotie individuelle des lignées clonales et leur fitness sera proposé, en considérant la taille des clones comme proxy de fitness. Si la diversité génétique d'une population influence sa démographie, ce volet permettrait de tester l'hypothèse « la performance démographique d'une population est la résultante des performances individuelles des clones composants cette population ».
- ✓

Le dernier chapitre explorera l'impact des activités humaines sur la diversité et la structure génétique et/ou clonale des populations

L'étude du sens retour de la relation circulaire diversité/stabilité constitue l'objectif de ce dernier chapitre.

Ce chapitre sera composé d'un unique volet. En s'appuyant sur les populations de coraux profonds échantillonnées lors de campagnes océanographiques, les descripteurs de la structure génétique et clonale seront croisés avec des estimations de la démographie des récifs et de l'impact de la pêche profonde. L'objectif de ce volet est de tester l'hypothèse « les activités humaines influencent les

patrons de distribution de la diversité génétique et l'architecture clonales des coraux profonds ». Ce test d'hypothèse sera mené sur *Madrepora oculata* et *Lophelia pertusa*.

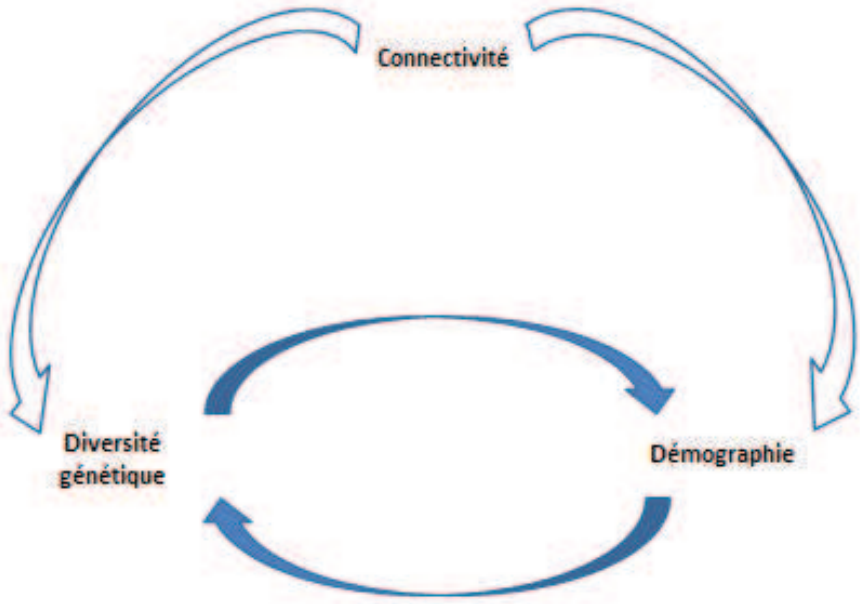

Figure 11 *Interrelations entre diversité génétique, démographie et connectivité. Cette organisation correspond au plan de la thèse*

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CHAPITRE 1. CONNECTIVITE PASSEE DES POPULATIONS DE MADREPORA OCULATA ET LOPHELIA PERTUSA DE MEDITERRANEE ET DE L'ATLANTIQUE NORD-EST : Y A-T-IL EU UNE RECOLONISATION POSTGLACIAIRE DE L'ATLANTIQUE DEPUIS LA MEDITERRANEE ?

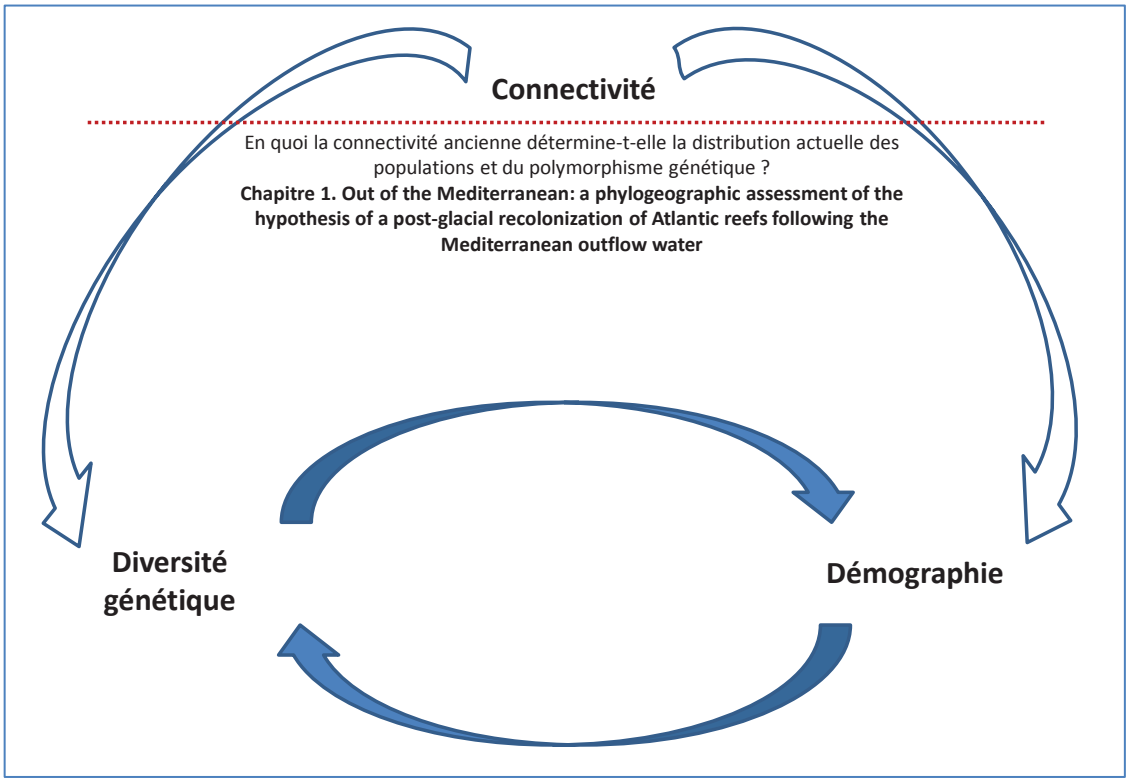

Figure 12 *Etude de l'influence de la connectivité ancienne sur la distribution géographique actuelle des populations et leur niveau de diversité génétique*

CONTEXTE & RESUME

L'hypothèse d'une recolonisation postglaciaire des récifs de l'Atlantique Nord-Est a été initialement émise par les géologues étudiant les récifs vivants et fossiles coraux d'eaux froides. Un arrêt de la formation récifale pendant les derniers évènements glaciaires (débutant il y a 2.58 millions d'années et prenant fin il y a environ 12 000 ans) a été confirmée par plusieurs études (voir les références dans le manuscrit ci-dessous).

La reprise de la croissance des récifs profonds de cette partie de l'Atlantique est récente, à l'échelle géologique, les datations isotopiques sont de l'ordre de grandeur de 10 000 ans. Les récifs les plus méridionaux apparaissent plus récents que les récifs atlantiques proches du détroit de Gibraltar, datant de 40-50 000 ans. Dans le Golfe de Gascogne, l'âge est estimé à environ 14 000 ans, tandis que les récifs au large de l'Irlande auraient environ 10 000 ans. C'est de cette estimation de gradient d'âge orienté Sud-Nord que découle l'hypothèse d'une recolonisation postglaciaire de l'Atlantique Nord-Est.

Par ailleurs, les récifs de coraux profonds ont une existence continue depuis au moins 500 000 ans, en Méditerranée. Cette mer est notamment caractérisée par une veine d'eau sortante, remontant le long de la marge continentale européenne. Ceci a amené De Mol *et al,* (2005) à poser l'hypothèse suivante : la source de recolonisation est la Méditerranée, dont la veine d'eau sortante à agit comme un vecteur de dispersion des larves de *Lophelia pertusa* et *Madrepora oculata*, les deux principales espèces constructrices de récifs profonds.

Les échantillons de *L. pertusa* et *M. oculata* collectés pendant cette thèse fournissent l'occasion de tester cette hypothèse, par le biais d'analyses phylogéographiques.

Le niveau de connectivité considéré ici est ancien, et concerne de larges échelles de temps et d'espace. Ce ne sont pas ces échelles qui sont impliquées dans les relations diversité-stabilité. Néanmoins, cette connectivité historique participe à la compréhension de la distribution actuelle des populations. De plus, l'histoire biogéographique peut permettre d'obtenir un aperçu de la sensibilité des populations aux facteurs contrôlant leur existence. Dans les cas des coraux profonds, ces facteurs sont profondément influencés par les variations climatiques anciennes. Dans quelle mesure est-il possible de quantifier la vulnérabilité des récifs face au changement global qui s'annonce ?

Les données supportent l'hypothèse d'une extinction en Atlantique, pour les deux espèces, qui montrent les signes d'une ré-expansion démographique récente. En revanche, les populations méditerranéennes ne révèlent pas un tel signal. Les données de *L. pertusa* sont en accord avec une source de recolonisation méditerranéene. En effet, l'haplotype majoritaire en Altantique est partagé avec les populations méditerranéennes, et le grand nombre d'haplotypes satellites semble être en accord avec un scénario d'un goulot d'étranglement suivi d'une ré-expansion démographique. Les données de *L. pertusa* supportent donc l'hypothèse de De Mol *et al,* (2005). En revanche, le patron biogéographique de *M. oculata* diffère par l'absence de cluster en étoile, et les valeurs de diversité génétique supérieures à celles de *L. pertusa*, en Atlantique. Ceci nous conduit à proposer un scénario différent pour cette espèce, qui pourrait avoir persisté en Atlantique à l'état résiduel, pendant les derniers évènements glaciaires.

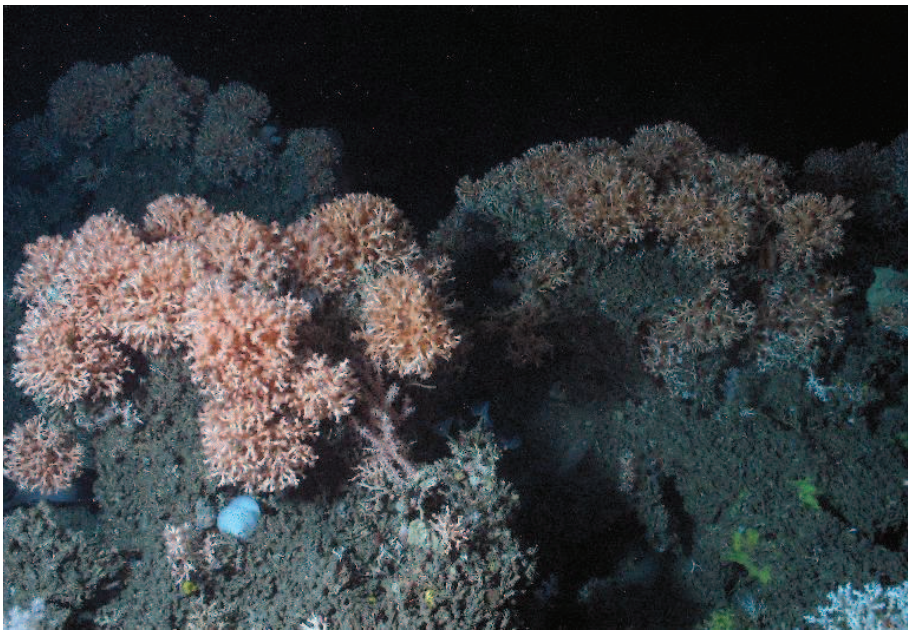

Figure 13 *Récifs de coraux profonds de Londsjup, en Islande (IceCTD, © Ifremer)*

OUT OF THE MEDITERRANEAN: A PHYLOGEOGRAPHIC ASSESSMENT OF THE HYPOTHESIS OF A POST-GLACIAL RECOLONIZATION OF ATLANTIC COLD-WATER CORALS FOLLOWING THE MEDITERRANEAN OUTFLOW WATER

R. Becheler, O. Mouchel, M. Choquet, A.-L. Cassonne, M. Taviani, Bourillet, J.-F. and S. Arnaud-Haond

Abstract

The cold water corals *Lophelia pertusa* and *Madrepora oculata* are two important deep reef builders in the deep sea. These key ecosystems represent hotspots for marine biodiversity and carbon cycling of continental margins. Their occurrence is largely driven by factors such as temperature and food supply, strongly influenced by climatic variations. The last glacial event yielded to the demise of reefs in the Northeast Atlantic, while Mediterranean may have played the role of glacial refugee. It was hypothesized that Atlantic recolonization occurred during the warming of Atlantic waters through the Mediterranean Outflow water. this scenario was tested using ITS-sequences amplified for both *L. pertusa* and *M. oculata*. Two slightly different phylogeographic patterns were observed for these species. Dominant haplotypes of *L. pertusa* are shared between Mediterranean and Atlantic, and signatures of a recent demographic expansion, notably the star-like cluster, were found for the majority of Atlantic location. The lack of structure among regions calls for a massive recolonization event from Mediterranean. Contrastingly, demographic expansions of *M. oculata* are less obvious. The strong regional structure and relatively high levels of haplotypic and molecular diversities suggest an alternative biogeographic history. Residual populations might have persisted during Ice Ages in NE Atlantic, before a recent demographic expansion around 12 000-10 000 years BP.

Keywords

Lophelia pertusa, Madrepora oculata, Cold-water coral, Ice Ages, Geographical expansion, Marine biogeography, Mediterranean Outflow water

Introduction

Cold water reefs are distributed worldwide in the deep-sea, along continental margins and seamounts (Roberts *et al*, 2006). The co-occurring species *Lophelia pertusa* and *Madrepora oculata* are two crucial reef-builders, enhancing the habitat complexity by the skeleton growth (Buhl-Mortensen *et al*, 2010). These cold-water corals (CWC) provide a feeding guild for a rich and abundant community gathering various taxonomic groups, such as sponges, gorgonians, bivalves and fishes (Baillon *et al*, 2012; Buhl-Mortensen *et al*, 2010; Freiwald, 2002). These key ecosystems also represent deep hotspots of carbon and nutrient cycling (Cathalot *et al*, submitted). Anthropogenic activities, including fishing, mining and oil industry, dangerously threaten them. Overall, the global change is likely to hardly affect the present distribution by altering biotic and abiotic factors controlling the growth of deep reefs. Among them, the advection of labile organic carbon (Davies *et al*, 2009), a water temperature generally comprised between 4 and 12°C (Freiwald, 2002; Freiwald *et al*, 2004; Roberts *et al*, 2009) and a significant hydrodynamic regime just above the seabed (White and Dorschel, 2010) are recognized to be largely influence by climatic variations. Studying the influence of past climatic variations on the distribution of CWC may provide information for assessing their sensitivity to future global change.

In particular, the successive periodic Ice Ages have affected the paleo-distribution of deep taxa (Bouchet and Taviani, 1992), by contracting Atlantic distribution toward warmer Mediterrannean water. The role of Mediterranean as glacial refugee was extensively demonstrated for terrestrial and freshwater taxa (Hewitt, 1999; Hewitt, 2004; Petit *et al*, 2003; Schmitt and Varga, 2012), as well as for marines ones (Ben-Shlomo *et al*, 2006; Maggs *et al*, 2008; Wares and Cunningham, 2001; Wilson and Veraguth, 2010). The Mediterranean Basin is an historical area of CWC (Di Geronimo *et al*, 2005; Freiwald *et al*, 2004; Taviani *et al*, 2005), where the occurrence of deep reefs has been recognized to be continuous for over 480 000 years (McCulloch *et al*, 2010). The last 50 000 years may correspond to an active growth period for reefs, in the western basin of the Mediterranean Sea, as well as in the Gulf of Cadiz and Moroccan margin (Schroder-Ritzrau *et al*, 2005). In the Mediterranean Sea, the temperature reached during glacial periods of Pleistocene (2.6 My – 12 000 years BP) was optimal for both *L. pertusa* and *M. oculata* growth (Delibrias and Taviani, 1984; Di Geronimo *et al*, 2005). Notably, the younger Dryas -the last cold oscillation of the Pleistocene, 12 400- 12 000 years BP- is a favorable episode of CWC growth in the eastern and western Mediterranean (McCulloch *et al*, 2010; Taviani *et al*, 2011).

The paleogeography in the Northern Atlantic contrasts with Mediterranean history, with an inferred active growth of reef between 2.6 to 0.5 My BP (Kano *et al*, 2007) whereas paleontological records suggest the disappearance of CWC in north Atlantic during the Pleistocene due to the intensification of the Northern Hemisphere glaciations (Kano *et al*, 2007). The modern colonization of northern Atlantic is estimated to have taken place after the last glacial maximum , about 12 000 years BP (Schroder-Ritzrau *et al*, 2005). In addition, a global age-gradient from south to north Atlantic (Fig. 14) was suggested from different studies using isotopic methods. For instance, in the Bay of Biscay, Shröder-Ritzrau *et al,* (2005) have assessed a beginning of growth since around 14 000 years, whereas at higher latitudes, such as offshore Ireland, modern deep-reefs would have settled between 12 000 and 10 000 years BP (Frank *et al*, 2005; Frank *et al*, 2009; Schroder-Ritzrau *et al*,

2005) and those from North of Norway between 8600 and 2000 years BP (Mikkelsen *et al*, 1982; Schroder-Ritzrau *et al*, 2005). This apparent gradient of age may be due to a progressive colonization of northern Atlantic, from southern populations, conjointly to the post-glacial warming of water masses (Fig. 14). Considered with the likely continuous occurrence of CWC in Mediterranean, these points led De Mol *et al,* (2005) to hypothesize that the Mediterranean has played the role of glacial refugee during the Ice Ages of the Pleistocene, and constitutes the source of the gradual northward recolonization of the Atlantic since 12 000-10 000 years, CWC larvae following the Mediterranean Outflow Water (Stumpf *et al*, 2010; Voelker *et al*, 2006).

Here, we aim at testing this biogeographic hypothesis, using phylogeographic reconstruction based on Internal Transcribed Spacer (ITS) on both *Lophelia pertusa* and *Madrepora oculata* samples collected from Mediterranean, to South of Iceland. We analyzed data asking whether samples beard the genetic signature of recent demographic expansions in NE Atlantic, and if so whether the pattern of genetic structure was congruent with a Mediterranean origin.

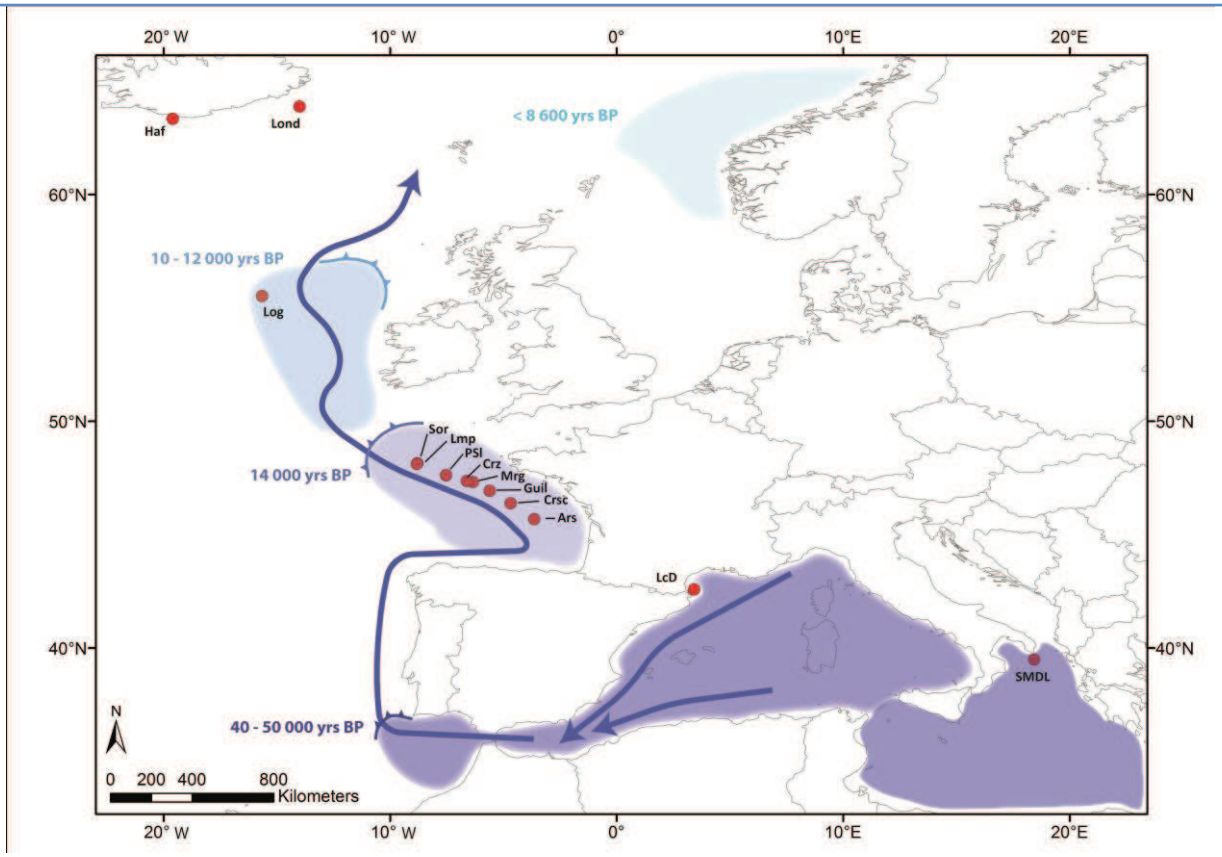

Figure 14 *Illustration of the scenario proposed by De Mol et al (2005). The post-glacial recolonization should happen step by step, from the strait of Gibraltar to northern locations, following the Mediterranean Outflow Water. The red circles indicate the locations of Lophelia pertusa and Madrepora oculata populations sampled during oceanographic cruises (see material and methods). The dates correspond to the age estimate of the beginning active growth of reefs for each region.SMDL: Santa Maria di Leuca. Canyons of: Lacaze-Duthiers (LcD), Ars (Ars); le Croisic (Crsc); le Guilvinec (Guil); Morgat (Mrg); Crozon (Crz); Petite Sole (PS); Lampaul (Lmp); Sorlingues (Sor). Logachev Mound (Log) and locations of Londsjup (Lond) and Hafadsjup (Haf)*

Materials and methods

2.1. Sampling locations

Samples of *Lophelia pertusa* and *Madrepora oculata* were collected from four geographical regions. In the Mediterranean Sea, the reefs of Santa Maria di Leuca (Ionian Sea) and the Canyon of Lacaze-Duthiers (western basin), were sampled using respectively grab and ROV (Remotely Operated Vehicle). Additional samples of *L. pertusa* and *M. oculata*, originating from the strait of Sicily and Southern Italy were also included in this analysis (Table 1). In the Bay of Biscay, samples were mainly collected during the BobEco cruise (September-October 2011), using ROV. Eight canyons were also explored and samples of both *M. oculata* and *L. pertusa* were collected, ranging from the Canyon of Ars (southern location) to the Canyon of Sorlingues (northern location). Additional samples from the canyon of Le Guilvinec were also included (Fig. 14). During the BobEco cruise, samples of *L. pertusa* and *M. oculata* were collected in a reef from Logachev Mounds (Irish Sea). In June 2012, two sites were sampled in the south of Iceland during the IceCTD cruise, for both *L. pertusa* and *M. oculata* . All details about samples are given in Table 1.

2.2. DNA extractions, amplifications of ITS markers and sequencing

L. pertusa samples were stored in Ethanol 90% until DNA extraction. Total DNA was extracted using the kit Fast DNA®SPIN for soil kit, according to the protocol provided by the manufacturer (MP Biomedicals, France). *M. oculata* samples from Mediterranean were stored in Ethanol 70%, and total DNA was extracted using the classical CTAB method (Doyle and Doyle, 1988). The DNA extractions of the Atlantic samples of *Madrepora* were performed directly on board on fresh tissues, using also the CTAB methods.

Internal Transcribed Spacer (ITS) was amplified using the universal primers Jo6 and TW5 (Diekmann *et al*, 2001). All PCR amplifications were performed in 50 µL volumes, containing 50 ng DNA templates, 1X reaction buffer (GoTaq, Promega, Madison, WI, USA) 0.2 mM of each DNTP, 2.5 Mm of $MgCl_2$, 0.6 µM of JO6 and TW5 primers and 2.0 U of Taq polymerase. A GeneAmp® PCR System 9700 (Applied Biosystems, Life Technologies, Saint-Aubin, France) was used for reactions of polymerization with the following program: 3 minutes at 94°C for initial denaturation, 7 cycles of 30 seconds of denaturation, 1 minute of annealing (the initial annealing temperature was 57°C and decreased of 1°C at each cycle) and 1 minute of elongation at 72°C. This touch-down step was followed by 29 cycles of 30 seconds of denaturation, 1 minute of annealing at 50°C and 1 minute of elongation at 72°C, and a final elongation of 5 minutes at 72°C. PCR products were initially visualized on gels of agarose (2%). When parasite fragments were observed, purification on gel was performed, before sequencing (GATC Biotech, Konstanz, Germany).

Table 1 *Genetic diversity parameters assessed for each population of both Lophelia pertusa and Madrepora oculata.*
n, sampling size; N, number of distinct haplotypes; N(15) and N(x), the estimate of the number of haplotypes for sub-samples of 15 and x individuals; k, the number of polymorphic sites, N_{ph}, the number of private haplotypes, that is found in a single population; h, haplotypic diversity; π_1, the mean number of pairwise differences; the π_2, nucleotidic diversity. The statistics of test of conformity the selective neutrality are also provided, with their associated p-values. D, statistic of Tajima's test; F, statistic of Fu & Li's test.

Region	Site	Species	n	N	N standard (15)	k	N_{ph}	*h*	π_1	π_2	D	F_s
Mediterrean	Santa Maria di Leuca (Med)	L. pertusa	21	5	4	6	2	0.49 ± 0.12	1.057	0.0009	-1.04 (0.152)	-0.91 (0.248)
		M. oculata	-	-		-	-	-	-	-	-	-
	Canyon ofLacaze-Duthier (Med)	L. pertusa	8	6	-	9	2	0.93 ± 0.08	3.179	0.0028	-0.86 (0.232)	-1.47 (0.120)
		M. oculata	-	-		-	-				-	-
	Additional (Med)	L. pertusa	9	3		2	-	*0.42 ± 0.19*	*0.611*	*0.0005*	*-0.583 (0.311)*	*-0.532 (0.136)*
		M. oculata	13	7		10	-	*0.79 ± 0.11*	*2.313*	*0.0014*	*-1.13 (0.144)*	*-1.86 (0.100)*
Açores	Açores	M. oculata	5	4		5	1	0.90 ± 0.16	2.2	0.002	-0.56 (0.39)	-0.85 (0.13)
Bay of Biscay	Canyon of Ars (BoB)	L. pertusa	2	1	-	0	0	-	-	-	-	-
	Canyon of Croisic (BoB)	L. pertusa	21	5	3.88 ± 0.03	5	1	0.35 ± 0.13	0.476	0.0004	**-1.87 (0.012)**	**-3.07 (0.001)**
		M. oculata	10	5		4	0	0.84 ± 0.08	1.224	0.0011	-0.52 (0.32)	**-1.76 (0.040)**
	Canyon of Le Guilninec (BoB)	L. pertusa	23	7	5.09 ± 0.03	7	1	0.52 ± 0.12	1.036	0.0009	**-1.53 (0.044)**	**-3.11 (0.012)**
		M. oculata	22	8		6	0	0.82 ± 0.06	1.55	0.0013	-0.19 (0.45)	**-2.90 (0.020)**
	Belgica (BoB)	L. pertusa	8	1	-	0	0	-	-	-	-	-
	Canyon of Morgat-Douarnenez (BoB)	L. pertusa	30	10	5.96 ± 0.04	15	4	0.63 ± 0.10	1.499	0.0013	**-1.60 (0.04)**	**-4.58 (0.006)**
		M. oculata	12	7		5	0	0.88 ± 0.08	1.79	0.0016	0.29 (0.66)	**-2.92 (0.019)**
	Canyon of Crozon (BoB)	L. pertusa	11	7	-	8	3	0.82 ± 0.12	1.891	0.0017	-1.57 (0.062)	**-3.06 (0.014)**
		M. oculata	14	4		4	0	0.76 ± 0.06	1.22	0.0011	-0.10 (0.48)	0.02 (0.466)
	Canyon of Petite Sole 1 (BoB)	L. pertusa	22	3	2.37 ± 0.02	3	1	0.18 ± 0.11	0.272	0.0002	-1.16 (0.16)	**-1.25 (0.039)**
		M. oculata	9	4		4	1	0.75 ± 0.11	1.56	0.0014	0.23 (0.673)	-0.13 (0.414)
	Canyon of Petite Sole 2 (BoB)	L. pertusa	28	5	3.42 ± 0.03	6	2	0.33 ± 0.11	0.682	0.0006	**-1.57 (0.035)**	**-1.71 (0.08)**
		M. oculata	13	6		6	1	0.85 ± 0.06	1.425	0.0013	-0.97 (0.19)	-1.78 (0.070)
	Canyon of Lampaul (BoB)	L. pertusa	7	3	-	2	0	0.67 ± 0.16	1.047	0.0009	1.34 (0.97)	0.11 (0.42)
		M. oculata	11	5		4	1	0.76 ± 0.11	1.20	0.0011	-0.44 (0.35)	**-1.61 (0.049)**
	Canyon of Sorlingues (BoB)	L. pertusa	2	1	-	0	0	-	-	-	-	-
Celtic Sea	Logachev Mounds (Irl)	L. pertusa	21	6	5.07 ± 0.02	5	4	0.61 ± 0.11	0.724	0.0006	-1.22 (0.09)	**-3.22 (0.003)**
		M. oculata	28	11		7	1	0.86 ± 0.04	1.6294	0.0015	-0.28 (0.43)	**-5.71 (0.002)**
Iceland	Londsjup 1 (Icel)	L. pertusa	8	4	-	7	2	0.64 ± 0.18	1.750	0.0015	-1.53 (0.35)	-0.11 (0.45)
		M. oculata	8	4		5	1	0.79 ± 0.11	1.86	0.0017	-0.17 (0.45)	0.005 (0.460)
	Londsjup 2 (Icel)	L. pertusa	20	7	5.91 ± 0.02	6	3	0.64 ± 0.12	1.121	0.0009	-1.25 (0.11)	**-3.12 (0.011)**
		M. oculata	34	2		1	0	0.34 ± 0.08	0.337	0.0003	0.56 (0.822)	0.97 (0.536)
	Hafadsjup (Icel)	L. pertusa	16	5	4.80 ± 0.01	7	2	0.53 ± 0.14	1.200	0.0011	-1.39 (0.076)	-0.96 (0.206)
		M. oculata	21	4		5	1	0.48 ± 0.12	1.07	0.0010	-0.69 (0.27)	0.41 (0.583)
	SW Iceland (Icel)	L. pertusa	5	3	-	3	2	0.71 ± 0.23	1.600	0.0011	1.22 (0.95)	0.28 (0.52)

ITS sequences were proofread and aligned using the Geneious software version 6.1 (Drummond *et al* 2009). Final lengths of ITS-sequences are 1130 bp and 1125 bp for *L. pertusa* and *M. oculata*, respectively. The position 366 in the ITS-sequences of *L. pertusa* was discarded from analysis due to recurrent ambiguity possibly due to incomplete concerted evolution, this site being characterizing by a systematic double peak. All singleton-sequences were screened, due to the potential occurrence of *in vitro* mutations. Corresponding individuals were re-amplified to ascertain the status of singleton. Corrections were done when needed.

2.3. Data analysis

Analyses were performed using Arlequin 3.0 software (Excoffier *et al*, 2005). The genetic differentiation of populations was assessed through the pairwise F_{st}-statistic, following the method of (Weir and Cockerham, 1984). In addition, an analysis of Molecular Variance (AMOVA) was performed, after having defined four groups following a geographical logic (Mediterranean, Bay of Biscay, Ireland and Iceland), to estimate the features of the distribution of genetic variability within and among groups.

Genetic diversity was estimated through three/four statistics: (i) haplotypic diversity (*h*) (Nei, 1987) being the probability that two randomly chosen haplotypes are different, (ii) the mean number of pairwise differences(π_1) (Tajima, 1983), corresponding to the number of nucleotidic positions differing within each pair of haplotypes, and average for each whole sampling set and (iii) the nucleotidic diversity (π_2) (Nei, 1987), computed as the probability nucleotides differ for a randomly chosen position. This set of statistics was assessed for each sampling location.

Departures from the mutation-drift equilibrium were tested through two statistics of tests of conformity: Tajima's D (Tajima, 1989) and F_s of Fu & Li (Fu, 1996), differing by their statistical power. Such departure from the selective neutrality may originate from events of selection, as well as bottleneck or demographic expansion, impacting the frequency of rare haplotypes. While bottleneck and balancing selecting negatively affect the amount of rare variants and generate positive values of both D and F_s, sweep selection and demographic expansion generate an excess of rare variants leading to negative values of conformity statistics. Because ITS markers are supposed neutral, such tests were performed to assess the null hypothesis of constant demographic size, with Arlequin 3.0, and these statistics were computed using 1000 simulations.

3.1. Genetic diversity of Lophelia pertusa and Madrepora oculata populations

The total number of haplotypes found among each species was proportionally higher for *Lophelia pertusa* than *Madrepora oculata* (42 haplotypes for 263 analysed *L. pertusa* individuals and 24 haplotypes for 200 *M. oculata* individuals). In addition, the number of private haplotypes (i.e. found in only one location) is also proportionally higher for *L.pertusa* (29 privates haplotypes, representing 69% of the total number) than *M. oculata* (12 private haplotypes, or 50% of the total number). For both species, these haplotypes are evenly distributed among the biogeographic regions included in this study (Mediterranean, Bay of Biscay, Ireland and Iceland, plus Acores for *M. oculata*). The highly dominant haplotype of *L. pertusa* (61% of analyzed individuals) is shared among all regions, and occupies a central position in the haplotypic network (Fig. 15), exhibiting a typical structure of star-like cluster. Around this dominant haplotype, 17 rare haplotypes are divergent by a single mutation, and 12 are divergent by two mutations. The second most common haplotype (18 individuals, or 7%) is essentially found in Logachev (Ireland) and represent a secondary center for an Irish star-like cluster. A contrasting pattern was observed for *M. oculata* with no dominant haplotypes in the dataset, nor any star like cluster. Seven haplotypes are widely presented, with 11 to 54 individuals while 13 are carried less than 4 individuals.

Levels of haplotypic and molecular diversities are variable among locations (Table 1). For *L. pertusa*, the haplotypic diversity *h* ranged from 0 (Canyon of Ars, where only two individuals were analyzed) to 0.82 (Canyon of Crozon) in Atlantic and reached 0.95 ± 0.10 in Mediterranean (Canyon of Lacaze-Duthier). The lowest diversity for canyon where the sampling effort was substantial, i.e. the first location of the canyon of Petite Sole, reached 0.18. This diversity is slightly lower in *M. oculata* populations, ranging from 0.34 (second location of Londsjup) to 0.88 (Canyon of Morgat). In Acores, *h* reached 0.90 ± 0.16 (four distinct haplotypes for 5 individuals). A comparable pattern of molecular diversity is observed for both species (Table 1). In North-East Atlantic, the mean number of pairwise differences π_1 ranged from 0.272 to 1.891 for *L. pertusa*, and from 0.337 to 1.790 for *M. oculata,* but is maximal for southern locations (3.333 in Mediterranean for *L. pertusa* and 2.2 in Acores for *M. oculata*). This descriptor π_1 and the nucleotidic diversity π_2 are highly correlated ($R^2 = 0.99$; $p < 0.0001$ for both species). In Atlantic, no significant relationship was observed between these descriptors of the genetic diversity and the distance of populations to the Gibraltar's straight, except for the haplotypic diversity of M. oculata ($R^2 = 0.47$; $p = 0.020$; negative slope).

Tableau 2 *Matrix of pairwise F_{st}, based on haplotypic frequencies, for Lophelia pertusa populations. The location for which sampling size was low (Canyons of Ars, Sorlingues and samples from Belgica cruise and SW Iceland) were not retained for this analysis. Bold values are significant at p < 0.05. Due to their low sampling size, the locations of Ars, Belgica, Sorlingues, Lampaul and SW Iceland were removed from the matrix.*

	SMDL	LcD	Crsc	Guil	Mrg	Crz	PSl 1	PSl 2	Log	Lond 1	Lond 2	Haf
SMDL	-											
LcD	**0.22**	-										
Crsc	0.03	**0.32**	-									
Guil	0.00	**0.31**	0.02	-								
Mrg	**0.01**	**0.21**	0.01	-0.02	-							
Crz	0.00	0.09	0.05	0.00	0.00	-						
PSl 1	**0.07**	**0.43**	0.00	0.06	0.04	**0.1**	-					
PSl 2	0.01	**0.30**	-0.01	-0.02	-0.01	0.03	0.02	-				
Log	0.06	**0.34**	**0.60**	**0.52**	**0.45**	**0.37**	**0.65**	**0.57**	-			
Lond 1	0.01	0.13	0.05	0.02	-0.01	0.00	0.07	0.03	**0.49**	-		
Lond 2	0.00	**0.18**	0.01	-0.02	-0.01	-0.01	**0.06**	0.00	**0.51**	0.02	-	
Haf	0.04	**0.20**	0.02	0.03	0.01	0.03	**0.05**	0.02	**0.50**	0.00	0.03	-

3.2. Genetic structure

The pattern of genetic structure differs between the two considered species. Within the *L. pertusa* dataset, two populations (Canyon of Lacaze-Duthier and Logachev) are clearly distinct from all other, all pairwise F_{st} being significant (Table 2). The position of Logachev in the haplotypic network (Fig. 15, green circles) is consistent with the related F_{st}-values. Within the Bay of Biscay, a weak genetic structure is observed, the large majority of F_{st} being non-significant. The AMOVA performed on this species (Table 3) indicates that 90.6% of the genetic variation occurs within populations. A rather regional pattern occurs within *M. oculata* dataset. While no significant genetic structure is observed within the Bay of Biscay, the strong and significant F_{st} between populations from distinct regions suggest a regional structure (Table 4). This is supported by the AMOVA indicating that 59% of variation occurs within population and 39% among regions (Table 5).

Tableau 3 *Results of AMOVA performed on Lophelia pertusa populations. The mutational model used here is the Kimura 2-parameters. The very large majority of variation (90.55%) occurs within populations. The regions defined for this analysis are the Mediterranean, the Bay of Biscay, Ireland and Iceland (see also the haplotype network).*

Source of variations	d.f.	Sum of squares	Variance components	P-values	Percentage of variation
Among regions	3	2.21	-0.002	0.249	-0.45
Among populations within regions	14	9.70	0.028	<0.0001	8.09
Within populations	244	77.59	0.318	<0.0001	92.36
Total	262	85.51	0.344	-	-

3.3 Demographic inferences

The tests of conformity to selective neutrality suggest slightly different demographic history for *L. pertusa* and *M. oculata*. The Mediterranean populations of *L. pertusa* did not lead to significant values of both D and F_s statistics, while significant and negative values were estimated in the Bay of Biscay, Ireland and one population from Iceland (the F_s for Londsjup 2). This is consistent with the star-like structure of the network (Fig. 15), supporting a demographic expansion for Atlantic populations. Only four populations of *M. oculata* indicate a demographic expansion, three in the Bay of Biscay (Canyons of Le Guilvinec, Morgat and Lampaul) and Logachev in the Irish Sea (see the Fu & Li F_s statistic, Table 2). No demographic expansion was detected in Iceland or Mediterranean populations.

Tableau 4 *Matrix of pairwise F_{st}, based on haplotypic frequencies, for Madrepora oculata populations. Due to the low number of Mediterranean samples, the locations of Santa Maria di Leuca and Canyon of Lacaze-Duthier were artificially merged into a single sampling set. Bold values are significant at $p < 0.05$.*

	Med	Ac	Crsc	Guil	Mrg	Crz	PSole1	PSole2	Lmp	Log	Lond 1	Lond 2	Haf
Med	-												
Ac	-0.04	-											
Crsc	**0.25**	**0.28**	-										
Guil	**0.20**	**0.19**	0.01	-									
Mrg	**0.25**	**0.24**	0.00	0.00	-								
Crz	**0.37**	**0.36**	0.03	0.07	0.00	-							
PSole1	**0.26**	**0.29**	0.05	0.02	-0.05	0.09	-						
PSole2	**0.35**	**0.35**	-0.01	0.07	0.02	-0.05	0.10	-					
Lmp	**0.31**	**0.32**	0.07	-0.01	-0.02	0.00	0.04	0.04	-				
Log	**0.37**	**0.39**	**0.11**	**0.23**	**0.21**	**0.16**	**0.27**	**0.09**	**0.23**	-			
Lond 1	**0.37**	**0.39**	**0.19**	**0.31**	**0.27**	**0.25**	**0.35**	**0.17**	**0.33**	-0.03	-		
Lond 2	**0.72**	**0.82**	**0.70**	**0.69**	**0.71**	**0.70**	**0.78**	**0.65**	**0.77**	**0.29**	**0.27**	-	
Haf	**0.51**	**0.57**	**0.43**	**0.50**	**0.47**	**0.46**	**0.55**	**0.40**	**0.54**	**0.11**	0.05	0.03	-

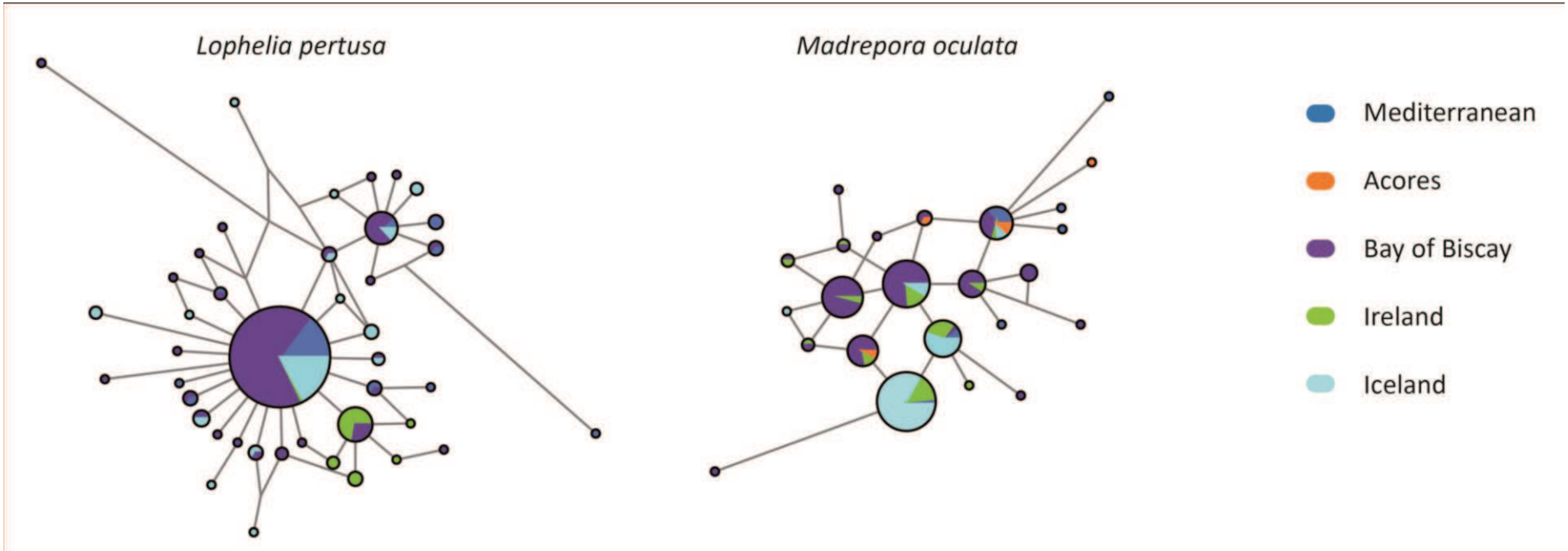

Figure 15 *Haplotype networks of Lophelia pertusa (left panel) and Madrepora oculata (right panel) populations, for ITS haplotypes. Each haplotype is represented by a single circle whose size is proportional to the frequency of the considered haplotype in the dataset. When a haplotype is found in more than one region, the pie charts illustrate the proportion belonging to each region. Length of links is proportional to the number of mutations separating two haplotypes, shortest ones representing a single mutation. Color legend of regions is provided on the right.*

Discussion

This study reveals two partially distinct biogeographic patterns of *Lophelia pertusa* and *Madrepora oculata*. Due to the systematic co-occurrence of these two species within Atlantic and Mediterranean deep reefs, such difference was unexpected, and either these species were not equally affected by glacial events, or their source and date of recolonisation of NE Atlantic differs. Data support the hypothesis of recolonization of north-east Atlantic from Mediterranean populations of *L. pertusa*. Whether the lack of Mediterranean sequences prevent to fully test the hypothesis of De Mol (2005), signs of recent demographic expansion were also found for *M. oculata* populations, but data suggest for this species a less intense impact of glacial events. An alternative hypothesis will thus be discussed.

Tableau 5 *Results of AMOVA performed on Madrepora oculata populations.*
The mutational model used here is the Kimura 2-parameters. The high percentage of variation among regions (38.59%) indicates a regional structure, and is congruent with F_{st}-matrix. An important part of the variation (59.19) occurs within populations, as observed for Lophelia pertusa. The regions defined for this analysis are the Mediterranean, the Acores, the Bay of Biscay, Ireland and Iceland (see also the haplotype network).

Source of variations	d.f.	Sum of squares	Variance components	P-values	Percentage of variation
Among regions	4	65.01	0.455	<0.0001	40.03
Among populations within regions	8	7.74	0.022	0.039	1.91
Within populations	187	123.43	0.6602	<0.0001	58.06
Total	199	196.18	1.137	-	-

4.1. Is there the signature of recent demographic expansion?

Both *L. pertusa* and *M. oculata* populations revealed signs of recent demographic expansions in the Bay of Biscay and Celtic Sea (Table 1), yet clearer in *L. pertusa* both through the typical star-like cluster and the higher number of samples showing significant tests for population expansion. These demographic features are in line with geological studies suggesting a break in deep reef growth in NE Atlantic during the last glacial period (Kano *et al*, 2007), supporting the hypothesis of a recent recolonization, at the Bay of Biscay and Celtic Sea.

However, the lack of star-like cluster, higher levels of haplotype and nucleotide diversities, and lower number of locations with significant test for demographic expansion (that should however be taken with caution due to the more limited sampling size in general for this species) observed for *M. oculata* (Fig. 15), suggest a distinct timing and/or the intensity for demographic expansion.

Several non-exclusive hypotheses may explain these discrepancies. First, these high levels of diversity and the relative homogeneity of haplotypic frequencies (Fig. 15) could indicate a weaker effect of genetic drift on *M. oculata* in NE Atlantic. One explanation may be the persistence of small populations of *M. oculata* in the Atlantic during the last glacial event, having allowed maintaining a larger number of distinct haplotypes, as suggested by the diversity of haplotypes and the absence of largely dominant one.

In case of extinction-recolonization event, a massive input of recruits initiating the recolonization may explain the maintenance of high levels of diversity. Such intense input might limit the founder effects, preventing the apparition of star-like clusters and explaining the lower amount of demographic expansion observed in this species. Nevertheless, this hypothesis relies on high dispersal abilities and such scenario may lead to a weak genetic structure among locations, notably between source and recolonized areas. This appears relatively unlikely due to the strong structure observed among regions (Tables 4 and 5), that exceeds the one observed for *L. pertusa*.

The observation of high diversity and strong structure with a limited, but detectable, signature of recent expansion events suggests the possible survival of small populations of *M. oculata* in the Atlantic.

Although recolonization is supported by most data for both species in the Bay of Biscay and Ireland, its source remains therefore ambiguous and may not necessarily be shared by both species.

4.2. Are the sources of recolonization identifiable?

The hypothesis of De Mol *et al.* (2005) implies that one or few Mediterranean haplotypes are shared between Mediterranean and recolonized Atlantic regions. In addition, a significant genetic structure is expected among Mediterranean and other regions only in the event of a limited amount of colonizer having led to a founder effect (i.e. leaving a signature of a bottleneck followed by expansion).

Two of the three more represented haplotypes within *L. pertusa* populations are shared between Mediterranean, Bay of Biscay and Iceland (Fig. 15), and constitute the center of the two star-like clusters. This may indicate that the recolonization of Atlantic was initiated by these two haplotypes, also most common in the Mediterranean. Yet, the population of Lacaze-Duthier is the only one Mediterranean population showing a significant genetic structure with Atlantic, whereas no such differentiation is observed with the reef of Santa Maria di Leuca. This global lack of structure and the fact that the large majority of genetic variability (92.4%, table 2) occurs within populations is in line with a recent colonization of Atlantic from the Mediterranean, the low amount of sampling sites available in the Mediterranean preventing a more detailed localization of the origin. The population of Logachev, however, appears divergent from all other with private haplotypes, suggesting another source of colonization that could not be inferred from available samples.

As for *M. oculata*, three well-represented haplotypes are shared between Mediterranean and Atlantic and due to the relatively low number of mutations separating these haplotypes (Fig. 15), a similar scenario cannot be rejected. However, a strong genetic structure observed within this species

suggests more limited dispersal among regions, making unlikely the hypothesis of a unique source of recolonization for the North Atlantic. Two common haplotypes seem to be typical from Iceland (Fig. 15), and shared with the Celtic Sea, which altogether with the lack of demographic expansion in Iceland may suggest a Southward recolonization pathway in the Atlantic, possibly combined with another Northward path originating in the Mediterranean. Alternatively the persistence of *M. oculata* in several Atlantic refugees, from which recolonization would have initiated, cannot be discarded and would fit well the balanced patterns of haplotypic and nucleotidic diversity as well as the observed pattern of regional differentiation

Examination of the status of Irish reefs may further help weighting those different scenarios of recolonization origin. The Irish populations of both *L. pertusa* and *M. oculata* occupy a particular status, showing strong signs of recent expansion (Table 1). For *L. pertusa*, the dominant Irish haplotype is shared with the Bay of Biscay (Fig. 15), and four satellite rare haplotypes are only found in this region. This may suggest a step by step South-North recolonization of the Atlantic with Celtic Sea occurring in a second time from a subset of individuals having first recolonized the Bay of Biscay as illustrated in the Figure 14.

Contrastingly, only one private haplotype was found for *M. oculata* (Table 1, Fig. 15) whereas 10 Irish remaining haplotypes are shared with others regions. The topography of the haplotypic network suggests that the Celtic Sea is a contact zone, Irish populations of *M. oculata* resulting from the convergence of populations from the Bay of Biscay (possibly themselves originating from the Mediterranean) and Iceland.

4.3. Conclusion

Lophelia pertusa and *Madrepora oculata* are the two reef building species in Northern Europe, co-occuring in most of the Mediterranean and Atlantic CWC areas. Signatures of recent demographic expansions were found for both species in the Bay of Biscay and Celtic Sea, which is consistent with the datations of beginning of reef growth (Schroder-Ritzrau *et al*, 2005) in these regions, following the Last Glacial Minimal. Yet, differences in biogeographic pattern occur between these species preventing to propose a unique scenario. This could results from ecological/physiological distinctions. Recent studies focus on physiological differences between *L. pertusa* and *M. oculata* (Kiriakoulakis *et al*, 2006; Larthaud *et al*, in press; Naumann *et al*, in press). Notably, the respiration and calcification of *L. pertusa* appear controlled by thermal acclimation while this limitation was not observed in *M. oculata* (Naumann *et al*, in press). The isotopic signature of fatty acids is also species-dependent suggesting either two feeding strategies or two distinct metabolisms (Kiriakoulakis *et al*, 2006). This highlights ecological differences between these species. The direct and/or indirect effects of climatic fluctuations, such as food supply and variations of temperature, would have differentially affected past populations of these species. This may in turn, partly explain the slightly distinct phylogeographic pattern of these corals.

The data fully support the hypothesis proposed by De Mol *et al*, (2005) for *L. pertusa*. The Mediterranean might represent glacial refugia for this species. Between 50 000-12 000 years BP, the populations were flourishing in this province (Delibrias and Taviani, 1984) and the Mediterranean Outflow Water (MOW) was intense. A first step of Atlantic recolonisation would have happened

around 40 000-50 000 years BP in the Gulf of Cadiz and Moroccan shelf (Fig. 14), a period of intense Mediterranean Outflow Water (Toucanne *et al*, 2012) acting as an agent of larval transport. Following the glacial events (after 10 000 yrs BP), a progressive northward recolonization of Bay of Biscay and Celtic Sea occurred.

However, the hypothesis of De Mol *et al,* (2005) is weakly supported by data for *M. oculata*. The glacial events have slow down the growth of these colonies and prevent the development of deep-reef, in the Bay of Biscay. Yet, residual populations may have persisted, forming largely scattered populations, at a demographic level sufficient to maintain initial haplotypic diversity. The warming of Atlantic waters following the last glacial minimal has allowed a demographic expansion, concomitantly to *L. pertusa*'s one. Populations of Celtic Sea may result from an admixture of southern and northern Atlantic locations.

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CHAPITRE 2. LES INTERACTIONS ENTRE DISPERSION ET CLONALITE CHEZ LES ZOSTERES MARINES ; INFLUENCE SUR LA DYNAMIQUE DES POPULATIONS ET L'ARCHITECTURE CLONALE DES HERBIERS MARINS.

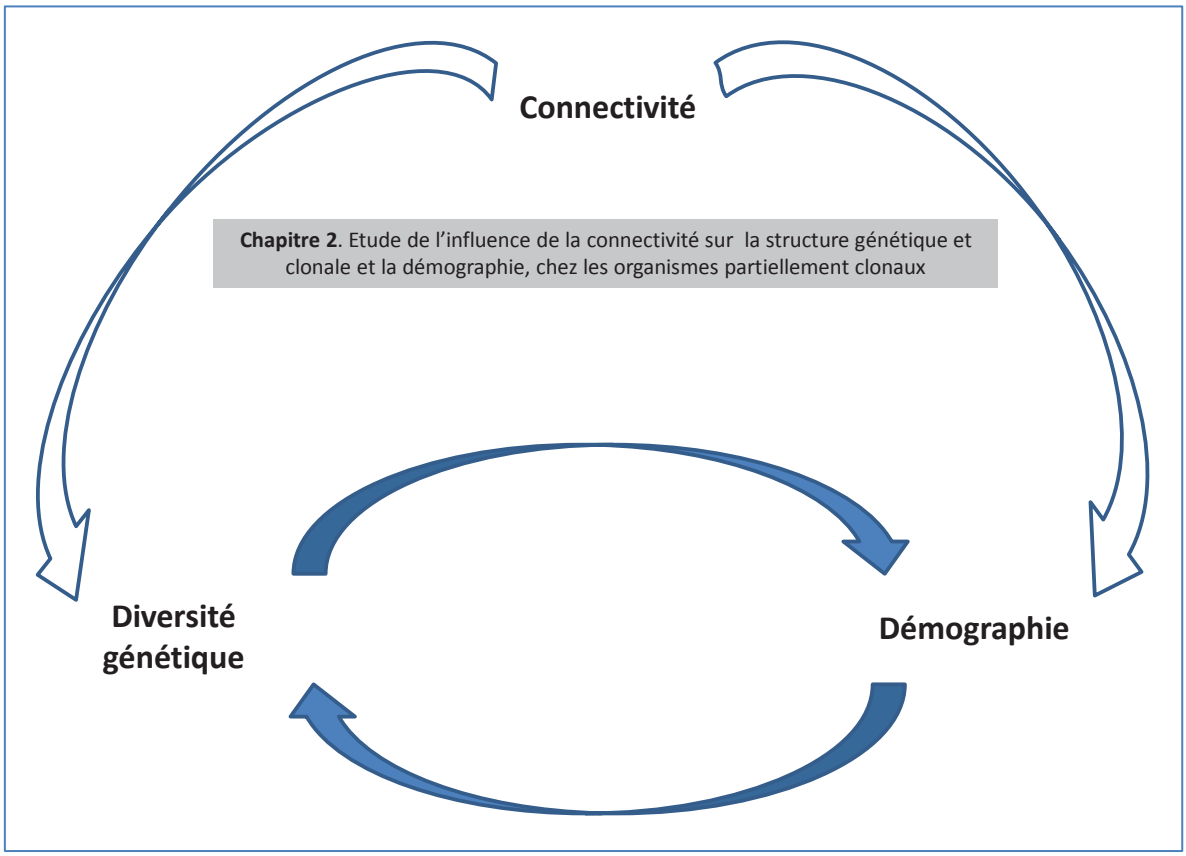

Figure 16 *Interrelation entre connectivité et structure génétique et clonale*

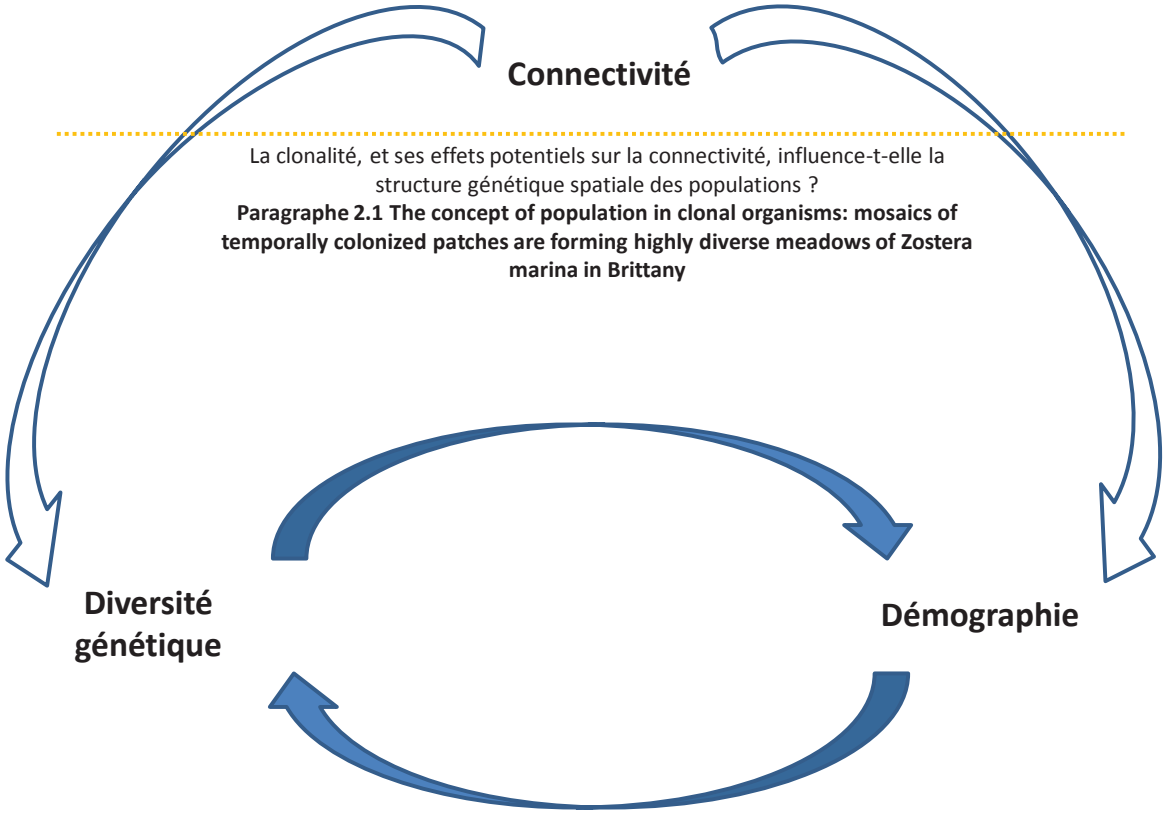

Figure 17 *Influence potentielle de la clonalité sur la structure génétique spatiale, via son effet sur la dispersion*

Contexte & résumé

La distribution spatiale de la diversité génétique est une information fondamentale en génétique des populations. La structure spatiale, correspondant à une distribution non aléatoire des variants génétiques, résulte de différents processus comme, par exemple, l'existence de pressions de sélection ou l'histoire des populations.

A fine échelle spatiale (i.e. à l'échelle intra-population), une telle structure spatiale révèle l'existence de patchs locaux composés d'individus proches génétiquement. Une restriction au flux de gènes est une source potentielle de structuration génétique à ces petites échelles d'espace. Chez les plantes terrestres, ce type structure à fine échelle est souvent observée, et s'explique par des restrictions au flux géniques, plus ou moins intenses (Vekemans and Hardy, 2004). En revanche, le milieu marin étant supposé plus dispersif, l'existence de telles structures à fine échelle est moins attendue sur le plan théorique.

Par ailleurs, la clonalité influence potentiellement la structure génétique par le biais de plusieurs phénomènes. Tout d'abords, la croissance clonale, qui correspond à la duplication d'un même individu génétique, génère *de facto* de la structure puisqu'elle se produit suivant un patron non-aléatoire (croissance linéaire ou sphérique). De plus, la compétition intra-spécifique est exacerbée, dans le cas des organismes partiellement clonaux, par la croissance végétative. Ceci conduit dans certains cas à l'exclusion, la croissance de certains clones se faisant au détriment de la survie d'autres. Enfin, la clonalité peut freiner l'efficacité de la dispersion, en limitant le recrutement des propagules par la compétition.

L'objectif central de ce sous-chapitre est l'exploration de la structure génétique spatiale des populations de *Zostera marina*, de l'échelle régionale à l'échelle intra-herbier, et d'évaluer l'effet de la clonalité sur cette structure. Pour cela, une stratégie d'échantillonnage particulière a été mise en œuvre. Au sein d'un même site, deux quadrats d'échantillonnage distants de quelques dizaines de mètres ont été dessinés.

Les principaux résultats sont les suivants :

- Les herbiers de Zostère marine bretons présentent un hotspot de diversité génétique, à l'échelle de l'aire de distribution
- Les architectures clonales variables, considérées conjointement à l'existence ou non de patrons d'autocorrélation spatiale, suggèrent la coexistence de deux stratégies de recrutements (initial versus répété, voir Eriksson 1993).

L'existence d'une structure génétique à fine, intermédiaire et large échelles spatiales révèle un schéma de mosaïques fluctuantes, les patchs de clones composant une prairie ayant probablement des origines différentes dans le temps et l'espace. Ce point nous a amené à discuter du concept de populations chez ces organismes, chez lesquels l'absence de délimitation de l'entité panmictique, liée à cette structure en mosaïque, rends le concept génétique de population peu approprié. L'un des concepts écologiques basé sur la continuité des prairies serait probablement plus adapté pour les espèces partiellement clonales, tout au moins les phanérogames.

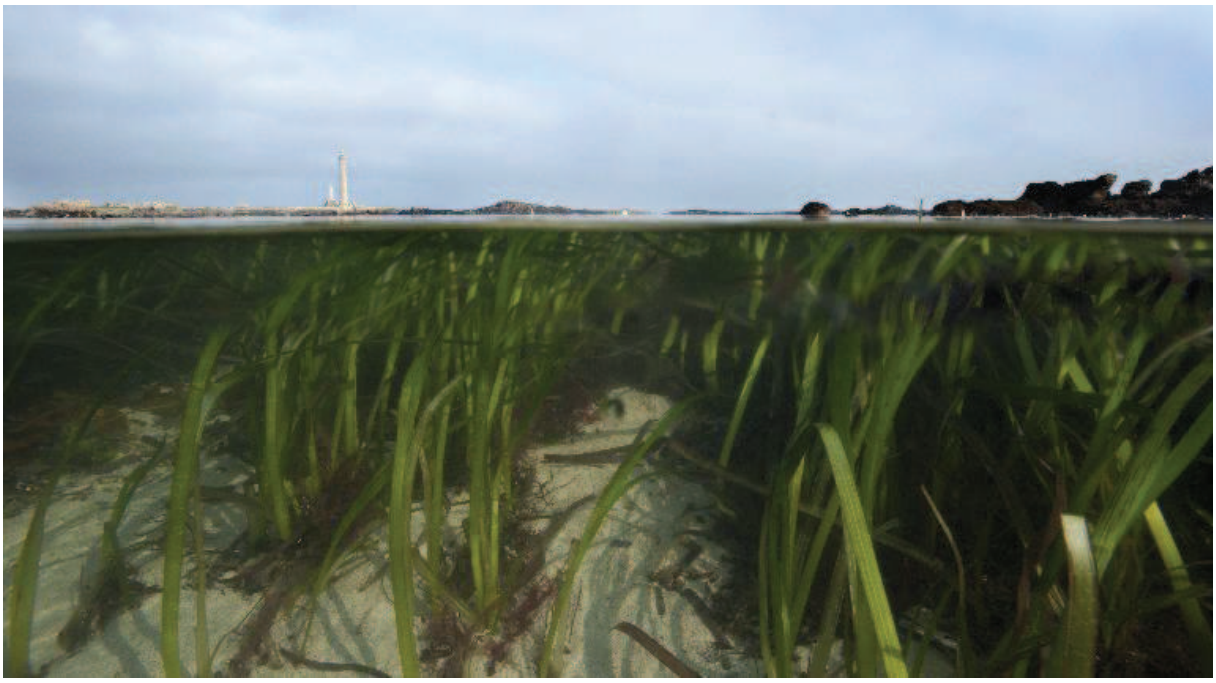

Figure 18 *Herbier de Zostera marina, à Lilia (Finistère).*
Les espaces vides constituent les sites potentiels de recrutement pour les propagules. La croissance clonale peut aussi combler ces zones libres (©photothèque Ifremer)

Information préalable : l'annexe 1 décrit la méthodologie pour l'étude de la clonalité, d'une façon plus pédagogique que celle utilisée dans les articles ou manuscrits d'article suivant.

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The concept of population in clonal organisms: mosaics of temporally colonized patches are forming highly diverse meadows of *Zostera marina* in Brittany

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Abstract

Seagrasses structure some of the world's key coastal ecosystems presently in decline due to human activities and global change. The ability to cope with environmental changes and the possibilities for shifts in distribution range depend largely on their evolvability and dispersal potential. As large-scale data usually show strong genetic structure for seagrasses, finer-grained work is needed to understand the local processes of dispersal, recruitment and colonization that could explain the apparent lack of exchange across large distances. We aimed to assess the fine-grained genetic structure of one of the most important and widely distributed seagrasses, *Zostera marina*, from seven meadows in Brittany, France. Both classic population genetics and network analysis confirmed a pattern of spatial segregation of polymorphism at both regional and local scales. One location exhibiting exclusively the variety '*angustifolia*' did not appear more differentiated than the others, but instead showed a central position in the network analysis, confirming the status of this variety as an ecotype. This phenotypic diversity and the high allelic richness at nine microsatellites (2.33–9.67 alleles/locus) compared to levels previously reported across the distribution range, points to Brittany as a centre of diversity for *Z. marina* at both genetic and phenotypic levels. Despite dispersal potential of several 100 m, a significant pattern of genetic differentiation, even at fine-grained scale, revealed 'genetic patchiness'. Meadows seem to be composed of a mosaic of clones with distinct origins in space and time, a result that calls into question the accuracy of the concept of populations for such partially clonal species.

Keywords: clonality, dispersal, ecotype, network analysis, population, Zostera marina

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Introduction

Seagrasses are the structural basis of key coastal marine ecosystems (Orth *et al.* 2006) supporting high biodiversity and biomass (Orth *et al.* 1984). These ecosystems provide a great number of goods and services, including primary production, the supply of food for megaherbivores, habitats for resident faunas and stabilization of sediments (Hemminga & Duarte 2000). Many of these are experiencing a decline on a worldwide scale, probably due to anthropogenic disturbances and climate change (Waycott *et al.* 2009). The ability of these species, and of the ecosystems they supply, to survive future environmental changes may largely depend on their genetic adaptability (Booy *et al.* 2000; Frankham 2005). The attempt to predict, and possibly prevent, changes in the geographical pattern of persistence, local extinction or range shifts should be based on a good understanding of the implications of genetic diversity for the resistance and resilience of local populations, as well as on reliable estimates of dispersal among geographic areas. Dispersal is likely to determine the balance between migration-drift and local adaptation,

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and in turn the likelihood of meadows to survive or to be naturally recolonized if locally extinct.

In temperate latitudes, meadows are generally monospecific, and the dominant family is Zosteraceae, which contains five monophyletic species, including the eelgrass *Z. marina*. This species is widely distributed in the northern hemisphere, and present in the Pacific and Atlantic Oceans, as well as in the Mediterranean and Black Seas. It is the dominant seagrass on the coasts of Brittany (France) where it shares (Den Hartog & Hily 1997) a dominant role with algae in structuring one of the most important coastal ecosystems and providing habitat for a large number of species (Hily & Bouteille 1999).

Eelgrass reproduces sexually through the production of propagules, and clonally through the vegetative production of ramets *via* rhizome elongation. This partial clonality has multiple biological and methodological implications. In particular, estimations of diversity in these populations require distinction between genet (genetic individuals, where all tissue has originated from one zygote; Eriksson 1993) and ramet (a potentially independent part of the genet that often represents the sampling unit; Eriksson 1993; Arnaud-Haond *et al.* 2007a). Clonal diversity has been shown experimentally to enhance resistance and resilience of experimental quadrats of *Z. marina* to perturbations (Hughes & Stachowicz 2004; Reusch *et al.* 2005; Ehlers *et al.* 2008). If these experimental results are extrapolated to natural populations (Hughes & Stachowicz 2009), the level of clonal diversity is found to be associated with an enhanced resistance to environmental perturbations. A large biogeographic survey (Olsen *et al.* 2004) reported moderate to high values of clonal richness for two locations in Brittany (Carantec: 0.54; Morgat: 0.90).

Genetic diversity, i.e. the allelic richness and/or heterozygosity observed in meadows, is influenced by numerous factors, such as effective population size, spatial pattern of dispersal and recruitment success of immigrant propagules (dependent on competition and local adaptation) and the biogeographical history of populations (Olsen *et al.* 2004). At the scale of its entire distribution, these authors reported an allelic diversity hotspot for *Z. marina* in the North Sea–Wadden Sea region, where populations are characterized by high allelic richness. This region is also a diversity hotspot for *Z. noltii* (Coyer *et al.* 2004). In contrast, East Atlantic meadows of *Z. marina*, including the two locations in Brittany studied here, exhibited much lower levels of allelic richness, suggesting a narrower adaptive potential. Yet those two particular samples from Brittany showed distinct levels of clonal and allelic richness, calling for further analysis of the spatial variability and fine-grained pattern of clonal and genetic composition that had thus far only been performed on samples from the Baltic Sea.

Moreover, the possibility of heterogeneity in intra-meadow clonal and genetic composition had never been explored, to the best of our knowledge, in such phylogeographic analyses on seagrasses (Coyer *et al.* 2004; Olsen *et al.* 2004; Arnaud-Haond *et al.* 2007b; Alberto *et al.* 2008). Some benthic marine invertebrates show contrasted patterns of genetic diversity and structure at a very fine-grained scale (Johnson & Black 1984; Arnaud-Haond *et al.* 2008), likely due to the chaotic nature of dispersal in the marine environment (Roughgarden *et al.* 1988). Given the large-scale dispersal potential of most marine angiosperms through seed or shoot dispersal, combined with their benthic nature and extensive clonal propagation once settled, a similar phenomenon might occur in seagrass meadows.

Finally, the occurrence of the variety '*Z. angustifolia*', already described in the UK, has also been seen in Brittany, particularly in the Morbihan Gulf (France, REBENT). Whether this particular morph corresponds to a distinct species, as suggested by some authors (Percival *et al.* 1996; Provan *et al.* 2008), or to an ecotype (Den Hartog 1970; De Heij & Nienhuis 1992), is still unknown as no genetic studies have been reported so far that address this issue. This topic is of central importance for understanding gene flow and local adaptation processes in *Z. marina* meadows across its distribution range.

In the present work, we used nine microsatellites to (i) identify the particular morph '*Z. marina v. angustifolia*', in order to test its status as a genetically distinct taxon or ecotype; (ii) investigate the genetic diversity and genotypic structure along the coasts of Brittany; (iii) test for the importance of fine-grained variation (intra meadow vs. regional scale) of these characteristics; and (iv) assess the dispersal potential at local (i.e. fine-grained) and regional scales.

Materials and methods

Sample collection

Eelgrass samples were collected in February to April 2009 from 7 intertidal meadows along the coast of Brittany (Fig. 1), stretching from Saint-Malo to Arradon. Distances between meadows ranged from 33 km (Molène—Roscanvel) to 442 km (Arradon—Saint-Malo). For each location, two 20 × 30 m quadrats separated by several tens of meters were chosen, located in continuous parts of the meadow monitored as part of the REBENT survey (REseau BENThique, a French network specialized in the survey of major coastal ecosystems including eelgrass meadows; http://www.rebent.org). Approximately 35 sampling units (SU) were collected according

Fig. 1 Locations of the seven intertidal meadows of *Z. marina*. For each location, two quadrats were determined for sample collection (approximately 35 sampling units collected from each quadrat).

Table 1 Locations, correspondence with the points surveyed by REBENT network and number of sampling units (SU). For Molène, we also give the number of haphazardly-sampled SU. The inter-quadrat distances were calculated with GPS coordinates

Site	Quadrate	Number of SU	Latitude	Longitude	Distance (meters)
Saint-Malo	Q1	35	48°38′923 N	02°01′992 W	85
	Q2	35	48°38′958 N	02°02′038 W	
L'Arcouest	Q1	34	48°49′428 N	03°01′162 W	70
	Q2	34	48°49′425 N	03°01′218 W	
Callot	Q1	35	48°41′064 N	03°54′968 W	30
	Q2	35	48°41′052 N	03°54′982 W	
Sainte- Marguerite	Q1	35	48°35′811 N	04°37′389 W	75
	Q2	35	48°35′830 N	04°37′443 W	
Molène	Q1	32 (12)	48°23′760 N	04°56′934 W	–
Roscanvel	Q1	35	48°19′934 N	04°32′209 W	100
	Q2	35	48°19′984 N	04°32′182 W	
Arradon	Q1	34	47°36′911 N	02°49′636 W	80
	Q2	35	47°36′914 N	02°49′574 W	

to randomly drawn coordinates (Table 1). In Molène, due to high patchiness of the meadow, only one quadrat was sampled, with 20 SU according to random coordinates and the 12 more collected at haphazardly in the patchy end of the meadow. Annual observations from REBENT indicated Saint-Malo as one of the sites where the variety '*angustifolia*' is observed across years. The field observations indeed showed the typical *Z. marina v. angustifolia* variety in both quadrats of this meadow, with dwarf shoots exhibiting narrow leafs almost comparable in size and shape to the dwarf *Z. noltii*.

The base of each leaf bundle, including the shoot apical meristem, was preserved in silica crystals until DNA extraction.

DNA extraction, isolation, microsatellite and ITS amplification and loci scoring

The Fast DNA®SPIN kit for soil was used for DNA extraction according to the protocol provided by the manufacturer (MP Biomedicals, France). Nine microsatellite loci (Genbank accession numbers: AJ009899, AJ009901, AJ009902, AJ009905 and AJ249303 to AJ249307; Reusch *et al*. 1999, 2000) were PCR-amplified using fluorescently labeled primers (GA12, GA19, GA20, GA17D, GA16, GA2, GA23, GA35 and GA17H). PCR products were visualized using an ABI-3100 FNVR automated sequencer (Applied Biosystems) and scored using STRand software (http://www.vgl.ucdavis.edu/informatics/strand.php). A double blind reading was made by two different users and gels were re-scored when discrepancies were recorded.

To standardize the samples at 30 individuals before estimations of clonal and genetic composition, excess individuals were removed at random.

To test whether the variety '*angustifolia*' corresponded to a species or to an ecotype, we also compared sequences of ITS markers (1100 bp) of two samples exhibiting the typical morphology of the variety '*angustifolia*' (Saint-Malo) with two samples from Arradon and from

Arcouest locations exhibiting the typical morphotype of *Z. marina*. ITS-PCRs were performed using the universal primers Jo6 and TW5 (White *et al.* 1990 in Diekmann *et al.* 2001).

Genetic and clonal data analysis

In order to identify genetic individuals (i.e. to discriminate genets from ramets on the basis of their Multi Locus Genotype, MLG), we used a 'barcoding' type approach based on nine microsatellite loci.

For clonal organisms, two questions must be answered: (i) do all the replicates of the same MLG really belong to the same genet (i.e. are they all issued from the same sexual reproduction event)? and (ii) does each distinct MLG really belong to a distinct genet?

To answer the first question, when the same MLG is encountered n times in a sample of N sampling units, the probability that the identical MLGs originate from different sexual reproductive events (p_{sex}) should be assessed (Arnaud-Haond *et al.* 2007a). Below a threshold value fixed at 0.01, identical MLGs may be considered as belonging to the same genet. Estimates of p_{sex} are derived on the basis of allelic frequencies estimated using the round robin method (Parks & Werth 1993), with a sub-sampling approach to limit the overestimation of the rare alleles. Allelic frequencies for each locus are estimated on the basis of a sample pool composed of all the MLGs discriminated, while excluding the loci for which allelic frequency is estimated. This procedure is repeated for each locus, taking into account Wright's inbreeding coefficient estimated after the exclusion of identical MLG (Young *et al.* 2002).

Once the clonal membership of identical MLG is ascertained using p_{sex}, slightly distinct MLGs belonging to the same genet may, nevertheless, still occur in the dataset, either due to the existence of somatic mutation or scoring errors (Douhovnikoff & Dodd 2003; Arnaud-Haond *et al.* 2007a). If ignored, this would lead to an overestimation of the number of clones in the sample analyzed. The two-step approach proposed by Arnaud-Haond *et al.* (2007a) was applied to test whether these slightly distinct (at one or two loci) MLGs belong to the same genet by: (i) screening each MLG pair presenting an extremely low distance; (ii) using p_{sex} on the set of identical loci in order to estimate the probability that the slightly distinct MLG could actually be derived from distinct reproductive events. When p_{sex} is lower than 0.01, the two identical MLG can be considered to belong to the same genet or Multi Locus Lineage (MLL; Arnaud-Haond *et al.* 2007a).

Estimates were calculated using the software GENCLONE 2.1 (Arnaud-Haond & Belkhir 2007).

For each quadrat, clonal diversity was estimated by:

$$R = \frac{G-1}{N-1},$$

where G is the number of MLLs in the sample and N is the number of SUs analyzed, as recommended by Dorken & Eckert (2001) and Arnaud-Haond *et al.* (2005). The minimum value for clonal diversity in a monoclonal stand is always 0, independent of sample size, and the maximum value is still 1 when each analyzed sample corresponds to a distinct MLL. The complement of the Simpson index (Pielou 1969) for genotypic diversity in each quadrat, representing the probability of encountering distinct MLLs when randomly taking two sampling units, was estimated as:

$$D = 1 - \sum_{i=1}^{G} p_i^2,$$

where p_i^2 is the frequency of the ith MLL (its estimation is given by: $p_i^2 = [n_i(n_i - 1)]/[N(N - 1)]$ where N is the number of ramets sampled and n_i is the number of sample units sharing the ith MLL). Moreover, we estimated the Simpson's evenness index to describe clonal equitability:

$$ED^* = \frac{(D - D_{min})}{(D_{max} - D_{min})}$$

with D_{min} and D_{max} being the approximate minimum and maximum values of Simpson's complement index given the sample size N and the sample clonal richness G, estimated as:

$$D_{min} = [\frac{(2N - G) \times (G - 1)}{N^2}] \times \frac{N}{N-1} \text{ and } D_{max} = \frac{(G-1)}{G} \times \frac{N}{(N-1)}.$$

The β of the Pareto distribution, representing the negative slope of the power law usually describing the distribution of ramets into groups of clonal size (Arnaud-Haond *et al.* 2007a), was also estimated as this metric seems less sensitive than other estimators to the relative density of sampling units vs. shoots in the sampled meadow. All clonal diversity and structure parameters were calculated with GENCLONE 2.1 (Arnaud-Haond & Belkhir 2007).

A single copy of each discriminated MLL was retained in the dataset used to assess genetic diversity and structure. Genetic diversity within quadrats was estimated as the mean number of alleles per locus (Â), with observed (H_O) and unbiased (H_E) multilocus heterozygosity (Nei 1978). Linkage disequilibrium (LD) was tested according to Black & Krafsur (1985). A permutation

procedure (1000 permutations) was used to test whether a particular estimate of the overall inbreeding coefficient (F_{IS}) or LD was significantly different from 0 ($P < 0.01$).

Genetic structure among populations was estimated with θ (Weir & Cockerham 1984). A Mantel test including geographical distances among populations was carried out to test for the two dimensional 'ISOLATION BY DISTANCE' (IBD) model (Rousset 1997). These parameters were estimated using GENETIX (Belkhir *et al.* 2004).

At the within-quadrat level, autocorrelation analyses were performed to test for the existence of restriction to dispersion at the intra-meadow scale, and to estimate the extent of clonality. We used the kinship estimator coefficient of Ritland (F_{ij}) as a genetic relatedness statistic (Ritland 1996). We performed regression analyses of mean F_{ij} against the Log_e of mean geographic distance, within each distance class. This allowed us to test the adequacy of IBD models in each quadrat. The autocorrelation analyses were performed using Ritland's coefficient of kinship: (i) first including all SUS, where it is mostly influenced by the spatial extent of clones/clonal lineages (i.e. the genetic neighborhood of SUS belonging to the same MLL) and (ii) using permutations (1000) in order to include only one ramet (and one of the possible corresponding coordinates, randomly chosen for each permutation step) from each genet at each permutation, in order to examine the dispersion through sexual propagules. The slopes of regressions (b) allowed us to calculate the Sp statistic (Vekemans & Hardy 2004). This statistic corresponds to the spatial autocorrelation profile, varying from 0 (no limitation to gene dispersal at the scale of the sampling) to $+\infty$ (theoretical case, where the structure is maximal). Its equation is the following:

$$Sp = \frac{-b}{1 - \hat{F}_{(1)}}$$

with $\hat{F}_{(1)}$ the kinship value for the first distance class. Autocorrelation parameter estimations were performed with GENCLONE 2.1 (Arnaud-Haond & Belkhir 2007).

The clonal subrange CR was estimated for each quadrat to describe the spatial components of the clonal population. It corresponds to the minimal estimates of the maximum distance between two identical genotypes belonging to the same clone, in meters, and is determined as the distance for which the probability of clonal identity becomes null (Harada *et al.* 1997; Alberto *et al.* 2005).

Network analysis

Network analysis is a graphic, holistic and non-parametric method that has proven useful in the illustration and analysis of population structure (Rozenfeld *et al.* 2007, 2008; Fortuna *et al.* 2009). In this study, networks based on genetic distances were used (i) to ascertain the relative position of the variety '*angustifolia*' (sampled in Saint-Malo) against other sampling locations, in a global network including all genets from all locations, and (ii) to illustrate the distribution of genetic distances at a finer scale (i.e. among genets from distinct quadrats within sampling locations). Individual-based networks of genetic distances were built at the global scale (all quadrats) and at local scales (for each sampling locality), illustrating the connection of some genets (agents) depending on their genetic distance (link). The distances used have proven successful in assigning unknown individuals to their correct subpopulations (Estoup *et al.* 1995) and is classically known as the 'Shared Allele Distance' (Chakraborty & Jin 1993), although it actually reflects the proportion of non shared alleles:

$$D_{SA_l} = 1 - \frac{\sum uS}{2\mathrm{u}}$$

with S the number of shared alleles and u the number of loci. D_{SA_l} spans from 0 to 1.

Networks are built including links for all distances, which are subsequently removed in decreasing order, until reaching the effective percolation point, Dpe (Stauffer & Aharony 1994; Rozenfeld *et al.* 2007), below which the network fragments into small clusters. This phenomenon can be interpreted as the first level of limitation to gene flow. The precise calculation of the Dpe is made with the standard methodology for a finite system, proposed by Stauffer & Aharony (1994) and consisting of calculating the average cluster size excluding the largest one:

$$\langle S \rangle = \frac{1}{N} \sum_{s<S\max} S^2 n_s$$

as a function of the last distance value removed. N is the total number of nodes not included in the largest cluster and n_s is the number of clusters containing s nodes. Once this effective percolation threshold is reached, we analyzed the network topology and its characteristics (Table 4; Fig. 4).

Global and local property estimates of the network

The clustering coefficient C_i of the node i is the ratio between the number of existing links with the maximal number of potential links within the cluster. It is defined as:

$$Ci = \frac{Ei}{Ei^{(\max)}} = \frac{2Ei}{ki(ki-1)}$$

with Ei the number of links existing among the neighbors of the node i, and the degree ki of a given node i

the number of other nodes linked to it. The clustering coefficient of the whole network <*CC*> is defined as the average of all individual clustering coefficients in the system. The clustering of nodes is interpreted as the existence of hierarchical substructure, with clusters of genets within which members are more closely related to one another than they are to other genets outside the particular cluster.

The *betweenness centrality* (Freeman 1977) of node *i*, *bc*(*i*), is the fraction of shortest paths between pairs of nodes that pass through node *i*. Let σ_{st} denote the number of shortest paths connecting nodes *s* and *t* and $\sigma_{st}(i)$ denote the number of those passing through the node *i*; then:

$$bc(i) = \sum_{s \neq t \neq i} \frac{\sigma st(i)}{\sigma st}.$$

Higher values of *betweenness centrality* in genetic networks have been interpreted as the importance of a given agent (a population or cluster of individuals) in relaying gene flow across the system, i.e. to and from other agents (Rozenfeld *et al.* 2008).

Results

ITS polymorphism in Z. marina and Z. marina v. angustifolia

Of six genets from the first quadrats in Saint-Malo, Arradon and Arcouest, only two haplotypes were observed, and these differed at only one nucleotide substitution out of 1100 base pairs of ITS 1 & 2. This difference distinguished one genet from Arradon from the five others, which shared the most common haplotype.

Clonal structure and diversity

The clonal diversity of the 13 quadrats showed high variability both within and among locations (Table 2). The minimum values were observed in the Molène meadow and quadrat 2 at Saint-Malo ($R = 0.48$; $\beta = 1.36$ and $R = 0.48$; $\beta = 1.54$, respectively), whereas quadrat 1 at Roscanvel and quadrat 2 at Arcouest showed highest clonal richness ($R = 1.00$; $\beta \geq 4.95$ for both). Mean clonal richness was 0.72. The largest discrepancies within location were observed in Arcouest ($R = 0.62$–1), Roscanvel ($R = 0.69$–1) and Saint-Malo ($R = 0.48$–0.62).

The clonal subrange was also highly variable (largest clones in quadrat 1 of the Saint-Malo meadow: CR = 18.61 m; shortest clones in quadrats 1 of Roscanvel and 2 of Arcouest: CR = 0.00 m). A high within-location variability was observed here also: those quadrats with the highest clonal diversity, and therefore a minimum clonal subrange, were found sharing a site with a second quadrat ranking among the highest in terms of clonal subrange (CR = 12.04 in quadrat 2 at Roscanvel and CR = 17.01 in quadrat 1 at Arcouest).

Genetic diversity and Hardy–Weinberg equilibrium

Genetic composition analyzed with a single representative of each MLL was highly variable among locations

Table 2 Parameters of clonal structure: for each quadrat; samples were standardized with 30 ramets. G: number of identified MLLs. R: clonal richness. D* and ED*: Simpson index and its equitability index. β: slope of Pareto distribution. Grey cells indicate values calculated following a procedure of minimal estimation. CR: clonal subrange. Parameters of genetic composition: the two parameters we assessed were heterozygosity and allelic richness. H_e: expected heterozygosity without bias (Nei 1978); H_o: observed heterozygosity. F_{IS} and LD values were estimated after 1000 permutations of alleles within the quadrat. The mean number of alleles per locus Â was also estimated. Grey values: ns; **P* < 0.05; ***P* < 0.01; ****P* < 0.001

		clonal structure							genetic composition				
Meadow	quadrate	N	G	R	D*	ED*	β	CR	He	Ho	Fis	Â	LD
Arradon	Q1	30	26	0.86	0.99	0.65	3.10	5.59	0.52	0.51	0.03	5.89	0.06
	Q2	30	21	0.69	0.97	0.86	2.09	14.56	0.54	0.52	0.03	6.00	0.00
Roscanvel	Q1	30	30	1.00	1.00	–	4.95	0.00	0.52	0.51	0.01	4.78	0.06
	Q2	30	21	0.69	0.97	0.88	2.40	12.04	0.50	0.52	−0.05	4.11	0.03
Molene	Q1	30	15	0.48	0.85	0.51	1.36	–	0.40	0.40	0.01	3.44	0.05
Sainte-Margue rite	Q1	30	28	0.93	1.00	0.52	3.97	2.50	0.54	0.50	0.08*	6.67	**0.50**
	Q2	30	28	0.93	0.99	0.00	4.01	3.04	0.69	0.60	0.14***	9.67	**0.58**
Callot	Q1	30	26	0.86	0.99	0.65	2.89	5.32	0.46	0.42	0.08*	5.78	0.06
	Q2	30	23	0.76	0.98	0.85	3.00	7.76	0.45	0.44	0.03	4.89	0.08
l'Arcouest	Q1	30	19	0.62	0.91	0.45	1.46	17.01	0.41	0.46	−0.13*	4.11	0.04
	Q2	30	30	1.00	1.00	–	4.95	0.00	0.40	0.45	0.12**	3.89	0.07
Saint-Malo	Q1	30	19	0.62	0.95	0.80	2.05	18.61	0.29	0.35	0.21**	2.33	0.07
	Q2	30	15	0.48	0.89	0.70	1.54	10.20	0.40	0.39	0.04	3.56	0.00

(Table 2), mostly due to the extreme composition of Saint-Malo (heterozygosity of 0.35 and allelic richness of 2.33 in quadrat 1) and Sainte-Marguerite (heterozygosity of 0.6 and allelic richness of 9.67 in quadrat 2). The estimates appeared more stable among quadrats within these locations, as well as among the other locations, despite high variance in clonal diversity estimates, which tends to support the idea of sexual and panmictic entities in the remaining quadrats, once replicates are removed.

Similarly, departures from HWE and the occurrence of LD were generally weak once replicates were removed, except for quadrat 2 in Sainte-Marguerite, showing heterozygote deficiency ($F_{IS} = 0.14$, $P < 0.001$) and quadrats 2 of Arcouest and 1 of Saint-Malo, with an excess of heterozygosity (respectively $F_{IS} = -0.12$; $P < 0.01$ and $F_{IS} = -0.21$; $P < 0.01$). Here most values were similar among quadrats within location except for Saint-Malo with the important heterozygote excess mentioned here in one quadrat and no significant departure observed in the other.

Slightly significant preferential matching between alleles was detected for the majority of quadrats, with 6.7–19% of LD values significantly different from 0. Here again, quadrat 1 of Saint-Malo and quadrat 2 of Sainte-Marguerite departed from the average with 50% and 58% of significant values, respectively. To assess whether significant values resulted from a 'locus' or 'population effect', we therefore removed these two quadrats. For each pair of loci (total of 36 pairs), no significant LD value was found for 11 pairs, 1 significant value was found for 15 pairs, 2 for 5 pairs, 3 for 4 pairs, and only the pair GA2 / GA17H showed 6 significant LD values, indicating a significant and possibly physical LD between these two loci. Finally, a positive and significant relation between F_{IS} and LD values was found ($R^2 = 0.31$; slope = 0.25; $P = 0.049$) indicating that most LD values may be statistical and due to departure from random mating rather than to physical proximity of loci, in agreement with the lack of significant results observed in a previous study with large-scale sampling across the distribution range of the species (Olsen *et al.* 2004).

Genetic structure among locations and differentiation

When the dataset was analyzed at the genet level (i.e. including only one copy of each recognized MLL), this revealed wide genetic differentiation among the 13 locations. All F_{ST} values per pair of sampling quadrats were significantly different from 0 ($P < 0.05$; Table 3). The minimum value was observed between quadrats Callot 1 and Saint-Malo 2 ($F_{ST} = 0.039$; $P < 0.05$) and F_{ST} reached 0.33 ($P < 0.01$) between Molène and quadrat 2 at Arcouest. Within locations, all quadrat pairs were also significantly different and sometimes exceeded some of the inter-location estimates. The minimum was observed among the quadrats at Roscanvel ($F_{ST} = 0.019$; $P < 0.05$) and the maximum among the quadrats at Callot ($F_{ST} = 0.12$; $P < 0.01$).

A Mantel test carried out among all pairs of populations was significant (slope : 0.04; $R^2 = 0.11$; $P < 0.01$; Fig. 2), but such significance cannot be interpreted as an indication of a strict IBD pattern, as it is mostly driven by the sampling scheme, which results in two clouds of dots representing the intra vs. inter location distances among quadrats. No relation was observed at either of these two scales (among pairs within location:

Table 3 Matrix of genetic distance (F_{ST}) and geographic distance (kilometers). The geographic distance is expressed in kilometers and ranged from 0.03 km for the two quadrats at Callot to 442 km between Saint-Malo and Arradon. F_{ST} values are calculated following Weir & Cockerham (1984), for each pair of samples. Grey values: $P < 0.05$. All other values are significant with a probability $P < 0.01$

		Arr 1	Arr 2	Ros 1	Ros 2	Mol	SMar I	SMar 2	Cal 1	Cal 2	Arc 1	Arc 2	SMal 1	SMal 2
Arradon	Ql	–	0.08	0.15	0.13	0.22	0.30	0.24	0.08	0.12	0.17	0.19	0.19	0.14
	Q2	0.08	–	0.14	0.13	0.25	0.28	0.21	0.12	0.06	0.13	0.13	0.21	0.14
Roscanvel	Ql	195	195	–	0.02	0.23	0.21	0.17	0.10	0.15	0.12	0.13	0.19	0.09
	Q2	195	195	0.1	–	0.24	0.23	0.20	0.09	0.14	0.13	0.14	0.23	0.11
Molene	Ql	198	198	33	33	–	0.14	0.14	0.14	0.28	0.28	0.33	0.28	0.22
Sainte-Marguerite	Ql	239	239	35	35	41	–	0.02	0.22	0.29	0.25	0.28	0.32	0.22
	Q2	239	239	35	35	41	0.08	–	0.20	0.24	0.20	0.23	0.25	0.17
C allot	Ql	299	299	134	134	101	60	60	–	0.12	0.10	0.16	0.10	0.04
	Q2	299	299	134	134	101	60	60	0.03	–	0.11	0.07	0.19	0.12
l'Arcouest	Ql	367	367	202	202	169	128	128	68	68	–	0.06	0.14	0.06
	Q2	367	367	202	202	169	128	128	68	68	0.07	–	0.17	0.10
Saint-Malo	Ql	442	442	277	277	244	203	203	143	143	75	75	–	0.07
	Q2	442	442	277	277	244	203	203	143	143	75	75	0.085	–

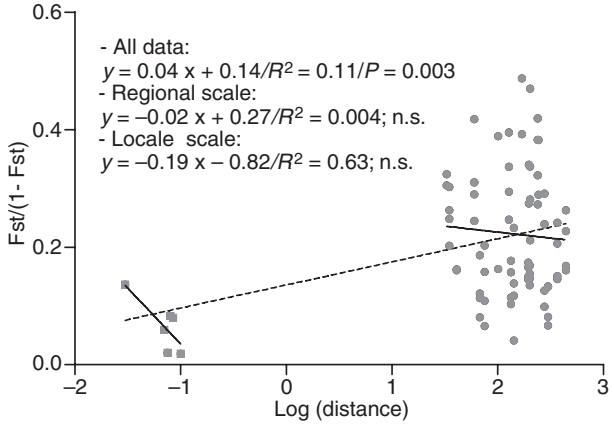

Fig. 2 Isolation-by-distance for *Z. marina*. The dashed line corresponds to the significant regression combining the two distance scales, indicating that pairs within a location are less distinct than pairs among locations. The left-hand full line corresponds to the regression with pairs within a location (local scale), and the right-hand full line to the regression with pairs among locations (regional scale).

slope = −0.19, $R^2 = 0.63$, n.s.; pairs among locations: slope = −0.002, $R^2 = 0.004$, n.s.), suggesting that distance may not be the predominant factor acting at the regional spatial scale.

The limitation to dispersal, as estimated through autocorrelation analysis, was indeed highly variable among meadows (for, an example, see Fig. 3). The value of *Sp* was significantly different from 0 for four out of 13 quadrats (quadrat 1 at Arradon, $Sp = 0.04$, $P < 0.001$; Arcouest, $Sp = 0.03$, $P < 0.001$; and Saint-Malo, $Sp = 0.02$, $P < 0.05$; and quadrat 1 at Roscanvel, $Sp = 0.02$, $P < 0.05$).

Network topology of Z. marina individuals

The effective percolation threshold (Dpe) was seen to be about 0.45 (data not shown), below which the network lost its integrity and the clusters broke down into two distinct clusters. On the global network just above this percolation threshold (Fig. 4) there is a first cluster composed of genets sampled in Molène and Sainte-Marguerite (on the top left part) and a second cluster of genets sampled the other localities. Within the giant cluster above the percolation point, as well as inside this secondary cluster emerging below it, the genets from Saint-Malo have a central position, along with the highest average value of *betweenness centrality* (<BC> = 0.0099) (global average value of BC is 0.0048, see Table 4 for network characteristics).

Network topologies at the fine-grained spatial scale (Fig. S1, Supporting Information) revealed two clusters for each location, related to the sampling quadrats, except for Roscanvel and Sainte-Marguerite, which showed clustering values that were half those of the four other locations (0.17 and 0.16 respectively vs. an average value of 0.32). It should be noted that no conclusion can be drawn about Molène as there was only one quadrat.

Discussion

Z. marina v. angustifolia: an ecotype rather than a protospecies?

Considered alternatively as an ecotype or as a distinct species, *Z. angustifolia* was first reported in the UK, the Morbihan Gulf and Arcachon Bay (France). The annual observations by the REBENT network also detected this

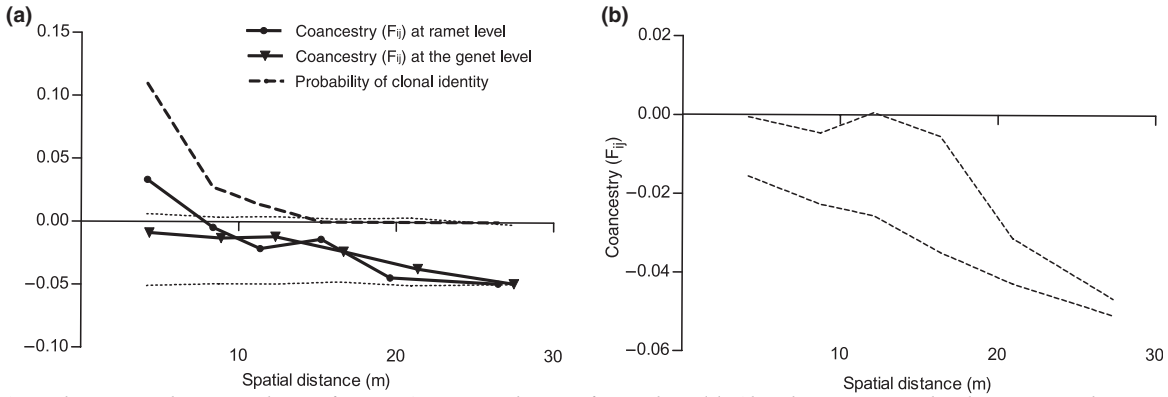

Fig. 3 Spatial autocorrelation analysis of *Z. marina* in quadrat 1 of Arradon. (a) Clonal structure and subrange. Kinship estimates from all ramet pairs (ramet level) or only for pairs between ramets showing a different multilocus genotype (genet level), and probability of clonal identity (proportion of pairs between ramets with identical MLGs), with confidence limits (for $P = 0.975$ and 0.025) based on 1000 permutations of spatial coordinates. (b) A single ramet per multiramet genet was randomly selected to create a 100-genet data file to generate the confidence limits for the correlogram.

Fig. 4 Network topology of the seven meadows of *Z. marina* studied, based on the Shared Alleles Distance between genets. Only links with distances smaller than or equal to the percolation threshold (Dpe = 0.45) are presented. For greater readability, nodes representing genets are not arranged according to their geographic coordinates. For each location, genets of quadrat 1 are represented by ellipses and genets of quadrat 2 by boxes. Colours correspond to sampling locations.

morph at Saint-Malo over a number of years. Its predominant occurrence in spring led us to consider it as an annual variety of *Z. marina* (Hily, personnal observation). No differences in ITS-sequence were observed between the variety *'angustifolia'* (sampling units from Saint-Malo) and the typical variety of *Z. marina* (SUS from other locations). The occurrence of private alleles was no higher in Saint-Malo than in the other locations (only two private alleles in the first quadrat). Also, pairwise F_{ST} values involving quadrats from Saint-Malo ranked among the average pairwise comparisons. Network analysis agreed with this result, showing a complete mixture of Saint-Malo MLLS with all other MLLS, and even highlighting a central position of genets from Saint-Malo (Table 4; Fig. 4). The values of *betweenness centrality* also suggest a higher genetic relatedness of genets from the meadow of Saint-Malo to those of most other locations. Our data therefore support *Z. marina v. angustifolia* in Saint-Malo as an ecotype, rather than a distinct species.

Table 4 Network values of Betweenness Centrality (BC) and Clustering coefficient (CC). Each value corresponds to one pair of quadrats from the same location. The BC column corresponds to the average value of location inside the global network; while the CC column corresponds to the average value of CC inside local networks

	BC(*100)	CC
Arradon	0.73	0.42
Roscanvel	5.93	0.17
Molene	5.03	0.33
Saint e-Marguerite	6.02	0.16
Callot	2.68	0.33
Arcouest	5.15	0.31
Saint-Malo	9.95	0.52
Average	4.9	0.32

It was not clear at this point in the analysis whether this morph with a cyclical 'bloom' arose due to the annual growing of shoots from persistent rhizomes or to annual episodes of germination of dormant seeds. The former seems to be a more likely explanation, considering the limited clonal diversity, high clonal subrange and heterozygote excess observed in Saint-Malo. These indices indeed tend to support the occurrence of large and persistent clones. In the neighborhood near the city (several tens of meters from the walls of Saint-Malo), the extreme exposure, together with a high level of local clam digging during equinox tides, suggests an increased anthropogenic influence on this meadow. The level of disturbance may therefore be high, allowing only the recruitment and persistence of specific genotypes able to cope with extreme conditions. Similarly, Diaz-Almela *et al.* (2007) highlighted an increase of CR in impacted populations of *Posidonia oceanica*, compared with reference non-impacted populations, and a higher resistance to disturbances resulting from fish-farming in meadows showing a high CR value. This suggests that large clones have a higher fitness, potentially conferring a competitive advantage for spatial colonization, and enhanced phenotypic plasticity (Diaz-Almela *et al.* 2007). This hypothesis is consistent with the excess of heterozygosity (in a scenario of heterosis), as well as with low clonal and allelic richness. Interestingly, *Z. marina v. angustifolia* is also generally observed in harsh conditions in the residual ponds within meadows of *Z. noltii* in Arcachon bay, France (Auby, *personnal communication*), where it is subjected to strong variations in temperature, salinity, pH and oxygen concentration during low tide. All these elements suggest that *Z. marina v. angustifolia* is an ecotype that is revealed above a perturbation threshold, leading to an extreme in the expression of the phenotypic plasticity of *Z. marina* that allows survival in such stressful and fluctuating environmental conditions. A further point of inquiry

would be to compare these two types in terms of resistance to 'wasting disease', a development of the slime-mold-like protist *Labyrinthula macrocystis* in *Zostera* leaves at various sites (Hily *et al.* 2002).

Clonal architecture and genetic variability

The clonal richness observed in Brittany is quite variable among sites but remains high (*R* ranges from 0.48 to 1) in comparison to previous studies (Reusch *et al.* 1999; Olsen *et al.* 2004). These values are comparable to those observed in the populations representing Brittany in the large biogeographic survey performed by Olsen *et al.* (2004) where the neighboring populations of Carantec and Morgat had R values of 0.54 and 0.90, respectively. The heterogeneity of clonal richness values for locations in Brittany indicates a notable variation in the pattern of investment in sexual vs. clonal reproduction at the regional scale.

Contrastingly, estimates of allelic diversity in the present study are strikingly different from those found by Olsen *et al.* (2004). A much higher allelic richness was consistently observed, ranging from 2.33 (quadrat 1 in Saint-Malo) to 9.67 (quadrat 2 in Sainte-Marguerite) alleles / locus (Table 2). Allelic richness evidenced here is comparable to, or even double, the highest values reported in the Wadden Sea (4.10 alleles per locus) that led these previous authors (Olsen *et al.* 2004) to consider this region as a hotspot of diversity for the species. The values are also at least comparable to, and sometimes strikingly higher than, those observed in the supposed center of origin located in the Northern Pacific (the mean of Â for this region reaches 5.89; from Olsen *et al.* 2004). The Wadden Sea–North Sea region exhibits a linear coastal distance equivalent to Brittany. Such a difference could be due to sampling strategy and scale, as the authors Olsen *et al.* (2004) took samples according to linear transects, a strategy that has been shown to be more prone to overestimate than underestimate diversity (Arnaud-Haond *et al.* 2007a). The higher values, which were observed consistently here, may therefore be attributable to the larger number of sampling locations analyzed. The sampling effort made by Olsen *et al.* (2004) was indeed greater in the region of the Wadden Sea (nine sampling areas) than in Brittany (two sampling areas), for which only one meadow was studied for allelic richness, potentially meaning that the sample in this previous study was not representative of the meadows at the scale of the Brittany coasts.

These new results reveal a hotspot of *Z. marina* genetic diversity in Brittany compared with other populations over the distribution range as a whole. Moreover, the discrepancy with the first estimates obtained from the neighboring location of Morgat points toward a possibly significantly heterogeneous distribution of genetic polymorphism at the regional scale. This was further confirmed by analysing the genetic structure at both regional and fine-grained spatial scales.

Mosaic pattern of genetic differentiation at regional and local scales

A rather highly structured pattern was revealed at both regional and fine-grained spatial scales. This significant and generalized differentiation was consistent even at the fine-grained spatial scale, among quadrats separated by less than 100 m, although these values tended to be smaller than those observed among locations (Table 3; Fig. 2). This was confirmed by network analysis, which showed the occurrence of two clusters in each location, corresponding to the two quadrats, except in Roscanvel and Sainte-Marguerite. F_{ST} values among quadrats agreed with this finding as they were also the smallest in these two locations. These results suggest a strong limitation to dispersal without a real pattern of gradual IBD, as shown by the lack of significance of the Mantel test at the regional scale.

Spatial autocorrelation analysis at the local scale allows a quantitative estimation of the spatial scale over which clonality affects the SGS (spatial genetic structure), as autocorrelograms performed at the ramet-level and at the MLL-level merge at the distance corresponding to the clonal subrange (Alberto *et al.* 2005). In agreement with the patterns of high SGS obtained when including all sampling units, the clonal subranges observed in Saint-Malo, Arradon, Roscanvel and Arcouest (Table 2) provide a minimal estimate of ten or possibly several tens of meters, as these estimates are confined to our sampling areas. This suggests that clonal propagation *via* rhizomatic elongation accounts for dispersal at the scale of up to several tens of meters, as previously reported for *Cymodocea nodosa* (Alberto *et al.* 2005) *Z. noltii* (Ruggiero *et al.* 2005) and *P. oceanica* (Arnaud-Haond *et al.* 2007b).

In the case of seagrasses, two modes of dispersal exist besides strict clonal elongation: (i) long distance dispersal *via* unrooted shoots, in species such as eelgrass that have easily breaking rhizomes (Harwell & Orth 2002; Hall *et al.* 2006; Orth *et al.* 2006) when exceptional climatic events such hurricanes possibly favor long distance dispersal (Kendall *et al.* 2004); and (ii) medium distance dispersal *via* seeds (Orth *et al.* 2006), with the formation of gas bubbles that adhere to the seed coat of *Zostera sp*, giving buoyancy (Churchill *et al.* 1985). These authors followed drifting seeds and reported a dispersal that may exceed 200 m, large enough to encompass distances similar to those among neighboring quadrats.

Considering these rather large estimates of dispersal potential and the lack of limitation to gene flow evidenced in nine quadrats, a relative genetic homogeneity may be expected at the local scale. Yet, SGS is detected in four quadrats and the genetic differentiation among quadrats of the same location is significant (Table 3) and appears clearly in network analysis (Fig. S1, Supporting Information). Such a combination of relatively high-dispersal potential and stronger or similar genetic differentiation at the very fine spatial scale compared to the regional one was previously described as the paradox of the chaotic genetic patchiness, and the pattern has been extensively reported for marine benthic invertebrates (Johnson & Black 1982; Watts *et al.* 1990; Johnson *et al.* 1993; Johannesson *et al.* 1995; Edmands *et al.* 1996; Arnaud-Haond *et al.* 2008), and fishes (Lacson & Morizot 1991; Hedgecock *et al.* 1994; Doherty *et al.* 1995). This pattern of genetic mosaic at both temporal and spatial scales may be explained by several hypotheses. Distinct origin or differential survivorship of recruits, as well as the 'sweepstake' hypothesis based on a differential reproductive success leading to instantaneous genetic drift (Hedgecock 1994), have been proposed. In the case of *Z. marina*, which is also a partially clonal species, other factors linked to the specific pattern of temporal recruitment and clonal growth are likely to be involved.

Recruitment dynamics and the concept of population

According to our results, dispersal does not balance the effect of genetic drift in eelgrass meadows. Three explanations can be advanced for this, the first being that (i) the hypotheses on which the estimates of autocorrelation patterns are based are not met, potentially leading to an overestimation of the dispersal potential. For example, the dispersion is assumed to be isotropic (i.e. equivalent in all directions of 2D space); this is a large assumption, particularly in coastal environments where current regimes are highly complex (Siegel *et al.* 2003). In cases where the conditions required to interpret spatial autocorrelation are met, this apparent discrepancy between expected and realized dispersal may be explained by (ii) low propagule production. This is not in agreement with the extreme clonal richness observed, which reveals an important implication of sexual reproduction in the quadrats of Roscanvel, Arcouest and Sainte-Marguerite. The third hypothesis (iii) is that of low recruitment success of dispersed propagules, possibly due to spatial competition exerted by already-established clones against drifting immigrant propagules. This hypothesis is consistent with previous studies, showing that recruitment in the sea may follow a chaotic distribution (Roughgarden *et al.* 1988) and that the more impacted areas in seagrass meadows (i.e. a lower density of shoots) exhibit greater recruitment success, probably due to a decrease in intraspecific competition (Reusch 2006). The influence of the outcompetition of migrants by some fitter clones is also supported by the observation of the highest level of clonal richness in the very recently (re)colonized meadow of Sainte-Marguerite.

Two dynamic strategies have been proposed for the settlement and growth of clonal plant meadows (Eriksson 1993): initial seedling recruitment (ISR) and repeated seedling recruitment (RSR). The colonization of an area results either mostly from the recruitment of an initial cohort occupying space through clonal growth (ISR) or from continuous colonization of patches (RSR). For a low level of environmental and demographic fluctuations, the predominant strategy may be ISR, due to the advantage for a seedling to be the first arrived and to acquire 'strength in number' by growing ramets to colonize space through clonal growth before another new recruit arrives. In this case of stable environmental conditions, and thereby demographic conditions, relatively low clonal diversity may also result from competitive exclusion of initially-settled clones, as suggested for species coexistence models (Huston 1979) and in the case of the dynamics of *P. oceanica* meadows (Arnaud-Haond *et al.* 2010). For an intermediate level of environmental and demographic fluctuations, which reduces the intensity of competitive exclusion, the number of free microsites favours the settlement of new recruits, thereby allowing the turn-over of patches and enhancing genotypic diversity, as described in the experimental approach by Reusch (2006). In such cases, the pattern of highest clonal diversity, and probably lowest genetic structure, would reveal the tuning of dynamic strategy toward RSR.

As for *Z. marina*, the range of clonal and genetic diversities at the regional scale therefore suggests that both strategies may apply in variable proportions depending on both the time elapsed since the last colonization and the levels of periodic disturbance in the meadows studied. As suggested for *P. oceanica* (Arnaud-Haond *et al.* 2007b), the heterogeneity of spatial and temporal patterns demonstrated here highlight a potentially serious limitation of the use of genetic differentiation as a tool to predict recolonization potential. Such results mean we should be cautious about drawing conclusions from genetic data alone in the absence of further ecological information about local adaptation and/or intra specific competition for space for example.

Finally, according to the genetic definition, the genetically differentiated quadrats of *Z. marina* would not be considered as belonging to the same population. Yet the pattern reported here leads us to question whether two quadrats belonging to the same continuous meadow at a distance of a few meters should be considered as

belonging to distinct populations. This population genetic concept was initially developed for species with exclusively sexual reproduction and may not be relevant for clonal organisms, as suggested by Bahri *et al.* (2009). The ecological population as defined by Camus & Lima (2002) ('a group of individuals of the same species that live together in a area of sufficient size to permit normal dispersal and/or migration behaviour and in which numerical changes are largely determined by birth and death processes'), based on their discrete distribution, may be a more objective concept for application to clonal organisms. It should however be noted that, in order to be meaningful in an evolutionary sense, such a concept would rely on the assumption that distance and fragmentation are the main *proxies* for assessing the efficiency of gene flow.

Conclusion

This is, to our knowledge, the first time that the detailed screening of within-meadow variance in clonal and genetic composition and differentiation has been performed. This work reveals:

- High heterogeneity of clonal and genetic diversities at the regional scale, and the possibility that Brittany (France) could be considered as a hotspot for the genetic diversity of *Z. marina* at the scale of the entire species distribution range.
- Strong genetic structure at regional scales revealing dispersal limitations that could potentially influence the future of *Z. marina* populations.
- Mosaic structure (genetic patchiness) at the local scale, supporting a RSR strategy that is likely driven by perturbations opening windows for recruitment.
- Large phenotypic plasticity, allowing *Zostera* development in a wide range of environmental conditions. As our results confirm the hypothesis that *Z. marina v. angustifolia* is an ecotype, this phenotypic plasticity is probably characteristic of highly stressful environments.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Network topologies of *Z. marina* genets at the local scale, based on the Shared Alleles Distance between genets. Only links with values smaller than or equal to the effective percolation distance (Dpe) are presented. Nodes representing genets of quadrat 1 are represented by ellipses and genets of quadrat 2 by boxes. The colour legend is the same as that used in Fig. 4. A, Arradon; B, Roscanvel; C, Molène; D, Sainte-Marguerite; E, Callot; F, Arcouest; and G, Saint-Malo.

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MOSAÏQUE GENETIQUE ET FLUCTUATIONS TEMPORELLES : DUALITE DE LA DYNAMIQUE DES CLONES ET DES PROPAGULES DISPERSANTES DANS LES PRAIRIES.

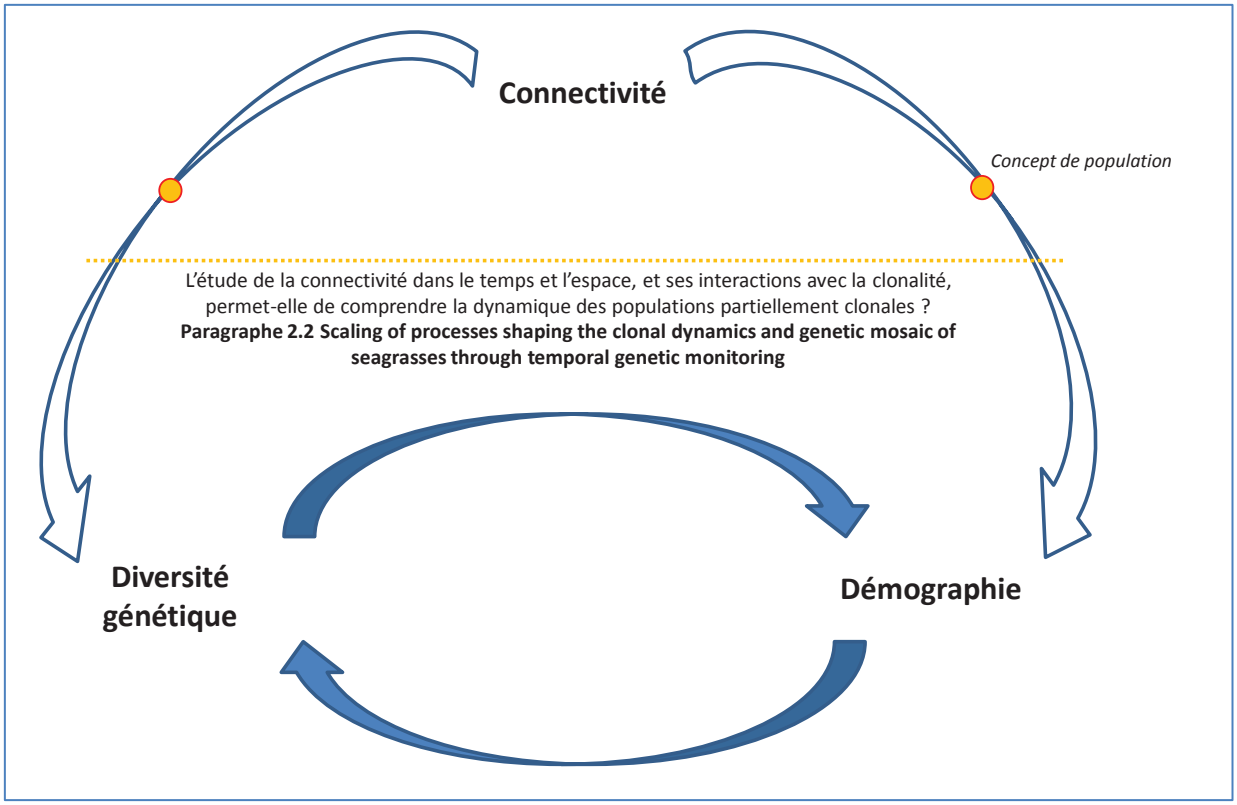

Figure 19 *Relation entre connectivité et clonalité dans le temps et l'espace*

Contexte & résumé

La structure génétique spatiale révélée dans le paragraphe précédent évoque un patron de mosaïques génétiques fluctuantes, fréquemment rapporté chez les invertébrés marins benthiques. L'émergence d'une structure se produit à une échelle spatiale inférieure aux capacités de dispersion de l'organisme considéré. Différentes hypothèses ont été avancées, pour expliquer ce patron contradictoire, basées sur la sélection ou sur des processus neutres. La sélection pré ou post-installation des propagules pourrait, dans le cas de conditions environnementales fluctuantes, générer une structure mosaïque dans le temps et l'espace. Si la sélection explique parfois une différentiation spatiale à certains locus, elle permet mal d'expliquer l'aspect multilocus de la « genetic patchiness ». Des processus neutres expliquent mieux cet aspect multilocus. Par exemple, l'hypothèse du tirage au sort (*sweepstake hypothesis*, dans la littérature), basée sur une forte variance du succès reproducteur, peut générer de la dérive génétique instantanée sur un évènement de reproduction&dispersion donné. Les différents nuages de propagules seraient alors génétiquement distincts. Un travail récent de Broquet *et al*, (2012) montre, par la modélisation, que

la « *genetic patchiness* » peut provenir des effets conjoints de la dérive génétique (petite taille efficace et succès reproductif différentiel) et de la dispersion collective de propagules apparentées (créant un ilot génétique à l'endroit où elles recrutent).

L'objectif de ce travail est de tester l'hypothèse nulle « la clonalité n'a pas d'effet sur les structures génétiques temporelles », dont le rejet permettrait de suggérer que la clonalité constitue un facteur favorisant l'émergence d'un patron de « genetic patchiness ». Pour cela, les herbiers de *Zostera marina* échantillonnés une première fois en 2009 ont été ré-échantillonnés trois ans après.

Les principaux résultats sont :

- une augmentation quasi-générale de la dominance clonale
- une structure génétique temporelle importante, excédant la structure spatiale.

Cette apparente contradiction a amené à proposer un modèle de dynamique des populations, basé sur la coexistence de clones éphémères (soumis à un fort turn-over dans le temps) expliquant la structure temporelle, et de groupes de clones persistants probablement responsables de la forte structure inter-patch.

Broquet, T., F. Viard, and M. Yearsley. 2012. Genetic drift and collective dispersal can result in chaotic genetic patchiness. Evolution **doi:10.1111/j.1558-5646.2012.01826.x**.

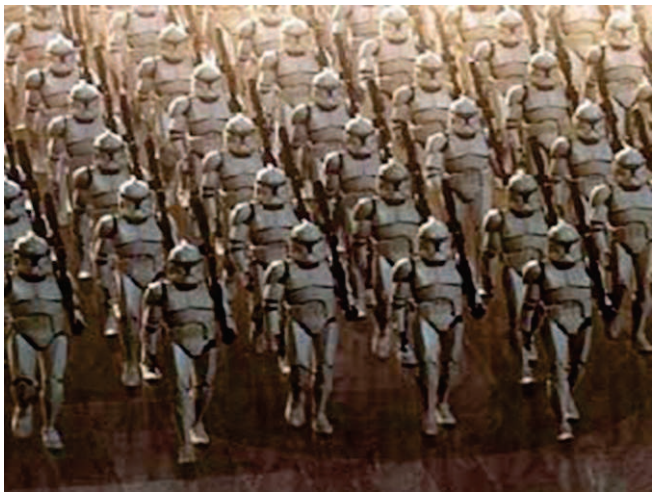

Figure 20 *La compétition entre lignées clonales est un mécanisme clé pour comprendre la dynamique clonale (un des 10 premiers résultats fournis par google-image, pour la requête « clone »).*

Heredity (2013), 1–8

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ORIGINAL ARTICLE

Scaling of processes shaping the clonal dynamics and genetic mosaic of seagrasses through temporal genetic monitoring

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Theoretically, the dynamics of clonal and genetic diversities of clonal plant populations are strongly influenced by the competition among clones and rate of seedling recruitment, but little empirical assessment has been made of such dynamics through temporal genetic surveys. We aimed to quantify 3 years of evolution in the clonal and genetic composition of *Zostera marina* meadows, comparing parameters describing clonal architecture and genetic diversity at nine microsatellite markers. Variations in clonal structure revealed a decrease in the evenness of ramet distribution among genets. This illustrates the increasing dominance of some clonal lineages (multilocus lineages, MLLs) in populations. Despite the persistence of these MLLs over time, genetic differentiation was much stronger in time than in space, at the local scale. Contrastingly with the short-term evolution of clonal architecture, the patterns of genetic structure and genetic diversity *sensu stricto* (that is, heterozygosity and allelic richness) were stable in time. These results suggest the coexistence of (i) a fine grained (at the scale of a 20×30 m quadrat) stable core of persistent genets originating from an initial seedling recruitment and developing spatial dominance through clonal elongation; and (ii) a local (at the scale of the meadow) pool of transient genets subjected to annual turnover. This simultaneous occurrence of initial and repeated recruitment strategies highlights the different spatial scales at which distinct evolutionary drivers and mating systems (clonal competition, clonal growth, propagule dispersal and so on) operate to shape the dynamics of populations and the evolution of polymorphism in space and time.
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Keywords: clonality; seagrass; spatio-temporal genetic structure; *Zostera marina*

INTRODUCTION

Clonality is a life history trait widely distributed among taxa and habitats, particularly in photosynthetic organisms. Partially clonal organisms are characterized by a mixed system allowing the combination of two reproductive strategies: the production of new genetically identical modules through vegetative growth or fragmentation and the production of new genetic individuals through sexual recombination. As a consequence, their population dynamics and evolutionary trajectories are profoundly affected by their rate and mode of clonal reproduction. Populations of clonal plants are composed of genetic individuals, or genets occupying space and dispersing locally through the production of modular shoots, or ramets (Harper, 1977). As genets are able to persist through time and space, the composition and evolution of populations of clonal plants is largely affected by the level of intraspecific competition (Eriksson, 1989, 1993; Pan and Price, 2001; Travis and Hester, 2005).

Depending on the turnover of genets and intensity of inter-genet competition for space, two extreme recruitment strategies have been defined (Eriksson, 1993): (i) the 'Initial Seedling Recruitment' (ISR) strategy, characterizing populations originating from a single event of colonization from one pool of seeds, followed by occupation of space, mostly through vegetative spread; and (ii) the 'Repeated Seedling Recruitment' (RSR) strategy, describing the continuous input of new genets.

In stable environments, ISR is expected to be the predominant strategy. Density-regulated populations are shaped by increasing competition among genets through time, the more ecologically competent excluding the less fit (Eriksson, 1989, 1993). This competitive exclusion among genets may theoretically result in a decrease of clonal richness through time, and is considered to be a major driver of the temporal evolution of clonal diversity (Soane and Watkinson, 1979; Eriksson, 1993; Watkinson and Powell, 1993). Such a strategy is similar to the mechanisms suggested by species coexistence models (Huston, 1979), and was proposed to describe the dynamics of meadows of the seagrass *Posidonia oceanica* (Arnaud-Haond *et al.*, 2010).

In contrast, a strategy closer to RSR is expected in areas that undergo frequent disturbances (Eriksson, 1993), where ecological events locally remove individuals and free microsites for new recruits. Populations of the seagrass *Cymodocea nodosa* have been proposed to persist due to an RSR strategy (Ruggiero *et al.*, 2005).

These strategies are two theoretical extremes observed in nature in proportions depending mostly on the species studied, and on the rate and intensity of environmental perturbations and demographic

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variations (Watkinson and Powell, 1993; Pluess and Stocklin, 2004; Douhovnikoff *et al.*, 2005). Their relative importance has strong implications for the evolution of populations and species, as spatio-temporal recruitment strategy affects both the preferential mechanism of space occupation and migration (clonal spread versus fragment or seed dispersal), and the predominant entity that persists will evolve under natural selection (the alleles or the clonal lineages, see Ayala, 1998). The pattern of spatial distribution of genetic and clonal diversities and their respective evolution in time will therefore be highly dependent on the balance between these two strategies. Numerous spatial studies have been carried out for diverse species, reporting highly variable levels of clonal richness that suggest a differential intensity between ISR and RSR strategies (Escaravage *et al.*, 1998; Coyer *et al.*, 2004; Olsen *et al.*, 2004; Pluess and Stocklin, 2004; Diaz-Almela *et al.*, 2007; Alberto *et al.*, 2008). The extreme and most demonstrative cases of ISR dominance are the monoclonal meadows of some seagrass species, including *Z. marina* (Reusch *et al*, 1999; Olsen *et al.*, 2004), *C. nodosa* (Alberto *et al.*, 2008) or *P. oceanica* (Arnaud-Haond *et al.*, 2007b, 2012). Meadows of the seagrass *Z. marina* surveyed simultaneously for their demographic evolution and genetic composition in Brittany were shown to be fluctuating mosaics of genetically differentiated patches with variable levels of genotypic and genetic diversity (Becheler *et al.*, 2010). The clonal and genetic composition of meadows suggested that both ISR and RSR strategies may apply in variable proportions as a function of the time elapsed since the last colonization and of the frequency and extent of disturbances (Becheler *et al.*, 2010). However, the thorough appraisal of the dynamics of these genetic mosaics and of the recruitment strategies operating at different spatial and temporal scales in natural meadows cannot be inferred from a single instantaneous snapshot. Such an approach requires the assessment of the temporal evolution of clonal and genetic composition of patches, an approach that has rarely been used on clonal organisms (see Hossaert-McKey *et al.*, 1996; Cronberg, 2002; Travis and Hester, 2005).

Here, we aimed to provide an estimate of short-term temporal variation in clonal and genetic composition at local and regional scales, in relation to the demographical changes recorded, particularly the time elapsed since the last recorded recolonization or major disturbance event. The seven meadows of *Z. marina* studied in 2009 were sampled again in 2012, following the same sampling strategy, and each ramet was genotyped using the same nine microsatellite markers. Our objective was to compare the spatial and temporal scales of evolution in clonal diversity and spread *versus* genetic diversity in order to infer the balance among recruitment strategies in natural meadows.

MATERIALS AND METHODS

Sample collection and field observations

Following the same sampling scheme and strategy as in Becheler *et al.* (2010), sampling units of *Z. marina* were collected in Spring 2009 and Spring 2012 from seven locations in Brittany, France (Supplementary Figure S1), stretching from Saint-Malo to Arradon. Distances between meadows ranged from 33 km (Molène–Roscanvel) to 442 km (Arradon–Saint-Malo). At each location, two 20×30 m quadrats separated by several tens of meters were chosen, which were located in continuous parts of the meadow being monitored by the REBENT survey (www.rebent.org), a national network of survey for major coastal ecosystems. Sampling units, corresponding to one ramet, were collected at 35 random coordinates within each quadrat, and their set of internal leaves were stored in silica crystals until DNA extraction. In Arcouest and Molène, due to patchiness of the meadow, the sampling area was not strictly $30 \times 20\,m^2$ because the total area covered by the patches had narrowed to less than this size. Deviation from random coordinate occurred when a gap was found on the field at the pre-identified coordinate (therefore corrected to the one of the closest existing ramet). The quadrat 1 of Arcouest was haphazardly sampled due to harsh meteorological conditions. In general, as only approximate GPS (global positioning system) positions were available together with visual cues, the quadrats for 2012 were placed as close as possible to the quadrat positions in 2009 but might have slightly shifted depending on the extent of the meadow and the moving of sediments. The estimated discrepancy between new and initial positions of quadrats was nevertheless estimated to be relatively weak and unlikely to exceed 10 m, except possibly at the Arcouest site, where the coastal landscape had significantly changed.

Microsatellite amplification and genotyping

Total DNA was extracted using the classic CTAB (cetiltrimetilamina) method (Doyle and Doyle, 1988). Using PCR conditions identical to those used by Becheler *et al.* (2010), the nine microsatellite loci used in 2009 were amplified in the present study. PCR products were visualized using the same ABI-3100 FNVR automated sequencer (Applied Biosystems, Life Technologies, Saint-Aubin, France), and scored using the microsatellite plugin of Geneious v5.6.4 (Biomatters, Auckland, New Zealand). Double-blind reading was used to minimize the occurrence of scoring errors and verify interpretation of the peaks.

To standardize the samples to the same number of sampling units ($n = 30$) before analyses, incomplete genotypes were removed and excess genotypes were randomly removed.

Genetic and clonal data analysis

The first step of the genetic analyses was the clonal discrimination, based on the probability that identical multilocus genotype (MLG) arise from distinct events of sexual reproduction, as described in Arnaud-Haond *et al.* (2007a). When $P_{sex(FIS)}$ (estimated taking departure from Hardy–Weinberg into account) falls below a threshold value fixed at 0.01, the two identical MLGs are considered as belonging to the same clonal lineage. The data set was also screened for the possible occurrence of scoring errors and somatic mutations, in order to avoid overestimates of clonal diversity. Multilocus lineages (MLLs) were defined as clustering slightly different MLGs likely belonging to the same genet, as detailed in Arnaud-Haond *et al.* (2007a) and in the Supplementary Information.

Clonal diversity was estimated with three parameters, previously used in Becheler *et al.* (2010) and recommended in Arnaud-Haond *et al.* (2007a), as this set fully describes richness and diversity of the clonal composition. R is the clonal richness, estimated as the ratio of the number of discriminated genets within the sampling set with the number of sampling units (Dorken and Eckert, 2001). The slope (β) of the Pareto distribution, describing the distribution of ramets within genets (see Arnaud-Haond *et al.*, 2007a for details), and sensitive to the presence or absence of dominant clonal lineages is also provided, as well as the Simpson's Index (Pielou, 1969), an index of diversity sensitive to dominant entities. Clonal discrimination and estimations of the parameters of clonal diversity and structure were performed with Genclone 2.1 (Arnaud-Haond and Belkhir, 2007).

A single copy of each discriminated MLL was retained in the data set used to assess genetic diversity and structure.

Genetic diversity within quadrats was estimated as the mean number of alleles per locus (Â), with observed (H_O) and unbiased (H_E) multilocus heterozygosity (Nei, 1978). A permutation procedure (1000 permutations) was used to test whether a particular estimate of the overall inbreeding coefficient (FIS) was significantly different from 0 ($P < 0.01$). Genetic structure among samples was estimated with θ (Weir and Cockerham, 1984) for 1000 permutations. This Fst-estimator was used to assess the spatial genetic structure among locations and between quadrats from a single location. In addition, a Mantel test including geographical distances among quadrats was carried out to test for the 2D-Isolation-By-Distance model, crossing the logarithm of the geographical distance (in kilometers) between quadrats and a derived index of genetic differentiation ($F_{st}/(1-F_{st})$), as recommended by Rousset (1997). For each sampling quadrat, this same estimator of F_{st} was also used on data sets gathered in 2009 (Becheler *et al.*, 2010) and 2012 (the present study) in order to assess the temporal genetic structure between identical sampling quadrats.

Autocorrelation analyses were also performed to estimate the evolution of the pattern of clonal extension and spatial genetic structure at the within-quadrat level. We used the kinship estimator coefficient of Ritland (1996) (F_{ij}) as a genetic relatedness statistic . We performed regression

analyses of mean F_{ij} against the Log_e of mean geographic distance within each distance class. The autocorrelation analyses were performed using F_{ij}, first including all sampled ramets and then using permutations (1000) in order to include only one ramet (and one of the possible corresponding coordinates, randomly chosen for each permutation step) from each genet at each permutation, in order to examine the dispersion through sexual propagules. The slopes of regressions (b) allowed us to calculate the S_p-statistic (Vekemans and Hardy, 2004). Autocorrelation parameter estimations were performed with GENCLONE 2.1 (Arnaud-Haond and Belkhir, 2007).

The clonal subrange (CR) was estimated that corresponds to the maximum distance between two identical MLGs belonging to the same clone, in meters, and is determined as the distance for which the probability of clonal identity becomes null (Harada *et al.*, 1997; Alberto *et al.*, 2005). It therefore provides an estimate of the minimal spatial extent of the largest observed clone in each quadrat.

The total number of genotypes occurring in the sampling quadrats in 2009 was approximated as follows: with R, the clonal richness (assessed from analyses), and with N_{tot}, the total numbers of ramets, within a quadrat (assessed using density counts in three subplots of 0.10 m^2 in the quadrat to estimate overall density across the entire surface of 30 * 20 m quadrats, see Arnaud-Haond *et al.* (2012) for a similar approach):

$$G_{tot} = R \times N_{tot}.$$

This allows an estimate of the expected percentage of each present MLG being sampled $\%G_{sampled}$, corresponding to the sampling density at the genotypic level:

$$\%G_{sampled} = (G/G_{tot}) \times 100$$

G being the number of MLGs detected in our sampling set of 30 ramets. This percentage estimate was used to appraise the likelihood of sampling the same MLG twice (both in 2009 and 2012). If R provides a reliable estimate of clonal richness, such percentage is extremely low, owing to the very low sampling density. Despite such low percentage, the repeated observation of persistent MLG sampled both in 2009 and 2012 suggests an overestimation of clonal richness through R estimates. This would imply the occurrence of a large dominance of space occupation by few clonal lineages, as showed in both seagrass species *P. oceanica* and *C. nodosa*, and confirm the consistent overestimation of clonal richness estimates based on extremely low sampling densities as usually performed in clonal plants (Arnaud-Haond *et al.*, 2007a).

Network analysis

Network analysis was performed on the basis of the totality of genets in the data set ($n = 289$) to compare the shape and properties of the networks in 2009 (Becheler *et al.*, 2010) and 2012 (the present work) on both local and regional scales. Nodes in the network represent genets, whereas links represent the genetic distance between two genets.

The genetic distance used is the 'Shared Allele Distance' (Chakraborty and Jin, 1993) based on the proportion of shared alleles between two individuals. It is estimated by:

$$P_{SA} = \frac{1}{2n_u}\sum_u S,$$

where the number of shared alleles S is summed over all loci u, and n_u is the number of loci.

Distance between individuals D_{SA} ranges from 0 to 1:

$$D_{SA} = 1 - P_{SA}$$

A 'fully connected' network was built, including all links among all genets, and then scanned to the percolation threshold (Stauffer and Aharony, 1994) as previously done for this type of data (Rozenfeld *et al.*, 2007; Becheler *et al.*, 2010; Moalic *et al.*, 2011). This method aims to analyze network topology at the minimal genetic distance, allowing gene flow to spread throughout a giant network. Under the effective percolation distance, a giant network collapses into smaller isolated clusters. Occasionally, when the system is not fully hierarchically structured, several nodes will prematurely disconnect and stand-alone outside of the giant cluster. This happens when genets are particularly genetically distinct (and therefore distant) from all other genets of the system. Only when a secondary cluster emerges, made of several nodes (genets), is the effective percolation threshold reached. The percolation threshold reveals the first significant level of limitations to gene flow within the system. Only links just above the effective percolation distance were used to analyze the topology and features of the network. The clustering coefficient C_i of genet i is the ratio between the number of existing links to the maximal number of potential links within the cluster. The clustering of genets reveals the existence of substructures, grouping the closest genets. It is defined as:

$$C_i = \frac{E_i}{E_i^{(max)}} = \frac{2E_i}{k_i(k_i-1)}$$

where E_i is the number of links existing among the neighbors of a given genet i, and the degree k_i of genet i is the number of other genets linked to it. The clustering coefficient of the whole network $\langle CC \rangle$ is defined as the average of all individual clustering coefficients in the system. Topologies and features of the current network were also compared with the network obtained in 2009. In order to visualize finer structures, the same methodology was performed to build a network for each location.

RESULTS

Short timescale variation of clonal diversity and architecture

All replicates of identical MLG showed a significant P_{sex} ($P < 0.01$) supporting the hypothesis they belonged to the same genet, and were therefore issued of a single event of sexual reproduction. In a single instance, two slightly different MLG were found to be likely derived from a single event of reproduction and differed at only one allele as a product either of somatic or of *in vitro* mutation, both MLG were merged into a single MLL (Arnaud-Haond *et al.*, 2007a) and considered as a single genet for further analysis.

Clonal diversity is variable in time depending on quadrat more than site (Table 1; Figure 1). In 2009, the mean clonal richness R was 0.76 with a variance of 0.03, ranging from 0.48 to 1. In 2012, the mean clonal richness had remained rather steady (0.71) with lower variance (0.01). The least and most diverse quadrats remained the same, respectively, Molène ($R_{2009} = 0.48$; $R_{2012} = 0.51$) and the quadrat 1 of Roscanvel ($R_{2009} = 1$; $R_{2012} = 0.89$). On the overall data set, the mean clonal richness of 2009 and 2012 are not significantly different (Wilcoxon test: $W = 102$; $P = 0.38$).

The Simpson Index values, D*, were very stable between 2009 and 2012 (Table 1), and no significant evolution was observed between 2009 and 2012 (Wilcoxon test: $W = 101.5$; $P = 0.39$). Contrastingly, the β-parameter of the Pareto distribution was the most variable of the descriptive parameters estimated (Table 1; Figure 1), and its quasi unidirectional evolution showed a consistent decrease of between 31 and 93% (except in Arcouest, quadrat 1, where there was an increase of 38%). The percentages of variation of β and the CR are highly correlated ($R^2 = 0.77$; $P < 0.01$; negative slope), indicating that an increase in the size of genets (CR) is related to reduced evenness in the distribution of ramets among genets (reflected by the β-parameter of Pareto). In the overall data set, the mean values of the β-parameter of 2009 and 2012 were significantly different (Wilcoxon test: $W = 139$; $P = 0.006$), indicating a consistent increase in clonal dominance.

In five quadrats of the 13 sampled, despite the possible shift in coordinates between dates and the low sampling densities, common MLGs were found in 2009 and 2012, with all P_{sex} values being < 0.001. One to six shared clonal lineages were found in five quadrats (Table 1). Assuming R provided a reliable estimate of genotypic richness (see Arnaud-Haond *et al.*, 2007a), the total number of genotypes expected to occur in each quadrat was estimated to be between 45 900 in Molène and 34 4172 in the quadrat 1 of Saint-Malo, resulting in a sampling coverage of 0.01–0.04% genotypes (Supplementary Table S1).

For most of the locations, the shoot density was found to be relatively stable between 2009 and 2012 (Supplementary Table S2).

Table 1 Parameters describing clonal structure and genetic diversity in 2009 and 2012

Locations	*Quadrat*	*Year*	*Clonal structure*								*Genetic composition*			
			N	*G*	*R*	*D**	*β*	*Sp*	*CR*	*Gsh*	*He*	*Ho*	*Fis*	*Â*
Arradon	1	2012	30	21	0.68	0.98	2.05	**0.02**	9.7	0	0.5	0.51	−0.02	4.6
		2009	30	26	0.86	0.99	3.10	**0.04**	5.6		0.52	0.51	0.03	5.89
	2	2012	30	22	0.72	0.98	1.45	0.00	13.0	2	0.5	0.47	0.05	4.66
		2009	30	21	0.69	0.97	2.09	0.00	14.6		0.54	0.52	0.03	6.00
Roscanvel	1	2012	30	27	0.89	0.99	2.14	0.01	6.4	0	0.47	0.44	0.06	4.44
		2009	30	30	1.00	1.00	4.95	0.00	0.0		0.52	0.51	0.01	4.78
	2	2012	30	22	0.72	0.97	1.45	0.003	5.0	1	0.52	0.56	−0.07	4.44
		2009	30	21	0.69	0.97	2.40	0.02	12.0		0.5	0.52	−0.05	4.11
Molène	1	2012	30	16	0.51	0.86	0.48	0.02	11.9	6	0.4	0.46	**−0.14**	3.22
		2009	30	15	0.48	0.85	1.36	—	—		0.4	0.40	0.01	3.44
Sainte–Marguerite	1	2012	30	20	0.65	0.97	1.32	0.02	11.2	0	0.48	0.52	−0.08	4.33
		2009	30	28	0.93	0.99	3.97	0.00	2.5		0.47	0.49	−0.04	5.00
	2	2012	30	24	0.79	0.99	2.05	0.01	6.0	0	0.5	0.46	**0.09**	5.00
		2009	30	28	0.93	0.99	4.01	−0.01	3.0		0.55	0.57	−0.04	5.55
Callot	1	2012	30	23	0.75	0.97	1.42	**0.04**	14.0	0	0.43	0.46	−0.06	4.33
		2009	30	26	0.86	0.99	2.89	0.00	5.3		0.46	0.42	**0.08**	5.78
	2	2012	30	17	0.55	0.96	1.11	**0.02**	18.9	0	0.44	0.47	−0.06	4.33
		2009	30	23	0.76	0.98	3.00	0.01	7.8		0.45	0.44	0.03	4.89
l'Arcouest	1	2012	30	23	0.75	0.98	2.02	—	—	0	0.41	0.44	**−0.09**	4.44
		2009	30	19	0.62	0.91	1.46	—	17.0		0.41	0.46	**−0.13**	4.11
	2	2012	30	19	0.62	0.84	0.34	0.01	19.1	0	0.33	0.34	−0.04	3.11
		2009	30	30	1.00	1.00	4.95	**0.03**	0.0		0.4	0.45	**−0.12**	3.89
Saint-Malo	1	2012	30	26	0.86	0.98	—	0.01	2.7	1	0.36	0.38	−0.07	4.11
		2009	30	19	0.62	0.95	2.05	0.02	18.6		0.29	0.35	**−0.21**	2.33
	2	2012	29	19	0.64	0.95	0.96	**0.05**	10.3	5	0.31	0.35	**−0.13**	2.77
		2009	30	15	0.48	0.89	1.54	0.01	10.2		0.4	0.39	0.04	3.56

Abbreviations: CR, clonal subrange in meters; D* and ED*, Simpson index and its equitability index; β, slope of Pareto distribution; G, number of identified MLLs; Gsh, number of clones shared between 2009 and 2012; He, expected heterozygosity without bias (Nei 1978); Ho, observed heterozygosity; MLL, multilocus lineages; R, clonal richness; Sp, statistic of spatial autocorrelation. Samples were standardized at 30 ramets. Parameters of genetic composition: the two parameters we assessed were heterozygosity and allelic richness. FIS-values were estimated after 1000 permutations of alleles within the quadrat. The mean number of alleles per locus Â was also estimated. Bold values indicate the significance of related parameters ($P<0.05$).

It should be noted that a full colonization event had happened between 2008 and 2009 in quadrat 2 of Sainte-Marguerite, offering the opportunity to follow the first year of clonal structure evolution, which could have affected the fluctuation in density.

Temporal variation of genetic composition of quadrats

Genetic diversity descriptors showed more stable patterns through time than clonal diversity ones (Table 1; Figure 1). The mean heterozygosity was steady with 0.46 in 2009 and 0.45 in 2012, associated with a constant variance (0.004 in 2009 and 2012). No significant differences in heterozygosity were found between 2009 and 2012 (Wilcoxon test: $W=98$; $P=0.50$). Similarly, values of allelic richness of 2012 are not significantly different from values of 2009 (Wilcoxon test: $W=104.5$; $P=0.32$).

Spatio-temporal genetic structure and differentiation among and within populations

Results in 2012 were similar to those observed in 2009 at local and regional scales. Spatial genetic structure was observed at all scales (Supplementary Table S3), all F_{st}-values being significantly different from 0 except between the two sampling quadrats of Arradon. This reveals a strong genetic differentiation on different spatial scales: from the regional scale, where F_{st}-values vary between 0.01 and 0.35, to the local scale, where the differentiation between the two quadrats produces F_{st}-values varying from 0 to 0.05. Temporal F_{st}-values were all significant and were systematically higher than inter-quadrat F_{st}-values (Table 2) ranging from 0.04 to 0.18.

A Mantel test carried out among all pairs of quadrats was significant ($R^2=0.06$; $P<0.05$), presenting a pattern very similar to the one observed in 2009. Yet, as explained in Becheler *et al.* (2010), this cannot indicate a strict IBD pattern, as the hierarchical sampling, resulting in two clouds of dots, drives this apparent tendency (Supplementary Figure S2). No such correlation was observed within each of the clouds corresponding to the pairs of quadrats from the same location ($R^2=0.03$; $P=0.73$) or to pairs of quadrats among locations ($R^2=0.001$; $P=0.71$).

Network analysis

The global network topology built on the base of genets in 2012 (Figure 2) was highly similar to the one obtained in 2009, with

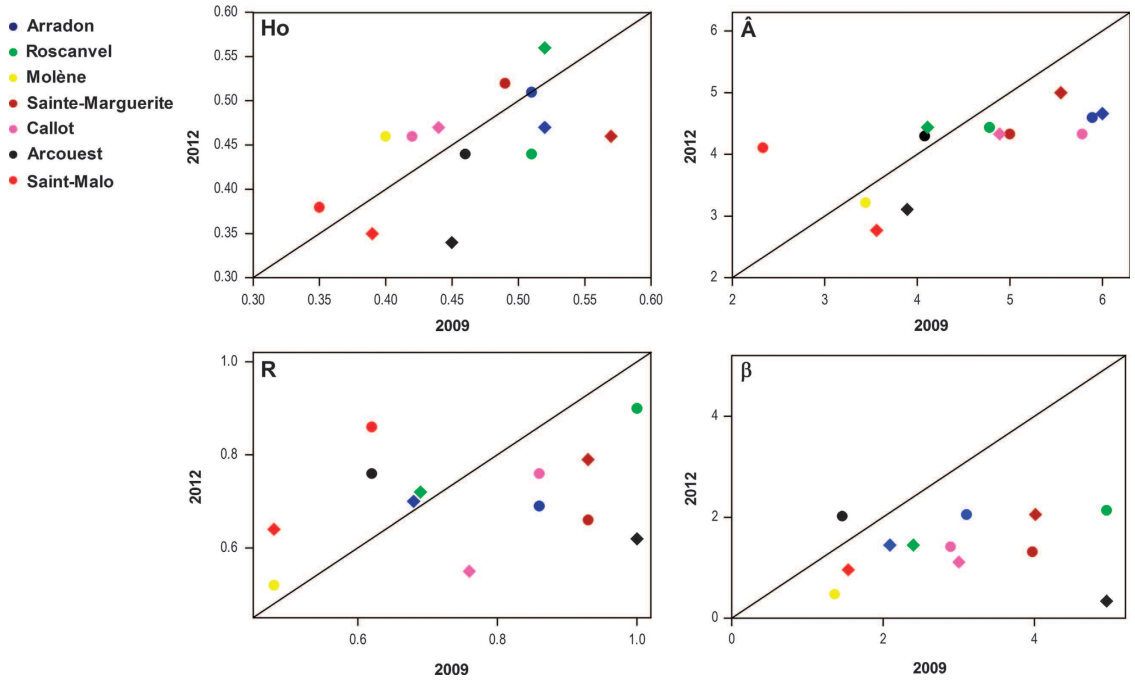

Figure 1 Diagrams of the temporal evolution of intraspecific diversity, for the observed heterozygosity (Ho), the mean number of alleles (Â), the clonal richness (R) and the β of Pareto's distribution, for each quadrat. *x* and *y* axes represent values from 2009 and 2012, respectively. For each site quadrat 1 is shown by a circle and quadrat 2 by a diamond.

Table 2 Genetic differentiation in space (between quadrats from the same location, inter-quadrat Fst) and in time (temporal Fst)

Locations	*Temporal Fst (2009 vs 2012)*	*Inter-quadrat Fst (2009)*	*Inter-quadrat Fst (2012)*
Arradon Q1	0.15***	0.08***	0.00
Arradon Q2	0.04***		
Roscanvel Q1	0.13***	0.02*	0.01*
Roscanvel Q2	0.10***		
Molène	0.11***	—	—
Sainte-Marguerite Q1	0.09***	0.01	0.04***
Sainte-Marguerite Q2	0.01*		
Callot Q1	0.18***	0.12***	0.01
Callot Q2	0.08***		
Arcouest Q1	0.10***	0.06**	0.02*
Arcouest Q2	0.12***		
Saint-Malo Q1	0.17***	0.07**	0.05***
Saint-Malo Q2	0.14***		

Fst-values were calculated after 1000 permutations (Weir and Cockerham, 1984). *$P<0.05$; **$P<0.01$; ***$P<0.001$.

identical percolation threshold (Dpe = 0.45). We observe the same organization with three central locations (Callot, Arcouest and Saint-Malo) and four peripheral ones (Arradon, Roscanvel, Molène and Sainte-Marguerite). The clustering coefficient 〈CC〉 was slightly higher than that recorded in 2009 (0.37 vs 0.30), suggesting an increase in the hierarchical differentiation among clusters (mainly corresponding to sets of samples within each location).

In order to see whether this result was because of an increase in differentiation at the regional or local scale, networks were also constructed at the levels of localities. This analysis also revealed an increase in clustering within every locality except for Saint-Malo (0.43 in 2012 vs 0.52 in 2009). Slight increases of a few percent were observed in Arradon and Roscanvel (+1 and +2%, respectively) and strong increases were observed in Molène and Callot (+34% for both), Sainte-Marguerite (+53%) and Arcouest (+47%). All values of 〈CC〉 are given in the Supplementary Table S4. In addition, no clusters of MLG corresponding to quadrats delineation are observed in 2012, in contrast to what was observed for most of the locations in 2009.

DISCUSSION

The snapshot of spatial genetic structure previously recorded at different scales suggested the existence of temporally fluctuating mosaics of genets shaping patches within meadows (Becheler *et al.*, 2010). This temporal survey aims to confirm this hypothesis. The comparison of clonal architecture and genetic composition between 2009 and 2012 provides insight into the possible dual dynamics of the meadows. First, an increasing dominance of large genets was observed, indicating the existence of a core of stable genets. This contrasts with the strong temporal genetic structure between the 3 years of monitoring, suggesting the concomitant occurrence of a turnover of genets. In the light of the recent demographic history of the quadrats, we propose a scenario explaining the evolution observed.

Prevalence of the ISR strategy: toward the emergence of large clones

Evenness of clonal distribution decreased in the large majority of quadrats (Table 1), whereas the CR showed an overall increase. These points highlight an increased dominance of large MLLs. The large MLLs varied in size, reaching at least 19 m in two quadrats (Table 1), and revealed the persistence of a stable core within the seagrass meadows, implanted for several years or decades, as horizontal rhizome elongation rate for *Z. marina* is estimated between 22 and 31 cm per year (Marba and Duarte, 1998). In addition, genets that

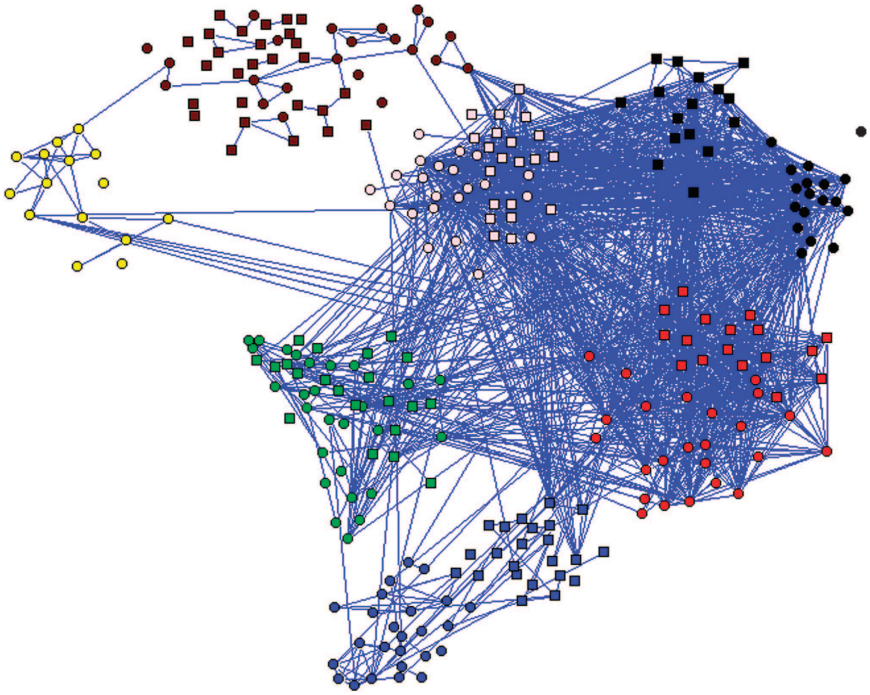

Figure 2 Network topology of the seven meadows of *Z. marina* based on the Shared Allele Distance between *genets*. Only links with distances smaller than or equal to the percolation threshold (Dpe = 0.45) are presented. For greater readability, nodes representing *genets* are not arranged according to their geographic coordinates. For each location, *genets* of quadrat 1 are represented by circles and *genets* of quadrat 2 by boxes. Colors correspond to sampling locations, according to the legend of Figure 1.

persisted in time were revealed by the resampling of identical genets across a 3-year period. Considering the low density of sampling and the low percentage of genotypes covered by our analysis, repeated sampling of some genets in time implies a rather strong pattern of clonal dominance. This dynamic of persistence and increased dominance of clones competing for space is exemplified by the analysis of quadrats showing stable density after a recent colonization, for example, in Sainte-Marguerite (Q2), where a decrease in clonal richness and evenness of respectively 15 and 49% was accompanied by an almost doubled CR. A very similar scenario occurred in the two quadrats of Callot, where a large increase of density occurred in 2008 (Supplementary Table S2), constituting a potential high input of new genets and leading to an increase in clonal richness for 2009. These elements highlight the prevalence of an ISR strategy (Eriksson, 1993).

A similar study was performed on a meadow of the terrestrial clonal plant *Lathyrus sylvestris*, studied in 2 successive years (Hossaert-McKey *et al.*, 1996), where a similar pattern of clonal dominance fitted with a predominantly ISR strategy (Figure 3a). In contrast to the present work, a large spatial but low temporal genetic structure was observed and interpreted as the result of successive colonizations at the edge of the population having formed mosaics of genets: (i) colonizing space through vegetative elongation and (ii) producing seedling through reproduction among flowers of the same genet or its relatives, recruiting closed to the maternal plants (Hossaert-McKey, 1988; Hossaert-McKey and Jarry, 1992; Hossaert-McKey *et al.*, 1996).

This pattern bears some similarity to the evolution through time of stable cores of large genets reported here, revealing a strategy of ISR and trimming of genets through the differential capacity of spatial spread and possible intraspecific competition in *Z. marina* meadows. Under relatively stable demographic conditions, differential clonal spread would therefore have a major role in shaping the clonal composition and differentiation of quadrats separated by only several tens of meters, as previously suggested by Becheler *et al.* (2010). The temporal component is, however, strikingly different in these two studies, whereas in case of *L. sylvestris*, the temporal differentiation was less marked than the spatial one, results show the opposite in *Z. marina* meadows. Such a contrasted pattern may be attributed to the contrasted mechanisms and dynamics of dispersal in the sea compared with on land. This leads us to propose a slightly different scenario (Figure 3b) to explain our findings, taking into account the large dispersal potential of seagrasses through drifting shoots or seeds in the marine environment (Harwell and Orth, 2002; Kendrick *et al.*, 2012).

A cloud of transient genets revealed by the temporal aspect of genetic patchiness

Reported in various marine taxa (Johnson and Black, 1982; Jones *et al.*, 1999; Arnaud-Haond *et al.*, 2008; Selkoe *et al.*, 2010; Hedgecock and Pudovkin, 2011), genetic patchiness is a paradoxical combination of high dispersal potential and strong genetic structure at local scales, characterized by three main features: (i) a fine-grained genetic structure comparable to or apparently exceeding that observed at a large scale; (ii) the fuzziness of population contours; and (iii) rapid temporal variations. The two first criteria were already met in for *Z. marina* meadows in Brittany (Becheler *et al.*, 2010) as well as in San Francisco Bay (Ort *et al.*, 2012), underlining the importance of clonality in favoring fine-grained genetic structure and spatial patchiness in organisms with mixed mating systems. Here, the temporal genetic structure exceeding the spatial one (Table 2) underlines a rather fast modification of genetic composition of patches, which is in line with the third characteristic describing genetic patchiness. The strong temporal F_{st} observed in this study emphasize

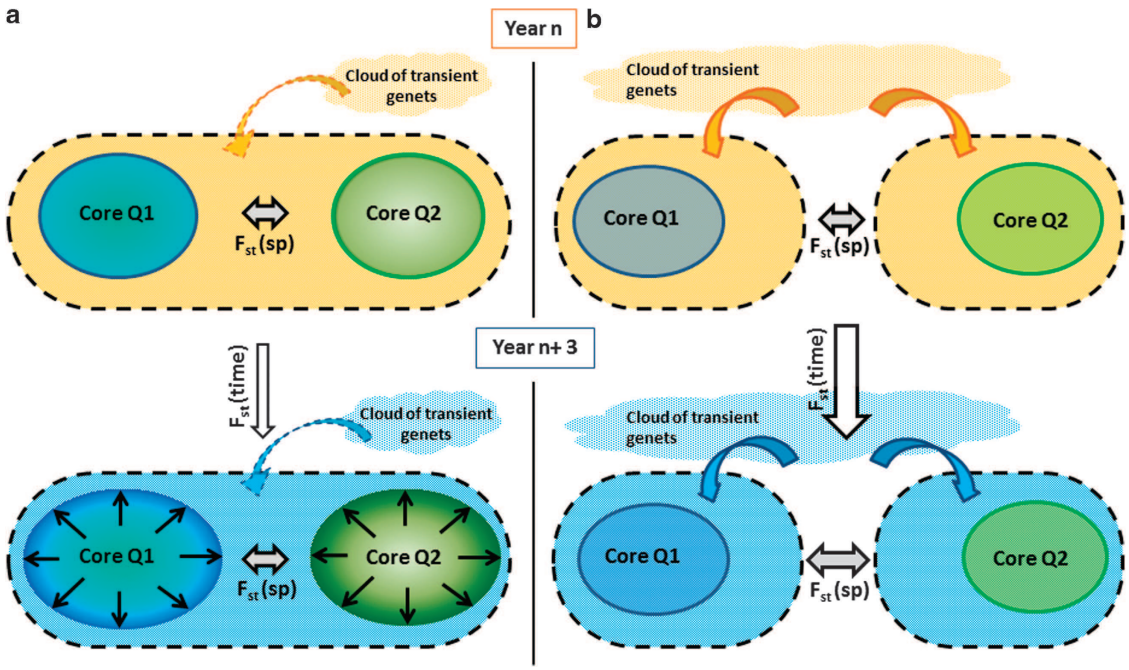

Figure 3 Conceptual illustration of scenarios proposed for explaining the spatio-temporal genetic structure. (**a**) Representation of the scenario described in Hossaert-McKey *et al*. (1996). (**b**) Representation of the scenario proposed in the discussion, which fits better with our results. The dual composition of the meadows (or sampling quadrats) contains cores of persistent genets (blue and green circles) and clouds of transient genets (orange and pale blue layouts, for 2009 and 2012 respectively). The divergence between cores is probably because of difference of genets having colonized both quadrats at the last event of drastic extinction, generating spatial structure. Annual migration and settlement provide pools of transient genets, of short lifespan, generating the temporal structure. In the scenario **a**, the flow of new genets is weak (weak temporal structure), and colonization of gaps is probably due to clonal elongation and mating among relatives (strong spatial structure). In the scenario **b**, annual input of transient genets is strong, maximizing the temporal structure.

the equal importance of dispersal and settlement of 'non-dominant clones', and lead us to hypothesize that a second compartment of genets could exist, subject to turnover within short time periods and generating significant variation in the genetic composition of quadrats through time (temporal F_{st}, Table 2).

Independent of longer-term meadow stability, the dynamics of *Z. marina* meadows are punctuated by annual variations in density, with a rather synchronized peak of recruitment of new ramets in early spring, filling gaps left by an annual winter drop in density (Marba *et al.*, 1996). Spring increase in density can be caused by the local clonal spread of genets that have persisted through winter, or new waves of recruits through ramet and seedling dispersal/settlement over broader spatial scales. Although the existence of persistent and widely spread genets reported here supports the first of these possible explanations, the temporal pattern of differentiation also suggests the co-occurrence of the latter. An annual cloud of recruits may disperse in synchronized waves settling over spatial scales encompassing both quadrats, tending to bring quadrats genetically closer. This likely annual turnover of transient genets illustrates the occurrence of a parallel RSR strategy (Eriksson, 1993) in *Z. marina* meadows. Following a typical pattern of genetic patchiness, the clouds of recruits may originate from a limited pool of plants or patches in the meadow and differ among years, therefore explaining the large temporal differentiation observed among quadrats. A recent work (Broquet *et al.*, 2012) analytically demonstrates that 'chaotic patchiness can be produced by neutral demographic processes alone', including collective dispersal (Selkoe *et al.*, 2006) and genetic drift. This is well illustrated by the successive recruitment events proposed to explain the empirical results reported here.

CONCLUSION

This study provides the first short-term temporal assessment of the dynamics of clonal and genetic composition of seagrass meadows at fine-grained, local and regional scales. Results revealed a typical pattern of genetic patchiness, with small-scale genetic structure despite large-scale dispersal potential, and high temporal differentiation. Some mechanisms underlying this observation appear slightly different from those of other marine species, however, as clonal growth and differential fitness of genets appear, as for terrestrial clonal plants, to be the root of the fine-grained differentiation observed between quadrats within meadows. At fine-grained scale, results support an ISR Strategy and the temporal persistence of part of the genetic pool. We therefore propose a scenario involving two compartments of genets to explain the results reported here (Figure 3b). The first compartment is a core of persistent and growing genets (ISR) contributing to the spatial differentiation within meadows, which may either result from a different time or source of colonization or from genetic divergence through differential exclusion of genets in the distinct quadrats. The second compartment is an annual cloud of transient genets, mostly alternating on an annual basis (RSR); although data reported here does not exclude the possibility that some of those genets would occasionally succeed on the longer term and finally contribute to the persistent core. These scenarios imply a central role on the frequency of perturbations and clonal life history traits, in particular, spatial spread and selection acting on clonal lineages, in the evolution of the clonal and genetic composition of meadows. These observations confirm the ecological concept of population based on distribution continuity as best adapted to clonal plants (Becheler *et al.*, 2010), but suggest the possible existence of a spatial scale larger than sampling

sites, and within which allelic frequencies may remain stable in time. These results underline the importance of addressing distinct interlocked spatial and temporal scales simultaneously in order to gain understanding of the dynamics of a population system as a whole. This is particularly important in those seagrass meadows apparently following permanent non-equilibrium dynamics at a local scale, possibly combined with long-term stability at a global scale.

DATA ARCHIVING

Data deposited in the Dryad repository: doi:10.5061/dryad.1vp70.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies this paper on Heredity website (http://www.nature.com/hdy)

Erratum

Les valeurs de F_{is} des quadrats de Sainte-Marguerite, Arcouest et Saint-Malo, pour l'année 2009, diffèrent entre les deux articles précédents. Les valeurs correctes sont consignées dans l'article publié dans *Heredity*. Une demande de publication d'erratum a été effectuée auprès de l'éditeur de *Molecular Ecology*.

Dans l'article de *Molecular Ecology*, des signes négatifs pour les quadrats d'Arcouest et Saint-Malo ont disparu lors de la mise en page finale. Les valeurs de diversité génétique et d'écart à l'équilibre d'Hardy-Weinberg des quadrats de Sainte-Marguerite (en 2009) ont été corrigées pour le suivi temporel, suite à la découverte tardive d'erreurs de génotypage.

CHAPITRE 3. ETUDE DE L'INFLUENCE DE LA DIVERSITE GENETIQUE *SENSU STRICTO* SUR LA STABILITE DEMOGRAPHIQUE DES POPULATIONS NATURELLES DE *ZOSTERA MARINA*

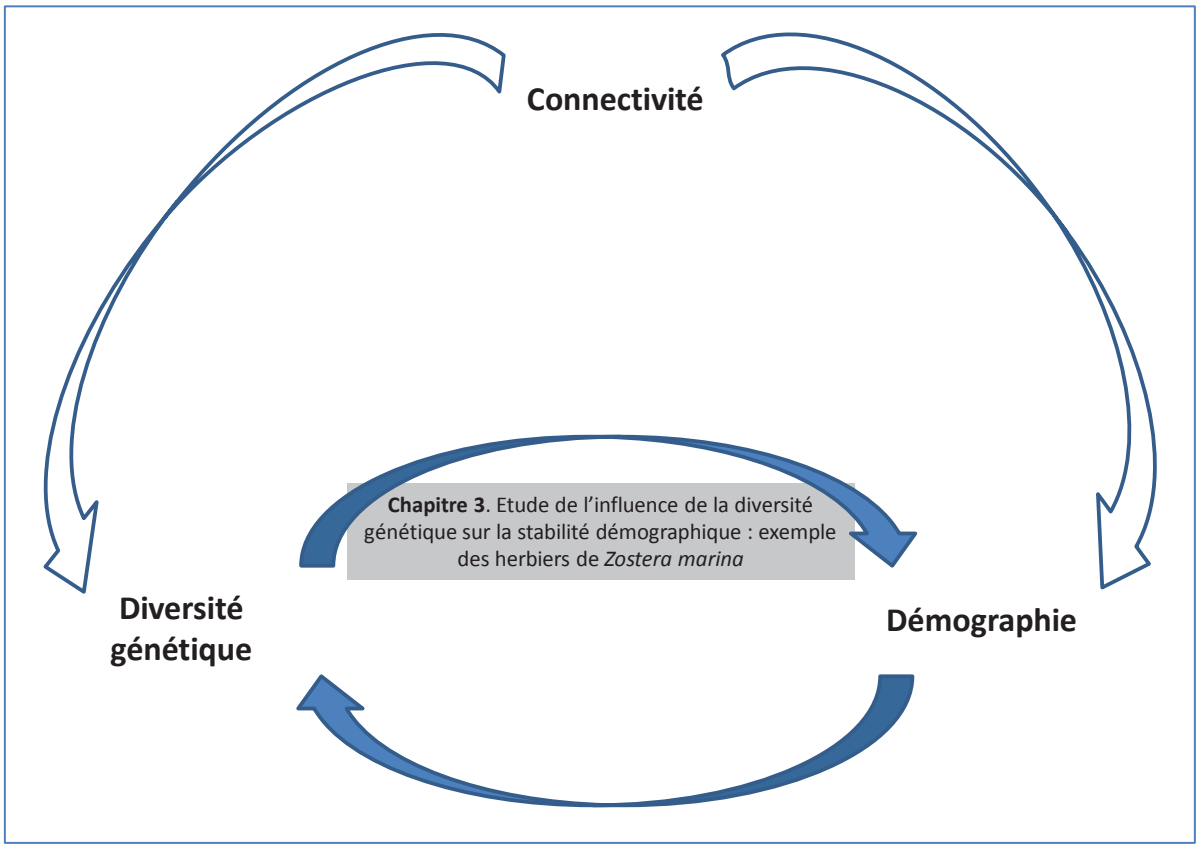

Figure 21 *La diversité génétique a-t-elle un effet positif sur la stabilité démographique de populations naturelles ?*

LA DIVERSITE GENETIQUE INFLUENCE-T-ELLE LA CAPACITE DE RESISTANCE DANS LES POPULATIONS NATURELLES ?

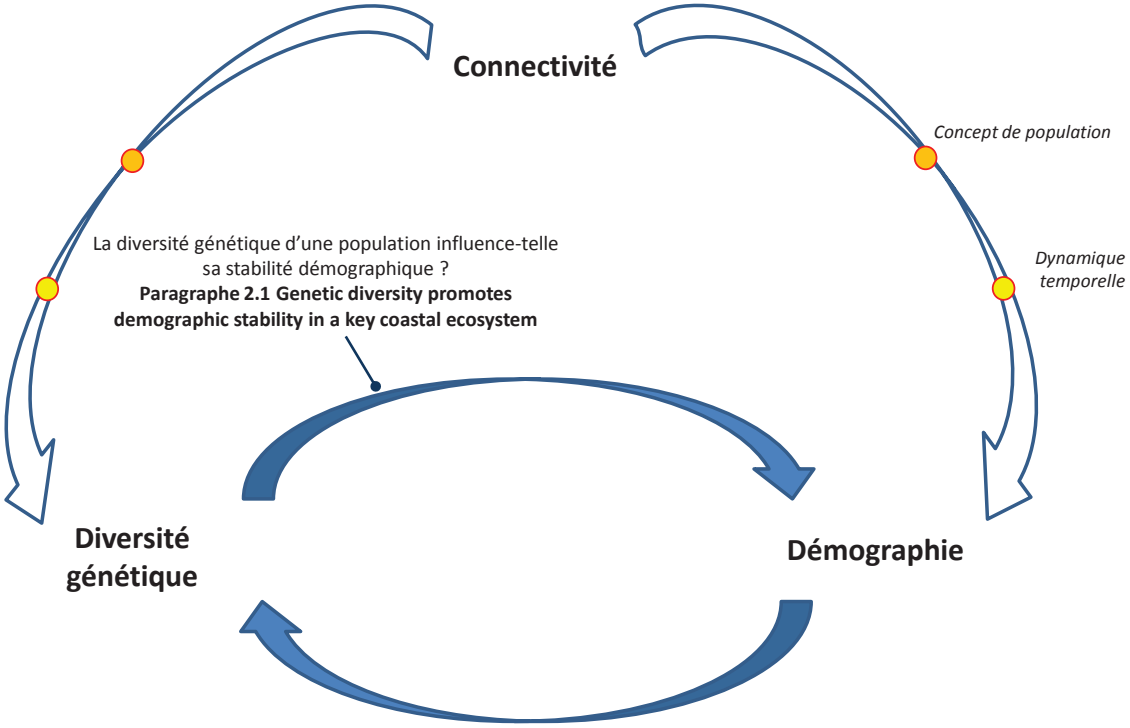

Figure 22 *Etude empirique de l'effet potentiel de la diversité génétique sur la démographie d'herbiers naturels*

Contexte & résumé

L'effet supposé de la diversité génétique sur la stabilité démographique manque de supports empiriques en populations naturelles. Parmi les raisons pouvant expliquer cette lacune, la dissociation des effets positifs réciproques entre diversité intraspécifique et stabilité de la démographie peut être particulièrement difficile. De plus, les populations naturelles se comportent généralement comme des systèmes ouverts, dont la démographie et les niveaux de diversité peuvent être influencés par leur degré d'interconnexion avec les populations voisines. C'est la raison pour laquelle une majorité d'approches expérimentales ont été réalisées à ce jour, dont toutefois les résultats ne peuvent pas être simplement extrapolés au fonctionnement des populations naturelles.

Par ailleurs, les approches expérimentales menées par Sonia Massa (Massa *et al*, accepté) révèlent une influence de la diversité allélique, masquée par la diversité clonale, sur les capacités de résistance des assemblages expérimentaux. Cette démonstration soulève des interrogations sur les interprétations des travaux précédents qui concluaient à une influence positive de la diversité clonale sur la stabilité démographique des assemblages expérimentaux (Ehlers *et al*, 2008; Hughes and Stachowicz, 2004; Reusch *et al*, 2005), sans avoir pris en compte la variation associée de richesses

allélique dans les quadrats expérimentaux. Les études sur les populations naturelles de *Posidonia oceanica* ont, par ailleurs, conduit à des résultats contradictoires, suggérant que la stabilité de la population pourrait davantage reposer sur la présence de grands clones, dont la taille révèlerait une performante écologique forte (Arnaud-Haond *et al*, 2010; Diaz-Almela *et al*, 2007).

Ainsi, l'hypothèse nulle de cette étude est la suivante : la diversité génétique, estimée par la richesse allélique et l'hétérozygotie moyenne de l'herbier, n'influence pas la stabilité démographique de l'herbier.

Afin de tester cette hypothèse, nous avons considéré les herbiers de *Zostera marina*, étudiés dans le chapitre 2. Depuis 2004, ces herbiers bénéficient d'un suivi de leur état écologique (réseau de surveillance REBENT, www.rebent.org). Notamment, la densité de pieds et des paramètres environnementaux (tels que le taux de matière organique, la biomasse d'épiphytes, la composition granulométrique du sédiment et le taux de nécroses des feuilles) sont estimés deux fois par an (équinoxe de printemps et d'automne). Les paramètres issus de ce suivi écologique ont été croisés avec les estimateurs de la diversité génétique et de la diversité clonale.

Une forte influence de la diversité génétique, *sensu stricto,* sur la démographie moyenne (à l'échelle de plusieurs années) a été démontrée, apportant un support *in situ,* à l'échelle des herbiers naturels, aux conclusions expérimentales de l'article de Massa *et al*, (accepté). Considérés conjointement, ces travaux supportent l'hypothèse que la stabilité d'une population dépend plus de la diversité de ses allèles que de la diversité de ses clones.

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GENETIC, NOT GENOTYPIC, DIVERSITY PROMOTES DEMOGRAPHIC STABILITY IN A KEY COASTAL ECOSYSTEM

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Abstract

Genetic diversity is recognized by the Convention for Biological Diversity as one of the three priority levels for conservation (ecosystem, species, gene). Yet, the influence of genetic diversity *sensu stricto* on populations durability remains a theoretical assumption poorly supported by empirical evidences, except in the extreme case of threatened and genetically impoverished populations. This may partly explain why actions for conservation dangerously neglect the genetic component of biodiversity. Here we focus on the seagrass *Zostera marina* structuring a key ecosystem threatened worldwide, by crossings genetic diversity –assessed at nine microsatellite markers- with long term survey of demography and environmental disturbances of eight meadows.

Seasonal correlations between genetic diversity and demography suggest a "parachute" effect of both allelic richness and heterozygosity on annual demographic variations, and an enhanced buffering in the response to four distinct disturbances. Such relations strongly support the role of genetic diversity *sensu stricto* as a driver of the long-term demography of seagrass populations, and thereby on the dynamics of the ecosystems based on those engineer species. These results show the critical importance of the genetic component of biodiversity for natural populations, and plead for its urgent integration into management plans.

Introduction

Biodiversity is undergoing its 6th wave of extinction, jeopardizing the durability of many ecosystems. This critical situation led the Convention for Biological Diversity (CBD) to recognize biodiversity protection as a priority at three levels: ecosystems, species and genes (www.cbd.org). Numerous ecological studies have explored the patterns and mechanisms linking species or functional diversity with ecosystem stability, and these have often established a positive relationship (Caldeira *et al*, 2005; Hector *et al*, 1999; Loreau and Hector, 2001; Naeem *et al*, 1996; Naeem *et al*, 1994; Tilman and Downing, 1994; Tilman *et al*, 2006). The potential influence of the genetic component of diversity on stability has been increasingly emphasized over the past 25 years, but has scarcely been demonstrated, except in very reduced populations that were often on the edge of extinction (Bijlsma and Loeschcke, 2012; Frankham, 2005b; Newman and Pilson, 1997; Schwartz *et al*, 2007). This gap may partly explain the alarming lack of action taken (Laikre *et al*, 2010) to protect this primary and crucial component of biodiversity.

To date, the role of the intra-specific diversity has been tested through the genotypic diversity, i.e. the number of distinct clonal lineages (but see box 1 in the Supporting Information), in natural and experimental populations. Experimental results demonstrate its positive effect on the resistance or resilience (Ehlers *et al*, 2008; Hughes and Stachowicz, 2004; Reusch *et al*, 2005) whereas field surveys delivered contradicting evidence depending on the species studied, the environmental fluctuations and the sampling scales and schemes (Arnaud-Haond *et al*, 2010; Diaz-Almela *et al*, 2007; Hughes and Stachowicz, 2009).

On the other hand, genetic diversity *sensu stricto* (heterozygosity and allelic polymorphism) is a level of diversity that is equally meaningful for both sexual and clonal organisms. It is also the parameter, particularly as estimated through allelic richness (Widmer and Lexer, 2001), most likely to influence the persistence of species and their evolutionary potential in the longer term. Most conservation genetics approaches are based on two main theoretical assumptions: (i) the positive influence of genetic diversity on the capacity of populations to face environmental changes (Booy *et al*, 2000; Frankham, 2005b; Widmer and Lexer, 2001), and (ii) a positive relationship between genetic diversity at supposedly neutral markers (the type the most frequently used so far), and the diversity of the genome as a whole, including genes potentially subjected to selective processes. Despite evidence of increasing risks associated with depletion of genetic diversity (Spielman *et al*, 2004a; Spielman *et al*, 2004b), these hypotheses still need to be formally tested, by a range of experimental and above all *in situ* case studies.

Seagrass are supporting one of the most important coastal ecosystems, in term of biomass concentration and worldwide distribution, but are suffering an alarming decline worldwide (Orth *et al*, 2006; Waycott *et al*, 2009). Here we performed an *in situ* assessment of the relationship between intraspecific diversity, assessed with 9 microsatellite markers and demographic stability in eleven meadows of the seagrass *Zostera marina*. To test for the hypothesis of a relationship between intraspecific diversity, as estimated with putatively neutral markers, and the demographic answer of meadows to forcing environmental parameters, we crosscut the descriptors of genetic and genotypic diversities for the eleven meadows with their demography and four environmental descriptors followed at spring and autumn during nine years. Our main question was the existence, *in situ*, of a

relationship between the genetic and/or clonal component of biodiversity as estimated through standard neutral markers classically used in conservation genetics, and the demographic stability of populations supporting an entire ecosystem. We also aimed at discriminating the driver of such relationship, if existing.

Material and methods

Species model and study sites

Zostera marina is a partially clonal marine plant, combining both sexual reproduction and vegetative elongation. This partial clonality induces particularities for population genetics study and leads to define two kinds of intraspecific diversities: i. the genetic diversity *sensu stricto*, reflected by allelic richness and heterozygosity revealing the level of polymorphism of a species genome within a given populations, and ii. the genotypic diversity, corresponding to the amount of distinct clonal lineages forming a population (Box 1 in the Supporting Information). In Brittany (France), seven locations supporting *Z. marina* meadows are followed by the French network REBENT (www.rebent.org), which goal is to provide long-term information about evolution and dynamics of these key ecosystems. Locations were chosen depending on their ecological features, along a gradient of swell and wind exposition. For each location, two sampling quadrats measuring 30*20 m² were designed for assessment of their levels of genotypic and genetic diversity levels as well as for demographic evolution and survey of different environmental parameters (Table 6; Fig. 26). The quadrats are separated by 30 to 100 meters. Sampling is performed during equinox low tides.

Tableau 6 *Information about the sampling quadrats (30*20 m²).*
N (2009) and N (2012) correspond to the number of sampled and genotyped Zostera units, during the genetic surveys in 2009 and 2012. Geographical coordinates correspond to the central point of patches within which sampling quadrats were designed. Aerial pictures of locations are available on www.rebent.org/medias/documents/www/contenu/FL05-Lieux_herbiers_zostera_marina.pdf. The inter-quadrat distances were calculated with GPS-coordinates.

Location	Quadrat	N (2009)	N (2012)	Latitude	Longitude	Distance (m)
L'Arcouest	Q1	30	30	48°49'428 N	03°01'162 W	70
	Q2	30	30	48°49'425 N	03°01'218 W	
Callot	Q1	30	30	48°41'064 N	03°54'968 W	30
	Q2	30	30	48°41'052 N	03°54'982 W	
Sainte-Marguerite	Q1	30	30	48°35'811 N	04°37'389 W	75
	Q2	30	30	48°35'830 N	04°37'443 W	
Molène	Q1	30	30	48°23'760 N	04°56'934 W	-
Roscanvel	Q1	30	30	48°19'934 N	04°32'209 W	100
	Q2	30	30	48°19'984 N	04°32'182 W	
Arradon	Q1	30	30	47°36'911 N	02°49'636 W	80
	Q2	30	30	47°36'914 N	02°49'574 W	

Genetic survey

Data used here were acquired to assess fine grained spatial genetic and clonal structure in 2009 (Becheler *et al*, 2010), and assess the temporal variation in those parameters three years later (Becheler *et al*, 2013) in order to bring information as to the contribution of clonal and sexual reproduction to the recruitment and dispersal in seagrass meadows. Two genetic surveys were

therefore carried out during spring 2009 and spring 2012 (Fig. 27). We attempted to replace sampling quadrats (30*20 m²) in the same place on the second series of sampling. This was done using eye reaper as GPS coordinates were not precise enough to ensure the exact superposition of temporal quadrats. It can therefore not be excluded that in some places changes in the landscape led to a slight shift in the positioning of the quadrats among years. For each sampling quadrats and at each time step, 35 sampling units (i.e. ramet: one shoot or two when connected with their rhizome) were collected according to randomly generated geographical coordinates. Due to the patchiness of several meadows (Molène and the quadrat 1 of Arcouest), some coordinates had to be adjusted to the nearest shoot on the field. The meristematic leaves of sampling units were immediately stored in silica crystals, until DNA extraction.

Environmental survey

The environmental of the REBENT network was performed twice a year starting in 2004 up to 2006, during the spring and autumn equinox tides. After 2006, only spring surveys were maintained annually (Fig. 27). We used the environmental values assessed annually between spring 2004 and spring 2012 and data for autumn available only from 2004 to 2006, except for density that was also estimated in autumn 2011 and 2012 for the present study (Table 7). More details can be found on the REBENT website (www.rebent.org).

Demographic parameters. The shoot density (number of shoots per square meter) was measured in 0.1m² quadrats (three replicates in each sampling quadrats) haphazardly positioned in the central part of each meadow. Temporal series of density are consigned in Table 7.

Wasting Disease Index. The Wasting Disease Index (WDI) is calculated as the ratio of the necrotic surface to the total surface of all leaves from 15 shoots haphazardly sampled in the meadow. The necrotic action of the marine slime-mold *Labyrinthula zosterae* is considered as a proxy for the stress suffered by the plant (Muehlstein, 1989; Muehlstein *et al*, 1988).

Epiphytic flora. At each station, the biomass of epiphytic algae (EB) was measured from 15 shoots haphazardly collected, by scratching the surface of all the leaves (n = 3 per site). These data, after normalization by the biomass of supporting leaves, were used as a proxy for the disturbance induced by the shading effect of epibiota, which limits light availability for *Zostera* leaf photosynthesis (Sand-Jensen, 1977) and contributes to the seagrass decline (Hughes and Stachowicz, 2004; Orth *et al*, 2006).

Organic matter. One core was sampled within each sampling quadrat to measure the Organic Matter content (OM) of the sediment, acting as a toxic for seagrass (Hemminga and Duarte, 2000b). The total Organic Matter is the rate (%) of weight lost by the dried sediment (48 hours at 80 °C) after burning (4 hours in a muffle furnace at 450 °C).

Sediment instability. One core was sampled within each sampling quadrat to analyse the grain size composition of the sediment. The dry sediment was sieved on a column with 12 mesh class sizes (from 63 µm to 2000 µm) to obtain granulometric curves. Using the abundance of each size class, we calculated the first and third quartiles (Q1 and Q3 respectively) of the size distribution. We then calculated the Trask's index $S0 = [Q3 / Q1]^{1/2}$ (Trask, 1930), as a sorting index of the sediment . The

minimal value is 1, characterizing sediment when only one size class is present. When this value increases, the sediment shows a greater diversity of granulometric classes. The temporal variance (TVSC) of the Trask's index is also as a proxy of granulometric instability, a disturbance that can lead to the uprooting of plants.

Tableau 7 *Evolution of Zostera marina densities for each sampling quadrates since 2004.*
Values correspond to the mean of the three counts (see material&methods) with the confident interval (95%). Grey values for the quadrat 2 of Sainte-Marguerite correspond to the value we excluded from the analyses due to the event of extinction-recolonisation occurring in 2008 (but see material & methods). For spring of 2009 and 2012, "GS" indicates that the genetic surveys were performed during these periods.

Location	Quadrat	2004		2005		2006		2007		2008		2009		2010		2011		2012
		Spring	*Autumn*	*Spring*	*Autumn*	*Spring*	*Autumn*	*Spring*	*Autumn*	*Spring*	*Autumn*	*Spring (GS)*	*Autumn*	*Spring*	*Autumn*	*Spring*	*Autumn*	*Spring (GS)*
Arradon	**1**	*27*	33,3 ± 11,3	14,7 ± 3,3	73,8 ± 4,6	15,3 ± 7,5	10,0 ± 3,0	11,7 ± 6,2	-	14,5 ± 7,5	-	10,8 ± 8,9	-	16,5 ± 5,8	-	23,8 ± 5,5	19,3 ± 4,0	6,7 ± 3,5
	2	45,0 ± 23,6	14,0 ± 4,9	14,3 ± 2,8	78,3 ± 17,6	11,7 ± 13,6	11,7 ± 7,3	17,7 ± 5,2	-	35,5 ± 23,9	-	13,8 ± 15,3	-	16,5 ± 12,0	-	29,5 ± 5,2	14,7 ± 5,1	40,0 ± 27,5
Roscanvel	**1**	43,0 ± 10,8	46,3 ± 3,3	30,3 ± 7,3	37,5 ± 2,5	22,0 ± 7,9	20,5 ± 5,1	22,7 ± 5,1	-	-	-	20,7 ± 4,0	-	20 ± 6,0	-	*29*	37,0 ± 7,4	-
	2	39,3 ± 8,0	32,7 ± 0,7	21,7 ± 5,3	44,0 ± 7,8	21,0 ± 7,9	21,0 ± 4,6	24,3 ± 10,5	-	*28*	-	18,3 ± 4,0	-	22,3 ± 3,3	-	*27*	29,5 ± 12	-
Molène	**-**	-	16,3 ± 2,4	12,0 ± 4,9	26,0 ± 15,9	19,7 ± 11,1	16,3 ± 6,7	32,3 ± 22,2	-	*0*	-	17,3 ± 10,5	-	21,0 ± 1,1	-	*24*	*28*	15,0 ± 2,0
Sainte-	**1**	46,7 ± 15,4	31 ± 1,1	14,5 ± 2,9	37,5 ± 16,9	18,5 ± 2,9	27,0 ± 4,1	15,8 ± 3,8	-	29,0 ± 1,1	-	19,3 ± 6,6	-	23,3 ± 12,5	-	*33*	*33*	-
Marguerite	**2**	-	*19,3 ± 1,3*	-	*44,5 ± 9,7*	*20,5 ± 1,0*	*30,0 ± 1,1*	*9,8 ± 0,9*	-	0	-	21 ± 1,1	-	23,3 ± 2,4	-	*26*	*36*	-
Callot	**1**	25,0 ± 4,1	*21*	18,7 ± 6,4	25,5 ± 3,1	30,3 ± 21,3	22,8 ± 8,3	37,3 ± 12,6	-	*72*	-	22,7 ± 1,7	-	14,3 ± 5,4	-	*21*	9,0 ± 3,3	27,5 ± 4,9
	2	25,7 ± 7,5	*20*	26,0 ± 6,3	59,8 ± 31,1	26,3 ± 14,6	16,5 ± 4,6	34,3 ± 20,4	-	*66*	-	32,3 ± 3,5	-	13,7 ± 9,2	-	*19*	15,0 ± 1,1	20,5 ± 2,9
Arcouest	**1**	48,0 ± 18,0	*65*	*44*	26,3 ± 2,0	30,3 ± 11,0	17,3 ± 6,8	33,3 ± 2,6	-	*38*	-	24,7 ± 1,7	-	19,3 ± 3,5	-	55,8 ± 14,4	7,0 ± 1,1	11,0 ± 2,0
	2	47,3 ± 17,7	*38*	29,3 ± 10,5	36,5 ± 16,5	27,7 ± 2,4	21,3 ± 4,0	35,0 ± 9,3	-	*33*	-	26,0 ± 4,9	-	28,3 ± 10,3	-	37,3 ± 14,8	11,0 ± 3,0	30,5 ± 2,9

Acquisition of genotypic and genetic data

Total DNA was extracted using the classical CTAB method (Doyle and Doyle, 1988). In 2009 and 2012, the same 9 microsatellite loci were amplified for this study (but see Becheler *et al*, 2010) and Becheler *et al* in press). PCR products were visualized using the same ABI-3100 FNVR automated sequencer (Applied Biosystems) and scored using microsatellite plugging of Geneious v5.6.4 (Biomatters®). Two readings were done blindly by two different readers in order to minimize the occurrence of scoring errors and ascertain the interpretation of peaks. Genetic individuals were recognized on the basis of their multi locus genotypes (MLG) and assigned to Multi-Locus Lineages (MLL) when needed (Arnaud-Haond *et al*, 2007a).

For each quadrat, the clonal richness $R = (G - 1)/(N-1)$ was estimated, where G is the number of MLLs in the sample and N is the number of sampling units in the sampling set. The minimum value for clonal richness in a monoclonal stand is always 0, independently of sample size, and the maximum value is 1, when all the different samples analysed correspond to distinct clonal lineages. The slope β of the Pareto distribution, describing the distribution of *ramets* within *genets,* is also provided. Low values of these parameters indicates the presence of large and dominant clonal lineages (see Arnaud-Haond *et al*, 2007a for details). All genotypic data analyses were performed with Genclone 2.1 (Arnaud-Haond and Belkhir, 2007).

After clonal discrimination, a single replicate of each clonal lineage was included for assessment of genetic diversity on a set of genetic individuals (genets) representative of each sampled quadrat. The first we used is the observed multilocus heterozygosity (H_o). This kind of heterozygosity reflects the proportion of heterozygote loci in the clonal lineages we have actually sampled. We also estimated the allelic richness (Â), corresponding to the mean number of alleles per locus.

Statistical analyses

We statistically compared the temporal variations of genetic diversity parameters and density. For this aim, the rate of variations of allelic richness and observed heterozygosity was assessed among years ($\Delta = [X_{2012} - X_{2009}] / X_{2009}$). Due to large seasonality effect, the evolution of density could only be clearly appraised when taking into account separately the evolution of spring and autumn densities. The temporal variation in meadows density was therefore assessed through four estimates i. the percentage of difference between the mean density of spring and the mean density of autumn; ii. The percentage of difference between the density in spring 2009 (first genetic survey) and spring 2012 (last genetic survey); iii. the percentage of difference between the density in autumn 2006 (last autumnal density estimated before the genetic survey in 2009) and the density in autumn 2011 (the more recent autumnal density before the second genetic survey); iv. Percentage of intra-annual difference of density for 2011, between the spring density and autumn density. The hypothesis of a similar pattern and extent of temporal variation at both genetic and demographic data was tested after an arcsinus transformation, using non parametric test (Kruskal-Wallis test) followed when significant by the pairwise post-hoc test of Behrens-Fisher (BF), providing non-parametric multiple comparisons. All analyses were performed using the free open source R Environment (R

Development Core team, 2010) with the Vegan (Oksanen *et al*, 2008) and non-parametric multiple comparisons (NPMC) libraries (Helms and Munzel, 2008). Linear regressions were performed between the mean densities –assessed for each season on the basis of all years for which data are available, see Table 7 and Figure 27- and parameters describing the genetic and genotypic diversities. Similar analyses were performed taken into account each year alone. Genetic and genotypic diversities were also crossed with the interannual variance of density for autumn and spring, and the total variance of densities (assessed on the basis of all available data between 2004 and 2012, including the two seasons).

Residual analyses were performed to assess the relationship between genetic/clonal diversity and the response of populations facing environmental disturbances, estimated through the parameters described above (OM, WDI, EB and TVSC), chosen as proxies for disturbance, as their potential to affect the demography of seagrass meadows has been previously demonstrated. Due to lack in the environmental dataset (Fig. 27), residual analyses were conducted using only the first autumns of the survey (2004, 2005 and 2006), affecting the statistical power of analyses.

Tableau 8 *Parameters of the linear regressions between the variance of densities and genetic/genotypic diversity.*
Spring Variance was assessed using all available data (2004 to 2011 or 2012 when available, see table 2). Spring Variance 'bis' and Autumn Variance were assessed using only the years for which both spring and autumn densities were also available (i.e 2004 to 2006 and 2011). Total Variance corresponds to the variance of the whole time-series, mixing both spring and autumn densities.

		Spring Variance		Spring Variance 'bis'		Autumn Variance		Total Variance	
genetic diversity	Ho	$r^2 = 0.04$	*p = 0.57*	$r^2 = 0.02$	*p = 0.69*	$r^2 = 0.06$	*p = 0.52*	$r^2 = 0.03$	*p = 0.66*
	Â	$r^2 = 0.07$	*p = 0.48*	$r^2 = 0.00$	*p = 0.91*	$r^2 = 0.31$	*p = 0.09*	$r^2 = 0.34$	*p = 0.07*
genotypic diversity	R	$r^2 = 0.06$	*p = 0.50*	$r^2 = 0.01$	*p = 0.80*	$r^2 = 0.03$	*p = 0.64*	$r^2 = 0.03$	*p = 0.61*
	β	$r^2 = 0.17$	*p = 0.23*	$r^2 = 0.00$	*p = 0.90*	$r^2 = 0.02$	*p = 0.72*	$r^2 = 0.04$	*p = 0.57*

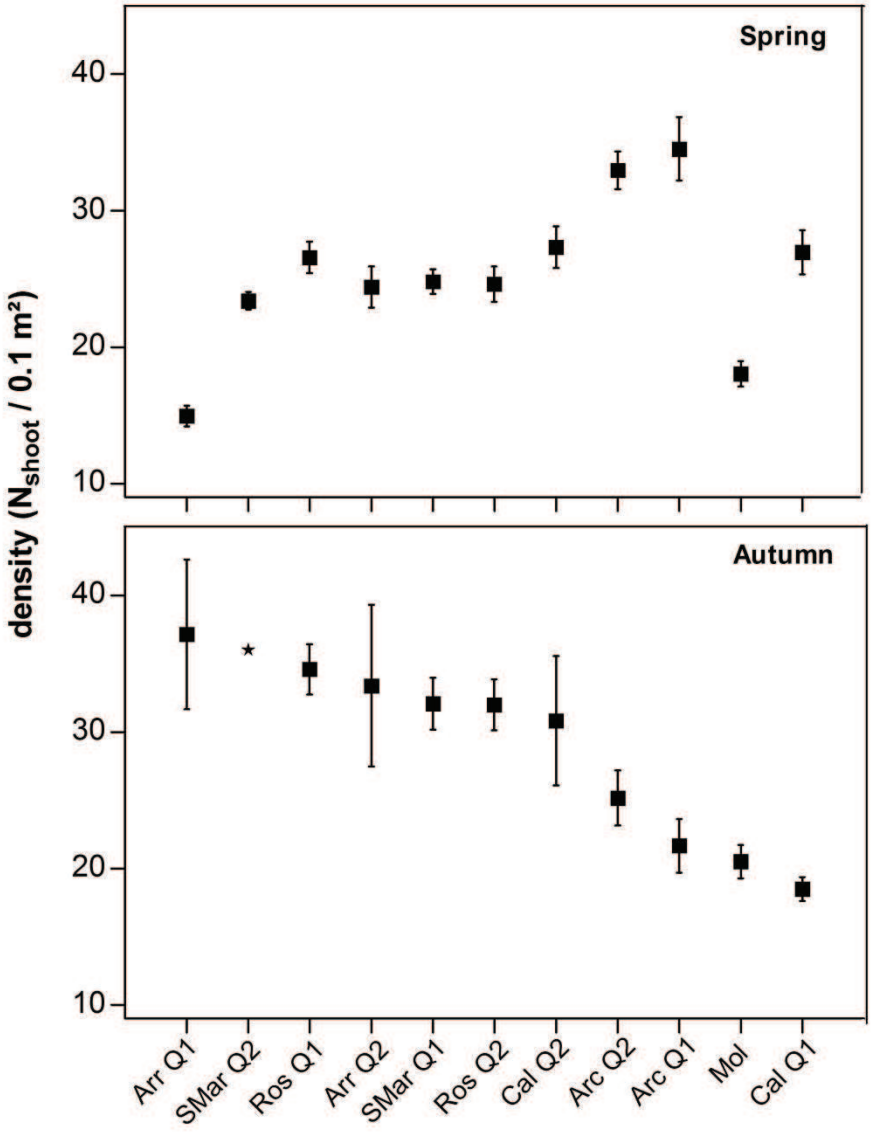

Figure 23 *Mean densities of each sampling quadrats between 2004 and 2012, with intervals of confidence (95%), for Spring and Autumn.*
The abbreviations Arr, Ros, Mol, SMar, Cal and Arc correspond respectively to the locations of Arradon, Roscanvel, Molène, Sainte-Marguerite, Callot and Arcouest. Q1 and Q2 correspond to the sampling quadrats 1 and 2 for each location. Spring and autumn densities are not correlated (r^2 = 0.16; p = 0.23). NB: for SMarg Q2, the star in Autumn gets no interval of confidence, due to a single count of density.

Results

Demographic evolution

Density in quadrats was highly variable in time and space, showing strong fluctuations both within and among years and both within and among locations (Table 7; Fig. 23). For each season, we estimated the mean densities using the available annual values recorded between 2004 and 2012 (Table 7). Densities were generally higher during autumn (season corresponding with the end of the growth seed germination season and characterized by a low erosion of meadows by storms) than during spring (season corresponding with the end of the winter period, characterized by a strong erosion of the meadows by hydrodynamism and associated to low growth of ramets); Table 7; Fig. 23). The seasonal densities appear less variable across year among spring than autumn periods. This difference could not be attributed to statistical bias due to the availability or annual data for spring compared to the large 2007-2010 gap for autumn data, as retaining the same years in spring than those available in autumn lead to similar results. Mean densities for the two seasons were not correlated ($r^2 = 0.16$; $p = 0.23$).

Temporal variability of densities and genetic diversity

Results of the Kruskal-Wallis test rejected the hypothesis of a similar temporal fluctuation of genetic diversity - i.e observed heterozygosity and allelic richness- and demography (expressed in % variation; $\chi^2 = 26.4$; $p < 0.001$). Following this result, the dissection of pairwise differences in the pattern of percentages of temporal variations in density and genetic diversities (both allelic richness and heterozygosity) was therefore performed. The tests revealed no significant differences of temporal fluctuations between observed heterozygosity and allelic richness (test; $p = 0.86$) but showed a significantly higher rate of temporal fluctuations of densities than any of the two estimators of genetic diversity. The p-values of these tests are consigned in the Table 9.

Relationship between genetic & genotypic diversities and density

Mean genetic diversity parameters were significantly correlated with the mean densities in autumn (Fig. 24), whereas no significant trend was detected with mean densities over spring periods. Significant correlations observed between mean heterozygosities and mean densities in autumn ($r^2 = 0.60$; $P = 0.005$; positive slope) disappeared in spring ($r^2 = 0.26$; $P = 0.11$; negative slope). A similar seasonal pattern was observed with allelic richness. In autumn, the relation is positive and nearly significant ($r^2 = 0.31$; $P = 0.074$; positive slope), but it should be noticed that this is due to a single outlier (quadrat 1 of Callot) without which the relationship would have appeared highly significant ($r^2 = 0.71$; $p = 0.002$). Similarly to heterozygosity, no significant relation was observed in spring ($r^2 = 0.13$; $P = 0.28$; negative slope).

Contrastingly, no significant correlations or relevant trend were retrieved between mean densities at any season and genotypic parameters ($r^2 = 0.007$; $P = 0.79$ and $r^2 = 0.009$; $P = 0.77$ for R and density

in autumn and spring respectively; r^2 = 0.014; P = 0.73 in autumn and r^2 = 0.007; P = 0.81 in spring for the β of the Pareto's distribution).

Considering independently each year, no significant relationship was found between density and genetic or genotypic diversities, neither in spring, nor in autumn. Finally, no significant relationship was observed between genetic and genotypic parameters and the variance of densities among years, neither for spring nor for autumn periods (Table 8).

Residual analyses crossing disturbance proxies, densities and genetic diversity

For both autumn and spring, the mean values of the proxies of disturbance targeted in the REBENT survey were uncorrelated among themselves (Table 10, above and below the diagonal), except the organic matter content OM and the sediment instability TVSC in spring (r^2 = 0.68; p = 0.003). For each proxy, the correlation between spring and autumn mean values was significant (Table 10, diagonal), except for the wasting disease index WDI (r^2 = 0.34; p = 0.077). These proxies are not correlated with mean densities, neither in spring nor in autumn (Table 11).

Residual analyses (algebraic distance between observed and expected values), performed independently for each proxy of disturbance and density, revealed a positive relationship between genetic diversity parameters and demographic residuals, for autumn periods (Fig. 25). All these tests are significant for observed heterozygosity in autumn, whereas no trend emerged for spring (Table 12). The relationship between observed heterozygosity and demographic residuals was stronger than with allelic richness.

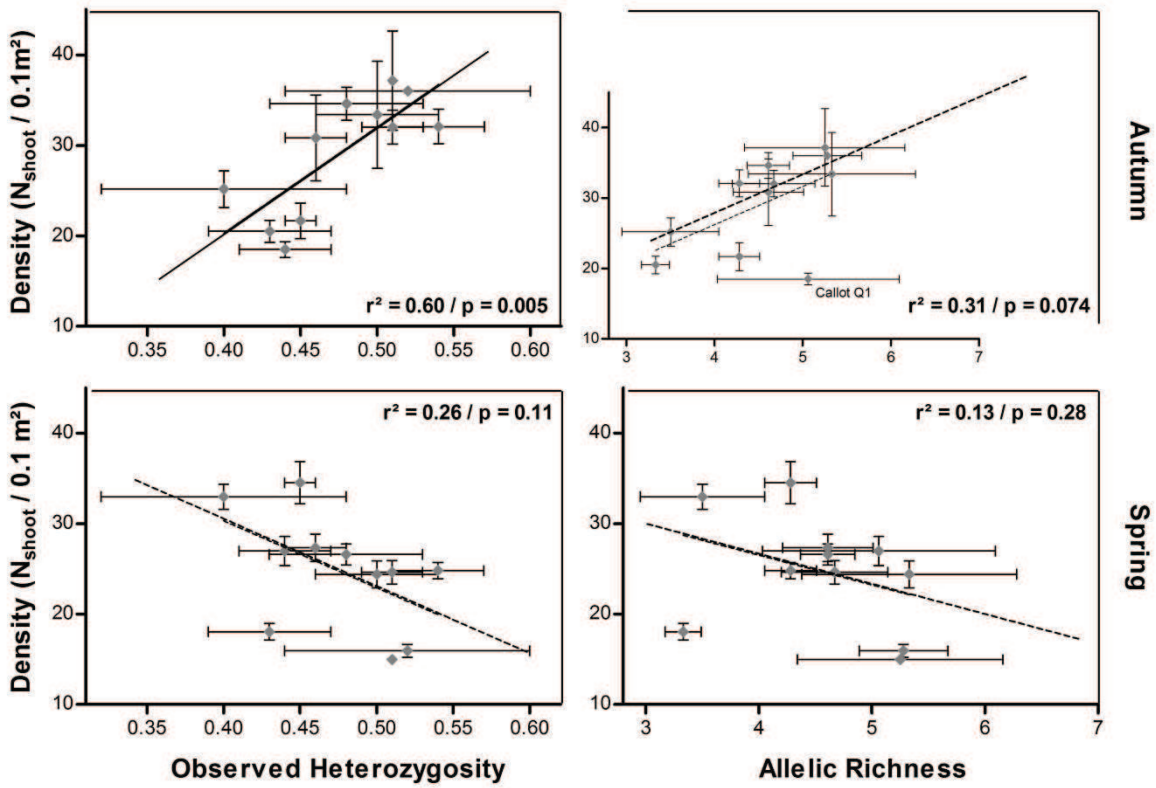

Figure 24 *Linear regressions between genetic diversity and meadow mean densities for each season. On the top, graphs show the relationship between genetic diversity and mean density in Autumn. A significant and positive correlation is obtained between the level of observed heterozygosity and the mean autumnal density. Allelic richness is positively correlated to the mean density, but the relationship is not significant. When removing the quadrat 1 of Callot, the correlation becomes highly significant ($r^2 = 0.71$; $p = 0.002$). At the bottom, parameters of genetic diversity are plotted with Spring mean densities. No significant correlations are observed. NB: the relationship between observed heterozygosity and allelic richness is positive and significant ($r^2 = 0.39$; $p = 0.041$).*

Discussion

This study provides to our knowledge the first empirical support to the hypothesis that the genetic diversity *sensu stricto* enhances the *in situ* demographic stability of populations facing environmental fluctuations and disturbances.

The main result emerging from this analysis is the occurrence of a strong seasonal relationship between the genetic diversity and the demographic answer of meadows to environmental forcing parameters. The usual stumbling block in identifying the driver of such a relationship *in situ* is due to feedback effects of diversity and stability on each other (Diaz-Almela *et al*, 2007) and the potential role of the dispersion as an external cause.

Here, the strikingly and the large temporal variance in demography of studied meadows, contrasting with their relative inter-annual stability in terms of genetic diversity, constitutes a key information to dissociate these feedback effects and suggest genetic diversity as the main driver of the relationship observed.

Densities of meadows are highly variable, among both years and seasons. The demographic dynamics is quadrat-dependent (Table 7), leading to different values of both mean and variance of densities, and a distinct ranking between seasons (Fig. 23). The general pattern of annual variations of demography in temperate seagrass is characterized by a maximum during ending summer and a minimum during winter. These demographic fluctuations are likely due to a combination of seasonal effect and environmental disturbances. The high variability observed from year to year in the date and the strength of the first storm in the autumn, and so the associated meadow erosion could explain this result. Another explanation could be the variability of the seed bank between locations which could induce various rates of new ramets from seed germination. The differences between autumn (season of decreasing density) and spring (increasing density), together with the absence of significant correlation between spring and autumn densities ($r^2 = 0.16$; $p = 0.23$; Fig 23), suggest variable dynamics of meadows either for growth during the spring or for buffering their decline during autumn. This strong variability, both within and among years, contrasts with the temporal stability of genetic diversity recorded across a three year period (Becheler *et al*, 2013; and see Table 6).

Indeed the relative stability of the genetic diversity *sensu stricto*, contrasts with the important variations of genotypic richness and clonal architecture of the *Zostera marina* populations (Becheler *et al*, 2013). The clonal architecture evolved during the three-years survey toward a reinforced pattern of clonal dominance, tending to diminish the clonal diversity through increased competition among clones. These persisting, large and competing clones constitute a stable core of genets within populations co-existing with a second set of yearly renewed transient genets. This may partly explain the relative stability of genetic diversity mostly influenced by these core genets and the local sources of annual recruits. Annual recruits, because they are less anchored in the sediment by roots and rhizomes, are more threatened by the erosion induced by storms and human activities (hand-fishing

for bivalves) than long living strongly anchored clones. This dual dynamic functioning highlights the evolution of genotypic diversity through the balance between recruitment through clonal growth versus dispersed seedlings. This may explain the lack of relationship observed here between clonal diversity and demographic performances in natural meadows.

Considering therefore the relative temporal stability of the genetic diversity, contrasting with the strong intra and interannual densities fluctuations, three non-mutually exclusive scenarios could be proposed to explain the relationship observed between long term mean densities and genetic diversity.

The dispersal as an external cause

Dispersal leads to both genetic and demographic connectivity, able to positively influence the level of genetic and genotypic diversity, as well as the demography of populations and may therefore be the real driver of an indirect relationship between genetic diversity and demographic stability. Yet, several orders of magnitude distinguish these two kinds of connectivity. While the genetic connectivity requires only few migrants per generation, the demographic one implies a stronger input of recruits. Yet, dispersal intense enough to influence demography would both homogenise allelic frequency and enhance the relationship between clonal richness and density, particularly for spring periods, corresponding the reproductive season. These expectations at odds with the almost systematic genetic structure reported here among quadrats together with the lack of correlation among genotypic diversity estimates and density at any season render this scenario unlikely.

The demography favours the genetic diversity

Large populations are expected to hold a larger genetic diversity due to a limited effect of genetic drift. A positive relationship between mean density and genetic diversity shall emerge with a strong influence of the minimal densities reached during the year (therefore close to the early spring densities). However, neither allelic richness, nor observed heterozygosity are significantly correlated with the minimal density ($r^2 = 0.02$; $p = 0.71$ and $r^2 = 0.25$; $p = 0.12$ respectively). The lack of relation between genetic diversity parameters and either maximal or minimal density render this scenario unlikely to explain the observations reported here.

The genetic diversity favors the demography

This scenario refers to the hypothesis of positive influence of genetic diversity on the resistance and/or resilience of populations. In a similar way, a similar seasonal dichotomy was found, and interpreted as the result of clonal richness as the driver of relationship during the winter (Hughes and Stachowicz, 2009), in a one-year study focusing clonal diversity at very fine grained scale ($1m^2$ quadrats). Considering the clonal richness constant during the year of survey, authors argued that the clonal richness was the driver of the relationship during the winter, because "*diversity positively affected shoot density only during periods of abiotic or biotic stress*" (Hughes and Stachowicz, 2009).

In our case also, the high annual demographic fluctuations and the relative stable genetic composition support genetic diversity as the predominant driver of the positive relationship emerging in this survey. This positive relationships between genetic diversity and stability therefore suggest a “parachute” effect of genetic diversity when a population faces the annual demographic decrease (Hemminga and Duarte, 2000b), supporting the role of genetic diversity in the resistance of a population. Despite this seasonal dichotomy, results are clearly distinct between both studies as no influence of the genotypic diversity was observed in our work. We suggest that this may be due to the hidden effect of allelic diversity that was not considered in previous studies although highly correlated with genotypic diversity when manipulated at low levels such as the one usually present in one meter square of natural meadow or experimental design (but see discussion below).

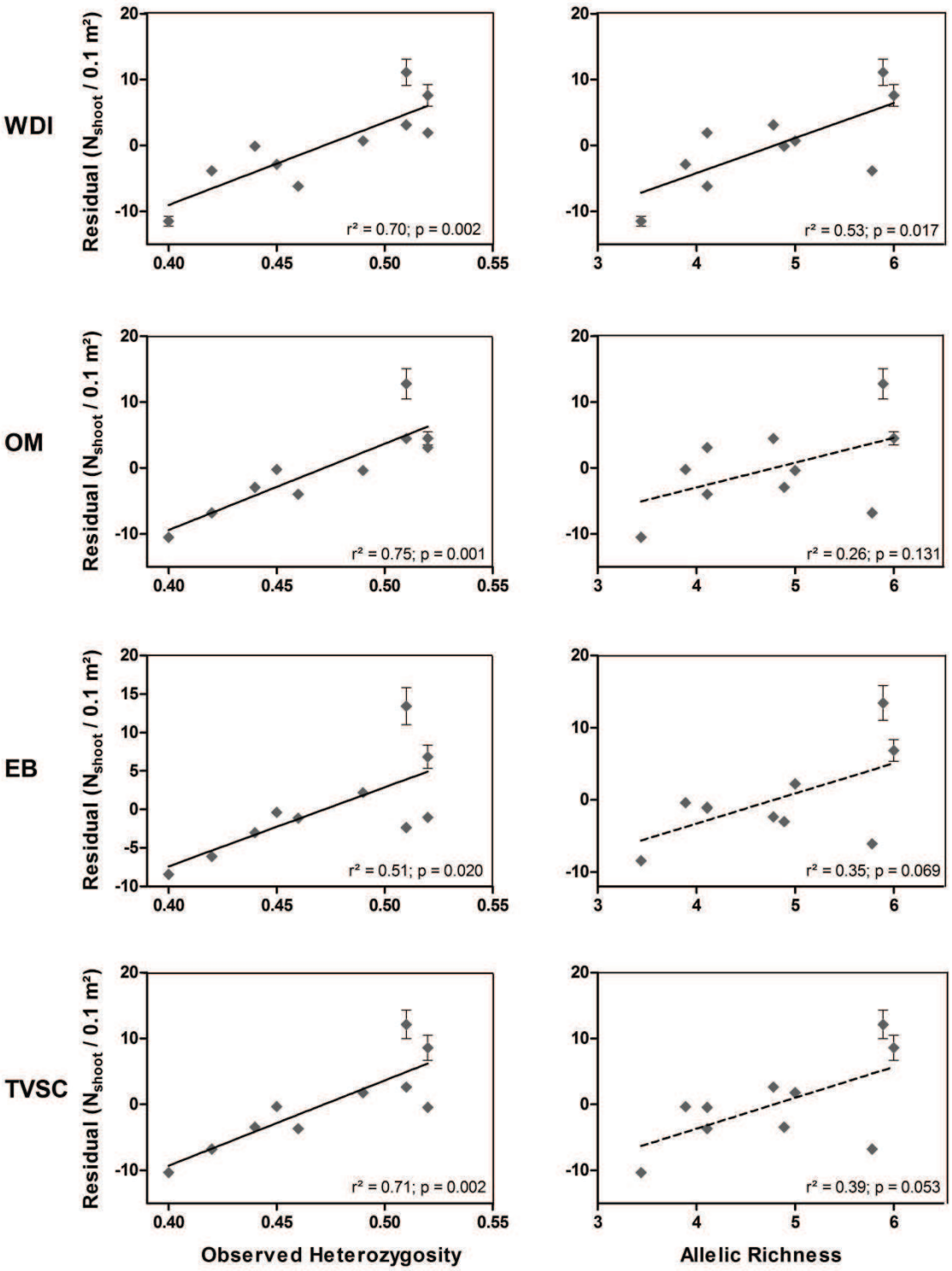

Figure 25 *Linear regressions between demographic residuals and genetic diversity.*
Residuals were calculated from the regression between one of the four disturbance proxies: OM, WDI, EB, TVSC) (Table 10). Significant correlations are illustrated by full regression lines, and non-significant correlation by dashed lines. Due to the lack of data for the autumns of 2007 to 2012, these tests were performed using the values of density and disturbance proxies of Autumn 2004, 2005 and 2006, and the values of genetic diversity obtained in 2009.

This last scenario seems to be the more likely, and is supported by a parallel study by Massa *et al.* (*submitted*), where experimental quadrats were setup with the sister species *Z. noltii* crossing increasing genotypic diversity and allelic richness in order to break the loop and assess the effect of each component on demographic resistance to perturbations. This experiment also demonstrated a positive effect of allelic diversity while no, or a slightly negative relationship was observed with genotypic diversity, when both parameters could be dissociated. In addition, for low level of genotypic richness, potential effects of both allelic richness and genotypic richness cannot be dissociated. The sampling quadrats designed for this study integrate a larger number of clonal lineages, preventing such entanglement of allelic and genotypic richness. These two kinds of intraspecific richness are not significantly correlated ($r^2 = 0.22$; $p = 0.15$).

Altogether, those results therefore strongly support the interpretation of the observed relationship as the outcome of a positive effect of genetic diversity *sensu stricto* on the demographic answer to perturbation.

This conclusion is supported by the residual analyses performed in this work. Among environmental factors potentially influencing demographic fluctuations, the four disturbance proxies analysed here (TVSC, OM, WDI, EB) are very different by nature and appear independent among themselves (Table 10) and from densities (Table 11). This therefore suggests a differential response of meadows to distinct environmental factors. Yet, results are very consistent with the previous ones. Residual analyses also revealed a seasonal dependence of demographic response of meadows in autumn to their level of genetic diversity (Fig. 25), whereas no trend emerged in spring (Table 12). The more genetically diverse meadows therefore exhibit an enhanced demographic buffering of environmental perturbations. These seasonal relationships bring further support to the role of genetic diversity as the driver of resistance for populations facing seasonal environmental disturbances.

These results support the tendency observed in large scale *in situ* analysis of *Posidonia oceanica* meadows (Arnaud-Haond *et al*, 2010), while contrasting with both experimental and very local *in situ* studies that have highlighted genotypic diversity as a driver of resistance or resilience of eelgrass (Ehlers *et al*, 2008; Hughes and Stachowicz, 2004; Hughes and Stachowicz, 2009; Reusch *et al*, 2005). Yet, the majority of these studies did not consider the relationship between clonal richness and genetic diversity, except in Reusch *et al,* (2005) reporting no significant relationship between the clonal richness and observed heterozygosity. Such discrepancy may be due to the fact that genetic diversity was a hidden variable not considered in those experiments, and that the experimental setup, although allowing a thorough exploration of rather narrow windows of space and time, may not entirely grasp the complexity of natural meadows (Arnaud-Haond *et al*, 2010). These studies reveal the response capacity of synthetically assembled entities (i.e. the genotypes) or short term surveys across small spatial scale, where the possibly entangled levels –and consequent effects- of allelic richness are unknown, and neither the interaction among genets through clonal growth, survival, competition and possible synergy, nor the sexual influence through recombination, could act during the time of the experiments. Contrastingly, longer term field surveys across large spatial scale provide estimates integrating mid and long term interactions between clonal growth, allelic recombination and environmental factors.

Recently, Stachowicz *et al,* (Stachowicz *et al*, 2013) have re-analysed former studies suggesting a positive influence of genotypic richness on demographic stability (Hughes and Stachowicz, 2004; Hughes and Stachowicz, 2009), taking into account the genetic relatedness among clones. Both in their experimental setup and *in situ* study, the genetic relatedness appeared a better predictor of demographic status, than genotypic diversity. Stachowicz *et al,* (2013) concluded that the relationships previously observed between clonal diversity and resistance/resilience of both natural and experimental populations may be due to a hidden effect, that of the genetic relatedness.

This point partly agrees with the work of Massa *et al* (submitted), highlighting a hidden effect of allelic richness in experimental setups. Altogether, these two studies (Stachowicz *et al*, 2013; Massa *et al*, submitted) and the present work strongly question the claimed influence of genotypic (i.e. clonal) richness on demographic stability, and call for a reappraisal of the relationship between diversity and stability at the intra-specific level, by standardizing the futures experimental and *in situ* studies:

1. By systematically considering both clonal and genetic diversities when studying clonal organisms, and systematically testing their statistical dependence.
2. By elucidating the limits of experimental approach, which could not integrate a number of clones similar to the number occurring in natural meadows. In particular, identifying the spatial scale at which conclusions are relevant for natural populations is crucial.
3. By considering the potentially very large differential of fitness among clones.

Beyond the differential persistence of clones in natural meadows, our results support the ecological importance of genetic diversity as estimated by heterozygosity and/or allelic richness. Based on allelic diversity characterized with a set of putatively neutral microsatellites, these results also support the classic underlying hypotheses that diversity at several unlinked neutral markers is a relevant *proxy* for the overall genome diversity - including genes potentially subject to selection. This work therefore supports the use and interpretation of neutral diversity in conservation genetics and confirms the value and accuracy of markers and data used so far for addressing conservation goals.

Conclusion

Altogether, these results have implications for our understanding of the dynamics and evolution of clonal organisms in general, and for the application of knowledge to conservation strategies. Although the need to incorporate the intra-specific component of biodiversity into current ecological theory has been seriously put forward (Crutsinger *et al*, 2006), and genetic diversity recognized as a dangerously neglected conservation priority, there is an alarming gap between words and actions (Laikre *et al*, 2010). Unfortunately, such lack of action can have severe consequences for a large range of ecosystems, including key coastal ones based on seagrass meadows, which are undergoing an alarming worldwide decrease (Orth *et al*, 2006; Waycott *et al*, 2009). The present results therefore add support to the recent call for a comprehensive integration of the genetic component

of biodiversity into conservation policy, while confirming that both knowledge and tools are available for the development of accurate indicators and efficient monitoring. All that is needed now is action…

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Supporting Information

Removal of the location of Saint-Malo

The cyclical occurrence of the dwarf morphotype *Zostera marina* var. *angustifolia* was previously in the location of Saint-Malo (Becheler *et al*, 2013; Becheler *et al*, 2010). The different ecological features between this morph and the classical may bias analyses as the regulation of density of meadow is mainly determined by the size of ramets. This dwarf morph *Z. marina angustifolia* render cumbersome the comparison of density and epiphyte estimates with the regular *Z. marina* sites. This location was also removed from the analysis, in order to integrate only meadows where the classical morph of *Z. marina* was reported.

Box 1: Measuring intraspecific diversity in clonal species: a couple of definitions.

For such species**,** intraspecific diversity could be studied through two relatively independent components. The first, intensively used in populations genetic and conservation biology, is the genetic diversity, relevant for all organisms. The second is the clonal diversity, also called genotypic diversity, only relevant for clonal species. In order to avoid confusion between these terms, widely used in this work, we provide brief definitions, with a short explanation of adequate indexes for their assessment.

Genetic diversity

The genetic diversity, basal component of biodiversity, is supposed to reflect the evolutionary potential of populations, linked to allelic polymorphism. An amount of indexes is available, each of them having a different evolutionary meaning. Here, the two main indexes we used are:

Observed heterozygosity: proportion of heterozygote loci within the sample. This index is different from Nei's gene diversity, or expected heterozygosity (the probability that two alleles sampled at random from a population are different). The departure between these indexes corresponds to the non-respect of Hardy-Weinberg equilibrium, and is assessed by the inbreeding coefficient F_{IS} .

Allelic richness: a measurement of the number of alleles per locus. Allelic richness favours statistically the level of heterozygosity, even if different mechanisms such as selection, may confuse the relationship. Allelic richness is the more relevant proxy for adaptive 'account' (Widmer and Lexer, 2001).

Clonal diversity or genotypic diversity

Clonality is life-history strategy allowing organisms to produce offsprings without sexual reproduction, hence typically genetically identical – at the exception of possible somatic mutations- to themselves (Arnaud-Haond *et al* 2007).

Genet: entity composed of all the individuals issued from asexual reproduction of their common parent. In case of *Zostera marina*, the genet is the group of shoots connected or previously connected by the rhizome. For evolutionists and populations geneticists, the genet is the genetic individuals.

Ramet: sub-unity of genet. For *Zostera marina*, the ramet corresponds to the sampling unit. In ecology, the ramet is the basal entity, counted for demographic assessment.

Genotypic diversity: reflects the number of different genotypes in a clonal population. It is dependent of the extent of clonality, i.e the differential investment in sexual VS asexual reproductions. Several indexes were proposed for assessment of clonal diversity. Here we use the ones recommended by Arnaud-Haond *et al,* (2007a). Notably, **the clonal richness** R (see material & methods for its expression) is the ratio of the estimated number of genotypes in the population with the number of sampling units. The size of clonal lineages is taken into account in this work, through the **clonal subrange** (Alberto *et al*, 2005).

Supporting Figures

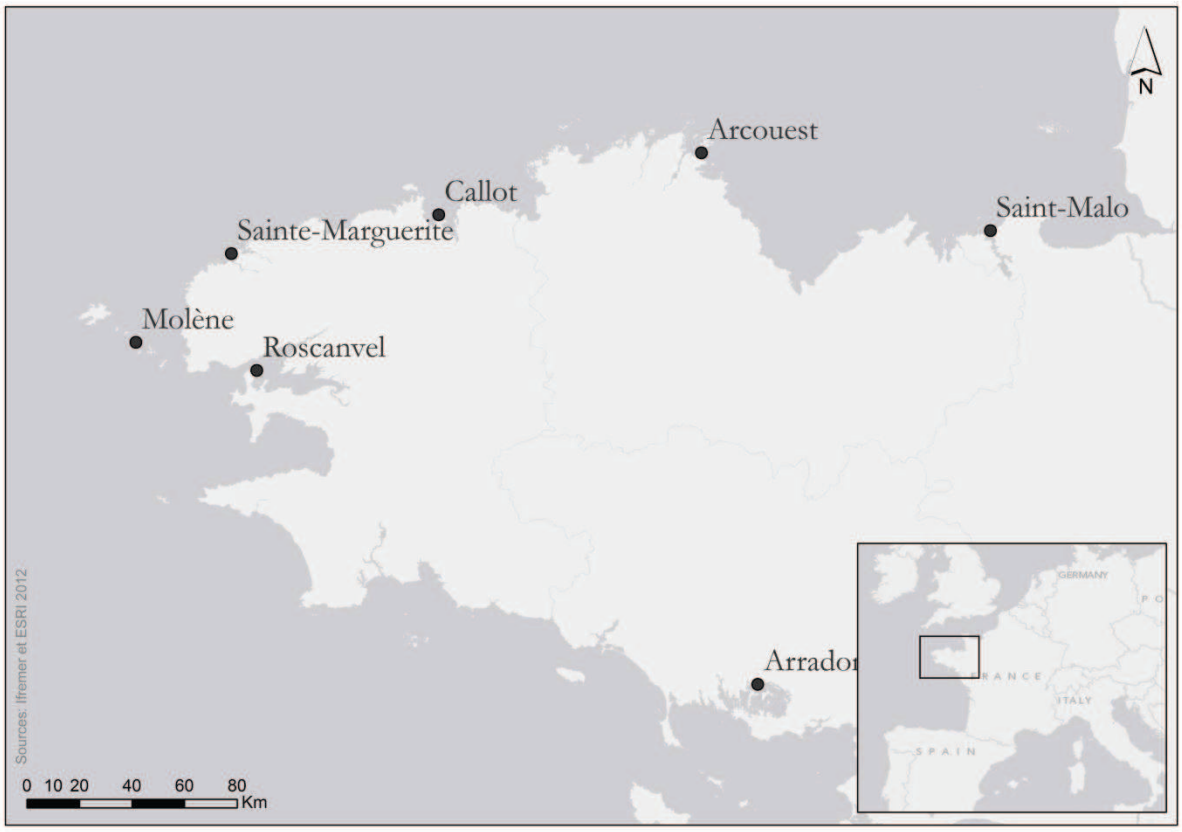

Figure 26 *Location of the seven Zostera marina meadows included in this study.*
*In each location, two sampling quadrats (30*20 m²) separated by several tens meters, were designed for both genetic and demographic/environmental surveys. The meadows of Saint-Malo were not included in this analysis (see above).*

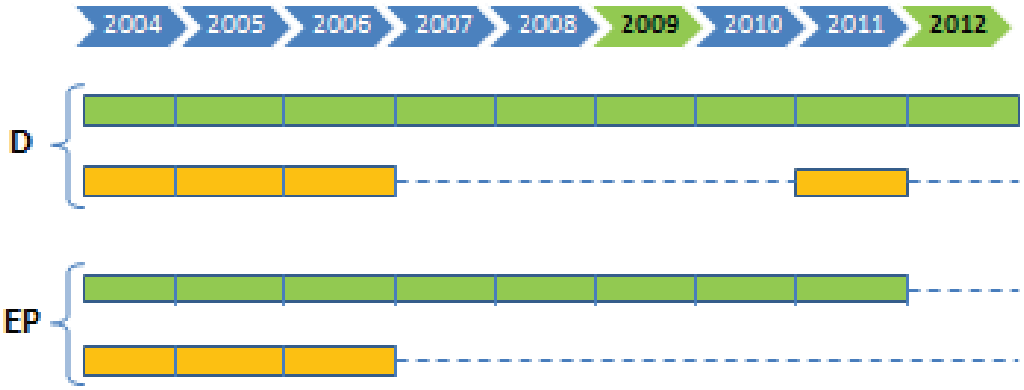

Figure 27 *Availability of demographic (D) and environmental perturbation (EP) data. The years in green indicates the two steps of the genetic survey (2009 and 2012) where the sampling was performed in spring. Green rectangles represent spring data series, while orange rectangles illustrates autumn data series*

Supporting tables:

Tableau 9 *Comparison of the temporal fluctuations of genetic diversity and density (see material & methods). Values correspond to the p-values of the pairwise Behrens-Fisher test. D1: percentage of difference Δ between the mean density of spring and the mean density of Autumn; D2: percentage of difference Δ between the density in spring 2009 and spring 2012. D3: percentage of difference Δ between the density in autumn 2006 and the density in Autumn 2011. D4: percentage of intra-annual difference Δ of density for 2011. %(Ho): percentage of variation of the observed heterozygosity between 2009 and 2012. %(Â): percentage of variation of the allelic richness between 2009 and 2012.*

	D1	D2	D3	D4
% (Ho)	<0,001	<0,001	<0,001	0,062
% (Â)	< 0,001	0,006	0,001	0,27

Tableau 10 *Correlations between the mean values of the disturbance proxies considered in this analysis.*
On the diagonal, the results indicate the correlation between the mean values of spring and autumn, for each proxy. Above and below the diagonal, the results indicate the correlation between the mean values different proxies for spring and autumn respectively.

	WDI	EB	OM	TVSC
WDI	$r^2 = 0.34$; $p = 0.077$	$r^2 = 0.10$; $p = 0.373$	$r^2 = 0.01$; $p = 0.815$	$r^2 = 0.02$; $p = 0.726$
EB	*$r^2 = 0.01$; $p = 0.785$*	**$r^2 = 0.47$; $p = 0.030$**	$r^2 = 0.20$; $p = 0.197$	$r^2 = 0.13$; $p = 0.307$
MO	*$r^2 = 0.03$; $p = 0.651$*	*$r^2 = 0.01$; $p = 0.751$*	**$r^2 = 0.74$; $p = 0.001$**	**$r^2 = 0.68$; $p = 0.003$**
TVSC	*$r^2 = 0.03$; $p = 0.633$*	*$r^2 = 0.08$; $p = 0.416$*	*$r^2 = 0.22$; $p = 0.168$*	**$r^2 = 0.87$; $p < 0,001$**

Tableau 11 *Correlation between the mean values of disturbance proxies and densities, for spring and autumn*.

	Density (Autumn)		Density (Spring)	
	r^2	p-value	r^2	p-value
WDI	0,07	0,46	0,05	0,53
EB	0,05	0,52	0,10	0,38
OM	0,14	0,28	0,00	0,90
TVSC	0,03	0,61	0,00	0,90

Tableau 12 *Results of the residual analyses performed with the mean densities of Spring. No significant correlation was observed. All slopes are negative, either with observed heterozygosity (Ho) and allelic richness (Â).*

	Residuals WDI			Residuals OM			Residuals EB			Residuals TVSC		
	r^2	*p-value*	slope	r^2	*p-value*	slope	r^2	*p-value*	slope	r^2	p-value	*slope*
Ho	0,14	*0,264*	< 0	0,23	*0,137*	< 0	0,18	*0,189*	< 0	0,23	0,134	< 0
Â	0,08	*0,402*	< 0	0,07	*0,425*	< 0	0,02	*0,68*	< 0	0,07	0,427	< 0

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LES CORRELATIONS HETEROZYGOTIE-FITNESS, CHEZ LES PLANTES PARTIELLEMENT CLONALES : EXPLIQUENT-ELLES L'EFFET POSITIF DE LA DIVERSITE GENETIQUE SUR LA DEMOGRAPHIE DES *ZOSTERA MARINA* ?

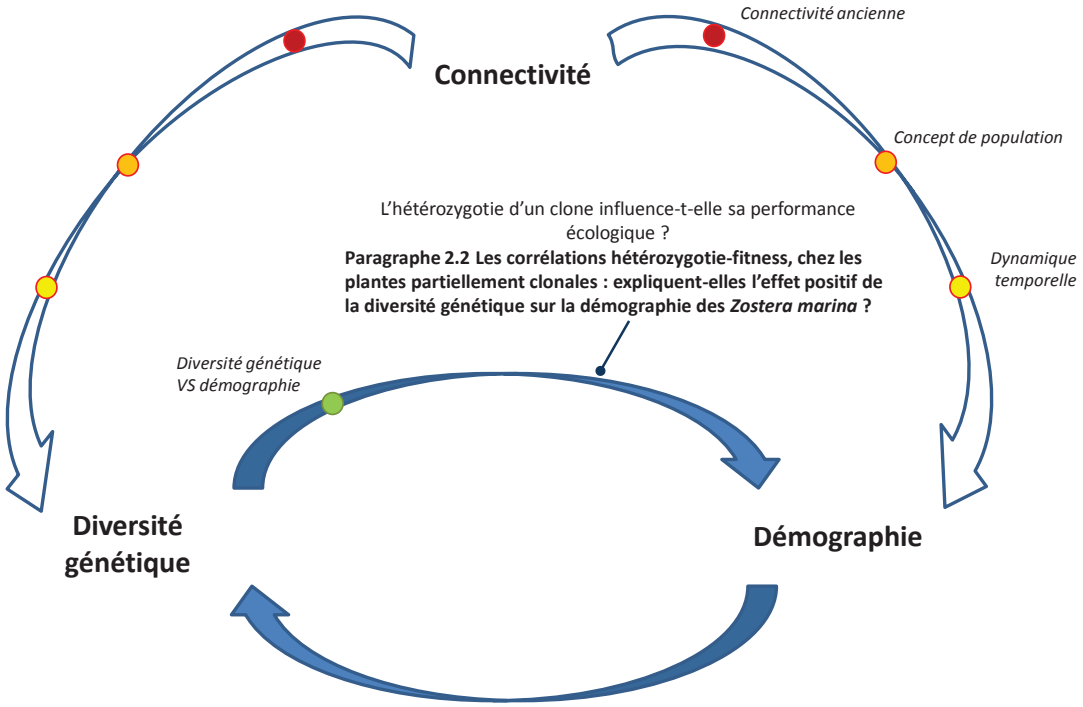

Figure 28 *Etude de l'existence possible de corrélation entre l'hétérozygotie d'une lignée clonale et sa taille, considérée comme proxy de sa performance écologique*

Ce volet ne constitue pas un paragraphe à part entière. Il s'agit plutôt d'un aparté visant à explorer les possibles bases génétiques des différences importantes de persistance et de taille (considérés comme des proxy de la fitness) observées chez les phanérogames marines.

Introduction

Les corrélations entre le niveau d'hétérozygotie et la fitness des individus ont été initialement détectées dans le cas de populations dont la démographie est fortement impactée (Frankham, 1995b; Laikre *et al*, 2010). La dérive génétique occasionnée par une réduction démographique importante conduit à :

- Une perte de variabilité génétique se traduisant, entre autres, par une diminution du niveau d'hétérozygotie des individus sur l'ensemble du génome
- L'augmentation du nombre de loci homozygotes, y compris en présence d'allèles délétères ce qui conduit à une diminution de la fitness.

C'est de ce contexte que peut émerger une corrélation entre hétérozygotie et fitness (David, 1998; Szulkin *et al*, 2010).

Fitness : la notion de fitness dépend de deux points. Le premier correspond à la probabilité d'un individu d'atteindre la maturité sexuelle ; le second correspond au nombre potentiel de descendants viables. *In fine*, ceci se traduit par la probabilité d'un individu de transmettre ses gènes à la génération suivante.

Lorsqu'un clone, ou individu génétique, possède de nombreux réplicats (c'est-à-dire lorsque le clone est de grande taille), le nombre de descendants potentiels augmente en conséquence. Sa fitness semble donc être fonction de sa taille. Ainsi, la taille du clone peut être considérée comme un proxy de la fitness individuelle (Pan and Price, 2001).

Dans la quasi-totalité des herbiers de phanérogames marines, on observe une variance de taille parmi les lignées clonales (Alberto *et al*, 2005; Arnaud-Haond *et al*, 2012; Arnaud-Haond *et al*, 2007b; Becheler *et al*, 2010; Diaz-Almela *et al*, 2007; Diekmann *et al*, 2005; Olsen *et al*, 2004; Reusch *et al*, 1999). Certaines lignées peuvent être dominantes en terme de surface occupée, tandis que d'autres lignées, généralement plus nombreuses, ne présentent qu'un faible nombre de réplicats. Dans les cas extrêmes, des lignées dominantes peuvent aller jusqu'à exclure toutes les autres lignées, et former ainsi des herbiers monoclonaux.

La question que l'on se pose ici est donc : existe-t-il une base génétique à ce différentiel de fitness individuelle, au sein d'un herbier ?

Une étude de Prugnolle *et al*. (2004) a suggéré que la dominance numérique de certaines lignées clonales de schistosomes était liée à leur niveau d'hétérozygotie. Leur conclusion est la suivante :

"In conclusion, in S. mansoni, the fittest clones are the most heterozygous (at least for females). This represents an unexpected consequence of clonality which probably leads

to increased maintenance of polymorphism in these parasite populations."

De même, il est fréquemment observé un excès d'hétérozygotie chez les plantes clonales (Arnaud-Haond *et al*, 2007b; Stoeckel *et al*, 2006). Une hypothèse avancée pour expliquer cet écart à l'équilibre de Hardy-Weinberg serait liée à la clonalité. Les lignées clonales de *Posidonia oceanica* présentes dans les milieux fortement impactés sont globalement de plus grande taille que dans les milieux contrôles (Diaz-Almela *et al*, 2007). Les auteurs de cet article émettent comme hypothèse, une relation entre la plasticité phénotypique des clones, leur conférant la capacité de croître de façon plus rapide et/ou de résister aux perturbations, et la taille que cet avantage leur permet d'atteindre.

Notre objectif est ici de tester l'existence d'un lien entre ces deux observations : un fort différentiel de taille, interprété comme un différentiel de fitness des clones, dans les populations naturelles, et par ailleurs un excès d'hétérozygotes plus souvent observé que dans des populations classiques non clonales. Sur la base des données microsatellites, marqueurs communément reconnus comme neutres (Jarne and Lagoda, 1996), nous avons donc testé ici l'hypothèse d'une super-dominance associative (Szulkin *et al*, 2010) conduisant à une corrélation entre le niveau d'hétérozygotie des clones et leur taille. Le résultat attendu sous cette hypothèse, est illustrée sur la figure 29. Les clones faiblement hétérozygotes seraient en moyenne de plus petite taille tandis les clones fortement hétérozygotes atteindraient potentiellement de grandes tailles.

Avec les marqueurs dont nous disposons ici (microsatellites), nos possibilités de test d'une relation entre ces excès en hétérozygotes et une origine génétique de ces différentiels de taille sont limitées. Il existe plusieurs limitations évidentes à notre approche, d'ordre technique et de l'ordre de l'incertitude biologique :

1) La puissance statistique offerte par un nombre de marqueurs limités ne permettant pas nécessairement d'obtenir un bon proxy de l'hétérozygotie du génome dans son ensemble.
2) Il peut exister, d'après la stratégie d'échantillonnage, des « effets bords » par lesquels certains clones verront leur taille sous-estimée du fait de leur présence en limite d'aire d'échantillonnage (voir correction ci-dessous)
3) On peut concevoir que certaines lignées clonales « jeunes » n'ont pas eu le temps nécessaire d'acquérir leur taille maximale potentielle. C'est cela qui génère le gradient de variance de taille présenté sur la figure 29.
4) Enfin, et plus important, les corrélations hétérozygotie-fitness (HCF) ont été montrées comme résultant de conditions biologiques particulières dans lesquelles nos prairies, quoique pour beaucoup en déclin, ne correspondent pas nécessairement : l'existence de consanguinité, de goulots d'étranglement récents, ou la remise en contact d'entités différentiées (Szulkin *et al*, 2010).

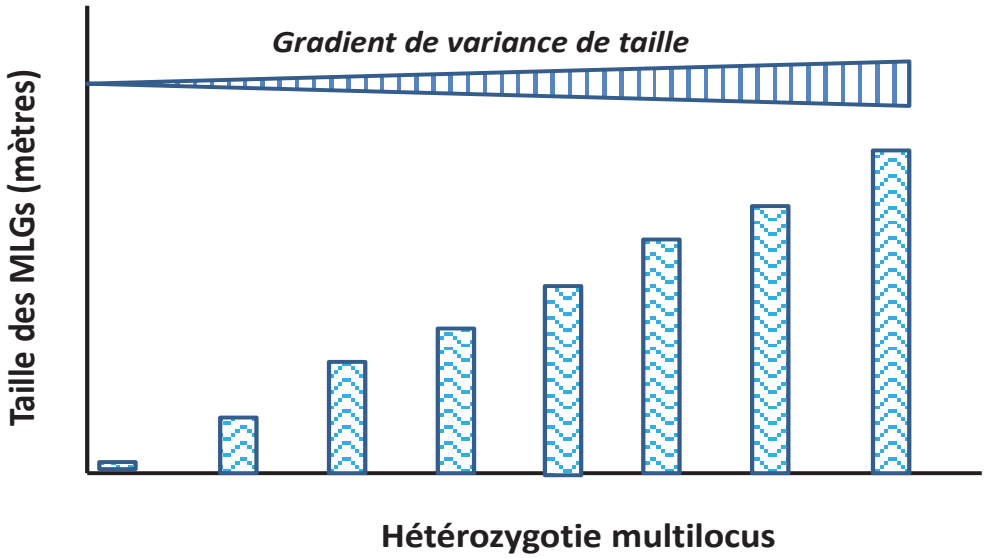

Figure 29 *Attendu théorique de la relation entre hétérozygotie multi-locus et taille en mètres, chez les plantes partiellement clonales, sous l'hypothèse d'une corrélation hétérozygotie-taille.*

Cette brève étude des relations hétérozygotie-fitness chez les phanérogames marines fournira quelques éléments de réflexion pour la discussion sur les relations diversité génétique/démographie.

Méthodologie

2.a. Les espèces étudiées et stratégie d'échantillonnage

Trois espèces de phanérogames marines ont été utilisées ici, pour lesquels les génotypes multilocus sont disponibles (marqueurs microsatellites), suite à des études précédentes : *Zostera marina*, *Posidonia oceanica* et *Cymodocea nodosa*. Les stratégies d'échantillonnages appliquées sont similaires. Pour la zostère, cette stratégie a été détaillée dans les paragraphes précédents (Becheler *et al*, 2013; Becheler *et al*, 2010). Des quadrats de 30*20 m² sont définis au sein des herbiers. Les unités d'échantillonnage sont prélevées suivant des coordonnées cartésiennes (X ; Y) générées aléatoirement, au préalable. Il en est de même pour la posidonie et la cymodocée, dont les unités d'échantillonnage sont prélevées au sein de quadrats dont la taille a été choisie en fonction de la taille approximative des clones estimées sur le terrain et par modélisation lors d'études précédentes, et mesurant respectivement 80*20 m² et 60 * 14m². Ainsi, pour la zostère, 13 quadrats ont été échantillonnés en 2009 sur le pourtour littoral breton. Ces mêmes quadrats ont été ré-échantillonnés en 2012. Des quadrats ont été définis au sein de 36 herbiers de posidonie, répartis sur l'ensemble de la Méditerranée (Arnaud-Haond *et al*, 2010). Distribués en Méditerranée, Golfe de Cadiz, et les côtes Nord-Africaines jusqu'en Mauritanie, 49 populations de cymodocée ont ainsi été échantillonnées (Alberto *et al*, 2008). Pour 25 d'entre elles, les coordonnées aléatoires sont disponibles.

2.b. Génotypage, discrimination clonale et estimations des hétérozygoties multi-locus

Neufs marqueurs ont été utilisés pour la zostère (voir le chapitre précédent), 7 pour la posidonie (Arnaud-Haond *et al*, 2007b) et 8 pour la cymodocée (Alberto *et al*, 2008). Pour chaque MLG défini dans ces études, les loci hétérozygotes sont dénombrés. Ceci détermine le niveau d'hétérozygotie multi-locus (HML) du clone considéré.

2.c. Réduction des effets de bords, et estimation des tailles des génotypes multi-locus

Les effets de bords conduisent à une sous-estimation des tailles des lignées clonales, qui augmente avec l'éloignement de l'unité d'échantillonnage par rapport au centre du quadrat d'échantillonnage (Fig. 30). Afin de limiter ce biais, une correction de l'effet bord a été opérée. Un sous-quadrat, de même centre que le quadrat initial, a été défini, de telle sorte que ses longueur et largeur valent les deux tiers des longueur et largeur du quadrat initial (Fig. 30). Les MLGs présents en un seul exemplaire sont (1) conservés dans l'analyse si leur unité d'échantillonnage a été collectée dans le sous-quadrat, (2) exclus de l'analyse dans le cas contraire. Les MLGs présentant plusieurs réplicats sont (1) inclus dans l'analyse si au moins un des réplicats appartient au sous-quadrat, (2) exclus de l'analyse dans le cas contraire. La figure 30 illustre cette méthodologie de limitation des effets de bords.

L'utilisation des coordonnées cartésiennes permet de calculer les distances entre unités d'échantillonnage. Le proxy de taille des MLGs utilisé ici correspond à la distance maximale entre deux réplicats d'un même MLGs, exprimée en mètres :

Soit : $D_{AB} = [(X_B - X_A)^2 + (Y_B - Y_A)^2]^{1/2}$

Avec A et B les deux réplicats les plus distants d'un MLG donné, et $(X_A ; Y_A)$ et $(X_B ; Y_B)$ les coordonnées cartésiennes des réplicats A et B respectivement. La taille des MLGs possédant un unique représentant dans l'échantillonnage est approximée à 0m.

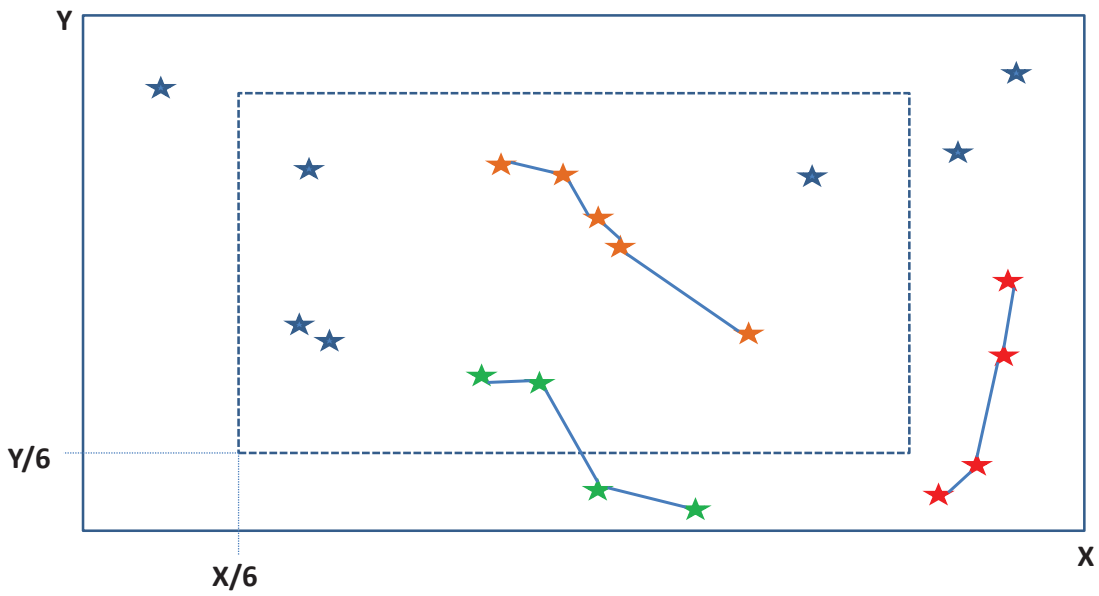

Figure 30 *Schéma de la méthodologie de sous-quadrat.*
Le grand quadrat dessiné en trait plein correspond au quadrat d'échantillonnage initial. Le quadrat intérieur dessiné en pointillé correspond au sous-quadrat, dont longueur et largeur valent les deux tiers des longueur et largeur du quadrat initial. Chaque étoile représente une unité d'échantillonnage. Les étoiles bleues correspondent à des génotypes multi-locus (MLG) présentant un seul réplicat dans l'échantillonnage. Les couleurs orange, rouge et verte symbolisent une lignée clonale chacune, et le trait bleu, les reliant entre elle, peut être vu comme le rhizome. Les lignées clonales orange et verte remplissent les critères pour être incluses dans l'analyse puisqu'au moins un réplicat appartient au sous-quadrat. Le clone rouge, lui, sera exclu. Les MLGs bleus extérieurs au sous-quadrat sont également exclus.

2.d. Sélection des MLGs particuliers

Plusieurs critères de sélection de MLGs particuliers, suspectés de posséder de fortes valeurs de fitness, ont été appliqués. Pour la posidonie et la cymodocée, les 10 plus grands MLGs ont été sélectionnés, et le niveau d'hétérozygotie multilocus (MLH) de ces MLGs est reporté sur la distribution des MLHs de l'ensemble du jeu de données. De plus, 5 herbiers de cymodocées sont monoclonaux, c'est-à-dire composés d'un seul clone géant. Le niveau de MLH de ces clones géants est également reporté sur la distribution globale des MLHs.

Enfin, pour la zostère, des MLG communs aux campagnes d'échantillonnage de 2009 et 2012 ont été observés dans les jeux de données et suspectés d'une capacité de survie accrue puisqu'ils semblent constituer ce que nous avons appelé le « cœur » de l'herbier. Leur niveau de MLH est également reporté sur la distribution globale des MLHs du jeu de données « zostère ».

3.a. Corrélation entre hétérozygotie multilocus et taille des MLGs

Après application de la méthodologie du sous-quadrat, 304 génotypes multi-locus de *Zostera marina*, 389 MLGs de *Posidonia oceanica* et 231 MLGs de *Cymodocea nodosa* sont retenus dans l'analyse. Les tailles des MLGs varient de 0 à 19.1m pour la zostère, de 0 à 78.1m pour la posidonie et de 0 à 55.9m pour la cymodocée. Sur l'ensemble des bases de données « zostère » et « posidonie », les corrélations entre hétérozygotie multi-locus et taille ne sont pas significative ($r^2 = 0.000$; $p = 0.82$ et $r^2 = 0.005$; $p = 0.18$; Fig. 31). En revanche, une corrélation significative est observée chez cymodocée ($r^2 = 0.024$; $p = 0.017$; Fig. 31). Le nuage de points, dans le cas de la cymodocée, présente bien l'allure triangulaire illustrée par la figure 29.

Ces analyses de corrélations ont été également réalisées population par population. Aucune corrélation significative n'a été observée.

3.b. Distribution des hétérozygoties multi-locus des génotypes dits particuliers

Quinze génotypes multi-locus persistants de *Z. marina* sont présents dans les jeux de données de 2009 et 2012. Le niveau moyen d'hétérozygotie multi-locus (MLH) de ces MLGs n'est pas significativement supérieur à l'hétérozygotie moyenne de l'ensemble du jeu de données (test-T unilatéral avec variance inégale : $p = 0.15$). Les 10 plus grands MLGs de *P. oceanica* présentent des tailles variant de 65 à 78.1 mètres. En moyenne, leur niveau de MLH est légèrement supérieur au niveau moyen de l'ensemble du jeu de données, mais cette différence n'est pas significative (test-T unilatéral avec variance inégale : $p = 0.15$). La même analyse menée chez *C. nodosa* conduit à une différence de MLH moyenne significative (test-T unilatéral avec variance inégale : $p = 0.02$). En revanche, la moyenne des MLH des clones composant les 5 prairies monoclonales de *C. nodosa,* n'est pas statistiquement supérieure à la moyenne globale de MLH (test-T unilatéral avec variance inégale : $p = 0.27$). Ces résultats sont illustrés par la figure 32.

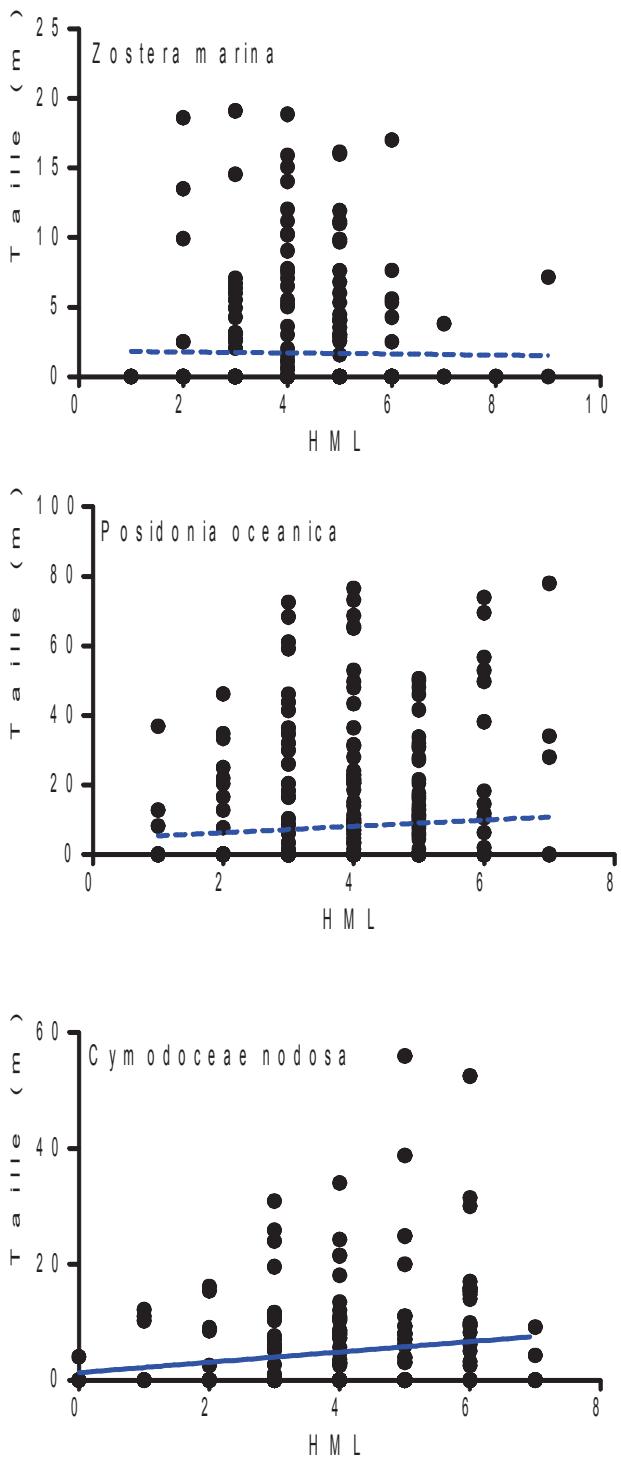

Figure 31 *Régression linéaire entre le niveau d'hétérozygotie multi-locus des génotypes et le proxy de taille de ces génotypes, pour les trois espèces de phanérogames marines considérées dans cette analyse.*

Les corrélations ne sont pas significatives pour Zostera marina et Posidonia oceanica ($r^2 = 0.000$; $p = 0.82$ et $r^2 = 0.005$; $p = 0.18$, respectivement). Pour Cymodocea nodosa, la régression est significative ($r^2 = 0.024$; $p = 0.017$) et l'allure du nuage de points correspond à la forme attendue (voir Figure 29)

Aucune relation claire ne se dégage de ces analyses entre le niveau d'hétérozygotie multilocus des lignées clonales et leur taille. Comme cela est fréquent dans ce type d'analyse biologique, il est difficile ici de conclure à l'absence de relation ou à l'absence de pouvoir statistique, compte tenu des différents éléments techniques et biologiques évoqués en préambule. Ces résultats peuvent donc être interprétés de plusieurs façons qui ne sont pas mutuellement exclusives :

1) Il n'existe pas de relation hétérozygotie-fitness dans ces populations car
    a. elles ne supportent pas de croisements consanguins,
    b. elles ne sont pas issues de remise en contact d'entités divergentes,
    c. le déclin avéré dans certaines prairies n'a pas atteint un seuil suffisant pour être qualifié de goulot d'étranglement au sens génétique du terme,
    d. La véracité de l'hypothèse nulle d'absence de corrélation implique par ailleurs que la clonalité partielle dans les populations ne les rend pas plus propices que les populations non clonales, à l'établissement de corrélations hétérozygotie-fitness en l'absence des trois conditions citées précédemment.

2) La taille n'est pas un bon proxy de la fitness des lignées clonales.

3) Le nombre de marqueurs neutres utilisé et l'échantillonnage ne permettent pas d'établir, quoiqu'elle existe, une relation hétérozygotie-fitness

Toutefois, les résultats significatifs obtenus pour l'espèce *C. nodosa*, et l'augmentation du nombre des clones persistants de *Z. marina* (Fig. 32) avec l'augmentation de MLH, ne nous permettent pas d'accepter l'hypothèse nulle (absence de relation HML/taille), au moins pour la Cymodocée, et incitent à pousser plus avant les analyses sur le sujet.

Le mécanisme fondamental qui génère des patrons de corrélations entre hétérozygotie et fitness est l'identité de déséquilibre (ID), qui correspond à la corrélation entre les niveaux d'hétérozygotie (ou homozygotie) des différents loci d'un génome (Szulkin *et al*, 2010). Contrairement au déséquilibre de liaison qui requiert de la dérive (ou goulot d'étranglement) ou une mise en contact de sous-populations divergentes (admixture en anglais), l'identité de déséquilibre peut se produire dans de grandes populations si des croisements consanguins ont lieu. Sans ce mécanisme, l'état homozygote/hétérozygote d'un locus est indépendant de l'état des autres locus du génome. Le niveau d'hétérozygotie multilocus, estimé au travers d'un jeu de marqueurs réduits, n'est donc pas informatif.

Pour poursuivre ce volet, la première étape consistera à mesurer l'ID dans les populations, suivant des procédures telles que les corrélations hétérozygotie-hétérozygotie (Balloux *et al*, 2004) ou la matrice g_2 (David *et al*, 2007) initialement développée en supposant que l'ID a pour origine l'autofécondation au moins partielle, mais qui reste valide pour d'autres sources d'inbreeding (Szulkin *et al*, 2010). Il est en effet intéressant de souligner que la seule des trois espèces pour laquelle un résultat significatif a été obtenu, est précisément la seule dioïque, chez laquelle l'autofécondation est donc impossible.

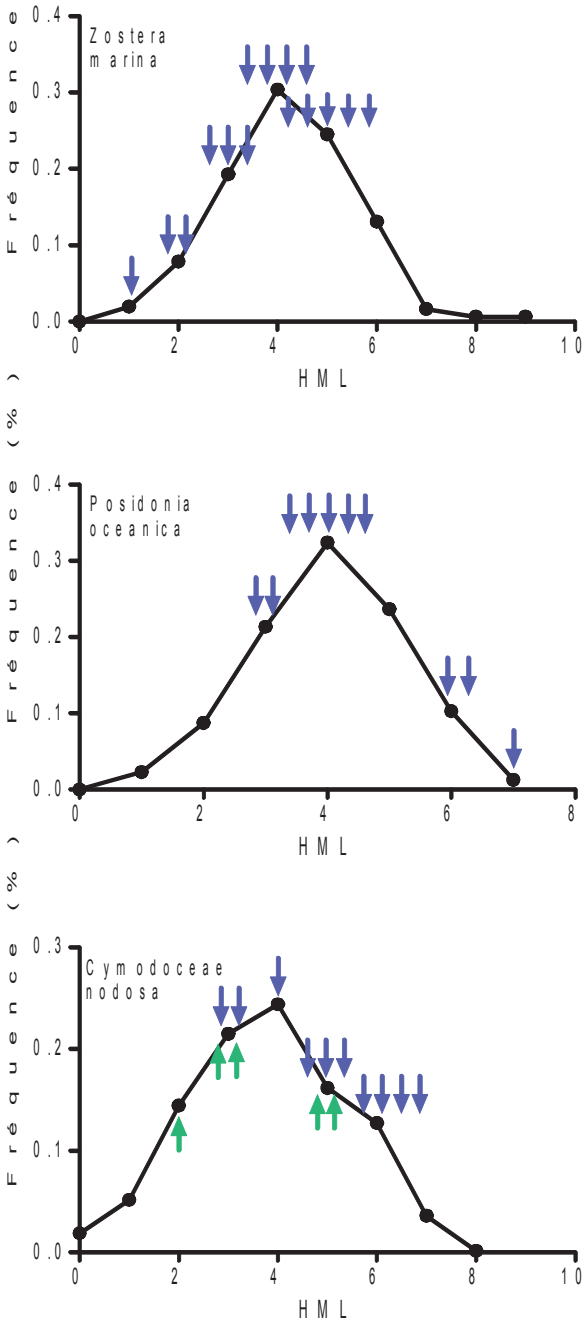

Figure 32 *Distribution des hétérozygoties multi-locus pour les trois espèces de phanérogames. Les flèches représentent les génotypes dits particuliers, et sont positionnées au niveau d'hétérozygotie de leur génotype associé. Pour Zostera marina, il s'agit des 15 lignées clonales partagées entre 2009 et 2012, dont l'hétérozygotie ne diffère pas significativement de l'hétérozygotie moyenne (test-T unilatéral : p = 0.15). Pour Posidonia oceanica, les génotypes sélectionnés sont les 10 plus grands de la base de données (entre 65 et 78.1m). Leur niveau d'hétérozygotie n'est pas significativement supérieure à la moyenne globale (test-T unilatéral : p = 0.15). Pour Cymodocea nodosa, les flèches bleues représentent les 10 plus grands génotypes des herbiers polyclonaux, tandis que les vertes correspondent aux génotypes formant les cinq prairies monoclonales du jeu de données. Dans le premier cas, l'hétérozygotie moyenne des grands clones est supérieure à la moyenne globale (test-T unilatéral : p = 0.15). Les clones géants formant les herbiers monoclonaux, quant à eux, n'ont pas une hétérozygotie moyenne significativement différente de la moyenne globale.*

Amalgamer les populations, comme cela a été fait, augmente artificiellement le nombre d'individus statistiques, mais les résultats manquent de fiabilité. En effet, présenter une hétérozygotie multilocus de 6 sur 9 loci ne signifie pas la même chose suivant si la lignée clonale provient d'une population de richesse allélique forte, ou à l'inverse faible. Une solution serait peut-être de standardiser le niveau de HML des clones par rapport à leur écart à l'hétérozygotie moyenne de leur population. En effet, conduits population par population, les tests menés ne comportaient pas suffisamment d'individus, et aucun résultat significatif, pour chacune des trois espèces, n'a pu être observé.

A partir des données disponibles, une piste à explorer par la suite reposera sur les possibilités de distinguer les lignées clonales, suivant leur appartenance au « core » ou aux « transients » chez *Z. marina*. Si cela s'avère faisable, comparer les HMLs moyens de ces deux catégories pourrait être informatif. Les seules lignées clonales que l'on peut catégoriser dans le « core » sont les lignées clonales qui ont été échantillonnées à la fois en 2009 et en 2012. Pour cela, on a vu que leur nombre se distribue effectivement en fonction du niveau de HML (Fig. 32), quoique l'apparent biais de distribution ne soit pas supporté statistiquement.

Ainsi, le différentiel de fitness entre lignées clonales semble être avéré, étant donné la diversité de taille observée. Est-ce lié à un eff*et al*éatoire ? Un scénario cohérent est le suivant : les premiers clones ayant colonisé l'espace auraient bénéficié d'une compétition interclonale réduite, et auraient pu acquérir une taille conséquente. Dans ce cas et en l'absence de taux de croissance différentielle (bien que nous considérions ce cas comme improbable), aucune origine génétique n'est en cause dans ce différentiel de taille.

Si l'on suppose néanmoins une origine génétique, elle peut se traduire, ou non, dans le niveau d'hétérozygotie des lignées. Le déclin de certaines prairies, leur composition hétérogène faites de clones potentiellement d'origine différente et la capacité de deux des espèces à s'autoféconder, constituent des facteurs pouvant potentiellement générer les HFC. Malgré cela, les conditions biologiques ne sont pas nécessairement réunies pour que la valeur sélective différentielle se traduise par un différentiel d'hétérozygotie.

Les résultats préliminaires issus de simulations indiquent, de plus, que la clonalité partielle implique, à taux croissant, une variance croissante du F_{IS} avec une distribution biaisée vers davantage d'excès en hétérozygotes (Stoeckel *et al*. soumis). Il est donc envisageable que les observations récurrentes d'excès en hétérozygotes dans les prairies ne recouvrent effectivement aucun phénomène sélectif lié à l'hétérozygotie.

Si néanmoins une telle corrélation entre hétérozygotie et fitness existe et que ce résultat est lié à une faible puissance statistique, il faudra trouver une méthode d'analyse permettant d'extraire le signal (qui semble relativement faible d'après résultats préliminaires). Il s'agira ensuite de comprendre le mécanisme sous-jacent. Les HFC, si elles existent pour plantes clonales, sont-elles liées exclusivement à l'inbreeding comme le suggère Szulkin *et al*. (2010) ? La clonalité constitue-t-elle une source potentielle d'HFC, comme le suppose Prugnolle *et al*. (2004)?

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CHAPITRE 4. L'ACTIVITE HUMAINE AU SEIN DES RECIFS DE CORAUX PROFONDS : LE CHALUTAGE DE FOND IMPACTE-T-IL LES NIVEAUX DE DIVERSITE GENETIQUE ET/OU CLONALE DES POPULATIONS DE *LOPHELIA PERTUSA* ET *MADREPORA OCULATA* ?

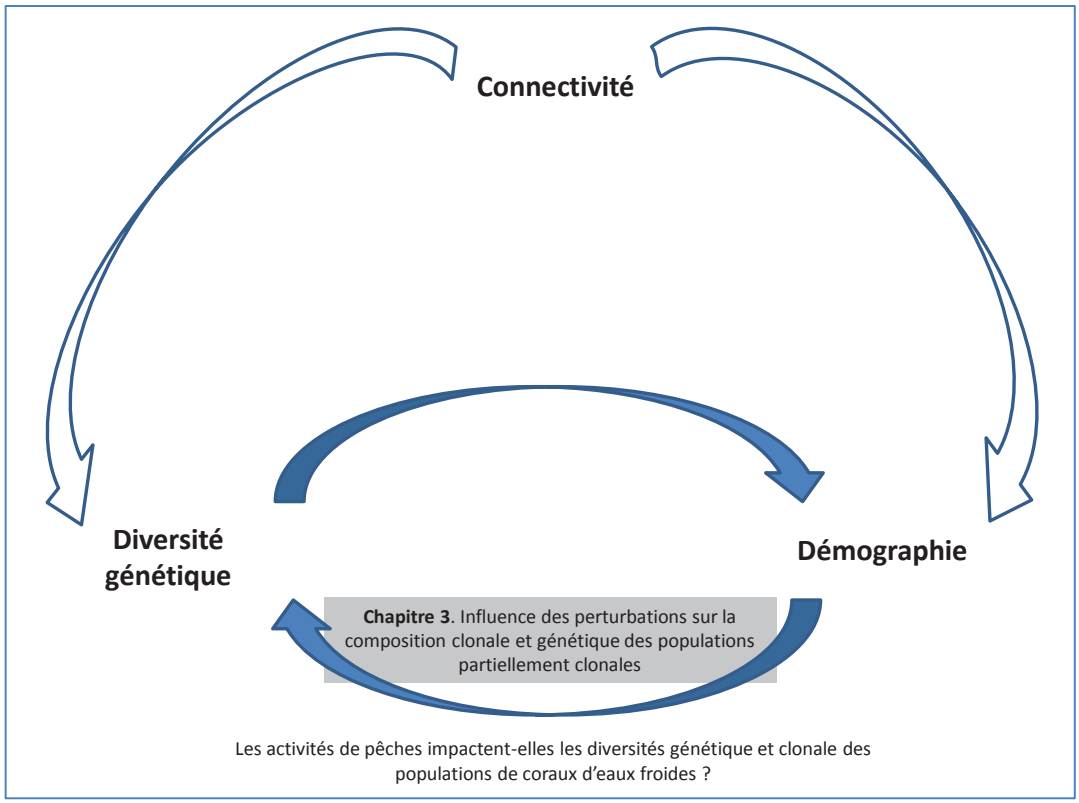

Figure 33 *Etape de l'exploration du schéma de thèse : le sens retour des relations diversité-stabilité*

Contexte & résumé

L'objectif central de ce chapitre est l'étude du sens « retour » des relations diversité-stabilité. En quoi les perturbations affectant la démographie, influencent indirectement les niveaux de diversité génétique et/ou clonale ?

Pour cela, les jeux de données sur les deux espèces de coraux profonds échantillonnées lors de cette thèse, réunissant des données génotypiques, démographiques et données d'impact fournissent un cadre adapté.

Dans le chapitre précédent, un effort particulier avait été mené pour dissocier les rétroactions entre diversité génétique et stabilité des populations. Si l'on souhaite étudier les conséquences des impacts humains sur les niveaux de diversités génétiques et/ou clonales, le signal peut, de la même façon, être masqué par un éventuel effet stabilisant de la diversité génétique (démontrée dans le cas du chapitre précédent). Ceci est particulièrement vrai dans le cas de perturbations faibles n'induisant pas des mortalités systématiques, pour lesquelles des génotypes résistants peuvent exister. Par exemple, il est tout à fait possible d'imaginer un clone de phanérogames plus résistant que la moyenne de la population à une baisse d'intensité lumineuse due à la turbidité de l'eau… Ici, le type d'impact considéré est l'activité de pêche, notamment le chalutage de fond. Face à cette force mécanique, les individus sont tous égaux, leur génome ne déterminant probablement en rien leur probabilité de survie (Fig. 34). Ce type de perturbations peu fréquentes mais extrêmes, dissocie de fait les rétroactions entre diversité génétique et résistance de la population, seule la résilience (capacité à recouvrer une démographie positive) étant potentiellement influencée par la diversité génétique.

En revanche, le degré d'interconnexion entre les récifs de coraux est mal connu et devra impérativement être étudié ici, puisqu'il représente une clé d'interprétation fondamentale des relations entre le niveau d'impact et la démographie ou la diversité intra-spécifique. Les conséquences d'une perturbation, et en particulier la résilience des populations locales, diffèrent en effet de façon évidente suivant que l'on se place en système ouvert ou fermé.

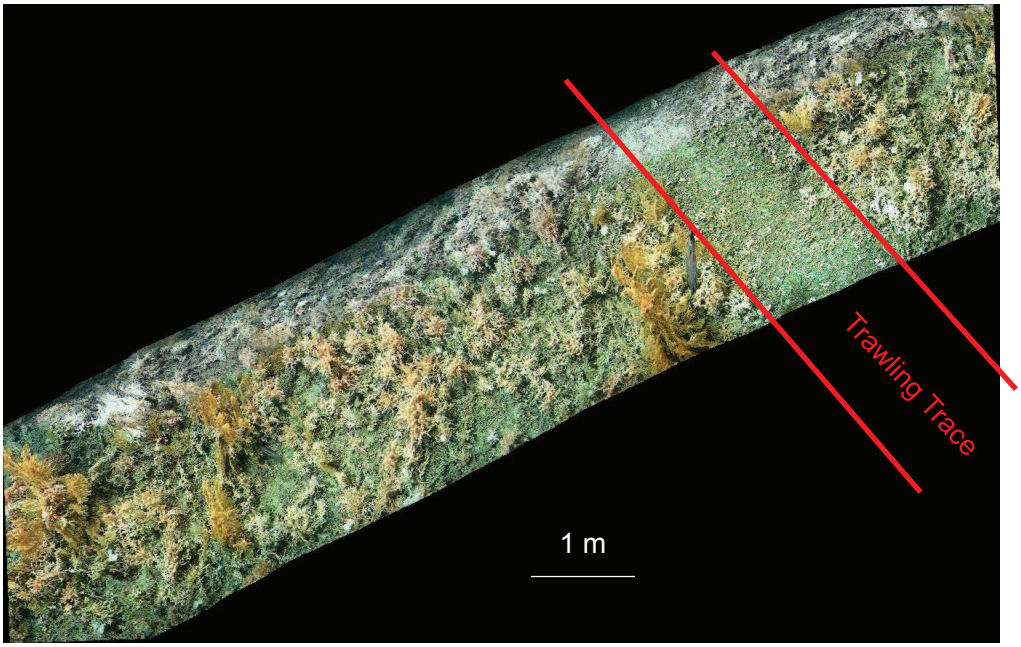

Figure 34 *Un récif du Golfe de Gascogne entaillé par les activités de chalutage. La zone impactée mesure environ deux mètres de large et s'étend sur plusieurs centaines de mètres. Quelles sont les conséquences de ces pratiques de pêche sur la trajectoire évolutive des populations structurant les récifs profonds ?*

CLONALITY, GENETIC DIVERSITY AND FISHING IMPACTS IN THE COLD WATER CORALS *LOPHELIA PERTUSA* AND *MADREPORA OCULATA*

Becheler R., van den Beld I. M. J., Mouchel O., Noël P. and Arnaud-Haond S.

Abstract

Human activities are expanding toward the deeper areas of the oceans, affecting a growing number of ecosystems. Cold-water coral reefs, representing hotspot both for marine biodiversity and carbon and nutrient cycling along continental shelf and slopes, can be impacted by the race toward deeper oil extractions along continental margins, but are above all strongly impacted by fishing activities, in particular bottom-trawling. This calls for an appraisal of human fingerprint on the levels of clonal and genetic diversity of the basal, reef-forming species, in particular *Lophelia pertusa* and *Madrepora oculata*, two important reef-builders. Additionally, the level of connectivity among reefs may provide information to appraise the likelihood of demographic and genetic replenishment in case of local extinction. Reefs from NE Atlantic were sampled, following a standardized sampling procedure, including geo-referencing of each sampling units. Levels of clonal and genetic diversity of both *L. pertusa* and *M. oculata* were assessed, and the degree of interconnection among locations was estimated through genetic structure analysis. These descriptors of intraspecific diversities were crossed with *proxies* of demographic density and fishing intensity. The rate of clonality of these species appears low, and clonal spread is probably very local, rarely exceeding several meters. Clonality through fragmentation may influence the resilience of damaged reefs, but at a rather limited scale. The *L. pertusa* sub-populations likely form a large panmictic unit including the Bay of Biscay, Ireland and Iceland. This revealed a potentially high dispersal ability, susceptible to positively influence resilience of reefs through recolonization. However, the recent or present day dispersal of *M. oculata* appears lower with regional differentiation. Due to the current lack of powerful *proxy* of the trawling intensity, we could only access very indirect proxy that did not allow a powerful test of fishing effect, and did in fact not deliver any significant result, without any possibility to argue for a lack of impact or a lack of statistical power in our analysis. Yet, spatial autocorrelation, only occurring in most preserved reefs, allow suspecting that human affect at least the equilibrium between clonal growth and dispersal.

Key words

Cold water corals, *Lophelia pertusa*, *Madrepora oculata*, clonality, connectivity, anthropogenic impacts

Cold water coral reefs are the structural basis of key deep ecosystems, forming important three dimension biological structures (Rogers, 1999). They support a high biodiversity and biomass (Freiwald *et al*, 2004; Roberts *et al*, 2006), providing habitat and nursery for numerous marine species (Baillon *et al*, 2012). Recently, it has been demonstrated these ecosystems constitute hotspot for the carbon and nutrient cycling of continental margins, far more intense than previously supposed (Cathalot *et al*, submitted). The deep reefs are distributed worldwide (Cairns, 1994; Zibrowius, 1980), along a large depth range in particular along the European Atlantic continental margin (Freiwald and Roberts, 2005), occurring at depth generally varying between 200 and 1000 meters but occasionally found as shallow as 35 and as deep as 4000 meter depth. The two main reef-builders are the species *Lophelia pertusa* and *Madrepora oculata,* co-existing in most of the reefs observed in the Northern Atlantic (com. pers.).

Increasing evidence of impact of human activities is provided by recent research efforts (Ramirez-Llodra *et al*, 2011; Roberts *et al*, 2006). The fishing activities and in particular bottom trawling are recognized as a major threat for CWC, especially in the North-east Atlantic Ocean (Roberts and Hirshfield, 2004). For example, 15 to 50% of the surface of four important Norwegian regions of *Lophelia pertusa* reefs have been shown as seriously damaged or entirely destroyed by trawling (Fossa *et al*, 2002). A recent work (Puig *et al*, 2012) has quantified the intensity of trawling on the upper continental slope of the Bay of Biscay -the typical habitat of *Lophelia/Madrepora* structured reefs. This revealed that scraping and resuspension of the sediment layer imposed by the trawling activity influences the bottom topography at an extent equivalent to that of the natural processes of sedimentation and erosion. Trawling through reefs demolishes the 3D-structures of corals, leading to an area of crushed remains of CWC (Fossa *et al*, 2002), a destruction particularly acute since the development of a method aiming at limiting damages to fishing gears and increasing (on the short term) fisheries efficiency by dragging chains across the bottom ahead of trawls (Roberts, 2002). The strong impact of bottom trawling combined to its frequency is potentially alarming when emphasized with the low growth rates of colonies, assessed between 5 and 25 mm/year (Freiwald *et al*, 1997; Mikkelsen *et al*, 1982; Mortensen and Rapp, 1998), and calls for a reliable assessment of both the impact of trawling on these populations and their capacity of resilience.

Nevertheless, very few is known about the evolutionary ecology of these structuring species and the connectivity among their populations, due to their relatively recent discovery of the remote ecosystems they support, and subsequently recent research activities (Roberts and Hirshfield, 2004).

The two widely distributed species of CWC, *L. pertusa* and *M. oculata*, are partially clonal species, able to alternate both sexual reproduction, through the production of gametes and larvae, and vegetative growth leading to the construction of deep colonies eventually forming reefs. Clonality is a life history trait deeply influencing ecological and evolutionary strategies of clonal, or partially clonal, populations and species, and an important component of population dynamics. The extent of clonality and the estimate of connectivity accounting for the multiple presences of identical

genotypes borne by distinct colonies requires geographical information for each sampling units, a real challenge for population genetics in the deep sea.

An overall assessment genetic structure of *L. pertusa* reefs in Northern Atlantic was reported from a pioneer work (Le Goff-Vitry *et al*, 2004), suggesting a reduced genetic connectivity among locations distributed among the Scandinavian fjords, the continental shelf of the Bay of Biscay and Ireland. Yet, the difficult access to these populations did not allow a systematic and rigorous sampling strategy, which may have impacted results and interpretation.

This work suggests very variable levels of clonal richness, but the highest rate of clonality was detected within populations, for which the sampling strategy was blind trawling rather than video guided collection allowed by Remote Operated Vehicles. Part of these observations may therefore accurately reflect of clonality variation, or may be due to the technical artifact of analyzing distinct parts of a single colony fragmented in the trawl or grab, leading to an overestimation of clonality and possibly to an overestimation of genetic structure among reefs. More generally, descriptors of clonality are largely influenced by sampling procedure (Arnaud-Haond *et al*, 2007a) and relevant assessment of clonal features requires standardized strategy. A priori, the rate of clonality of cold water corals may be low. The recruitment of larvae initiates the formation of a colony, likely corresponding to a unique genetic individual, as observed for the coastal gorgonian *Corallium rubrum* (Costantini *et al*, 2007a; Costantini *et al*, 2007b). Nevertheless, the fragmentation of colonies could be followed by dispersal and recruitment of the broken portion. This leads to a potential multiple occurrence of a genet in space, mimicking the phenomenon of clonal elongation, well known for terrestrial and marine plants (Arnaud-Haond *et al*, 2012; Douhovnikoff *et al*, 2005; Schmid and Harper, 1985). This was observed within a deep reef in Sweden where the estimated distance of clonal elongation reached tens or hundreds meters (Dahl *et al*, 2012). As a result, the extent of the rate of clonality for these species remains poorly understood, as no genetic study was performed on *M. oculata*, and the pioneer collection of *L. pertusa* could not be performed using standardized procedure (Le Goff-Vitry *et al*, 2004). Similarly, the way reefs may rely on each other demographically or for recolonization of depleted areas is still unknown. Here, we aimed at:

1. Characterizing the extent of clonality in both *L. pertusa* and *M. oculata* populations, at large scale and within distinct ecological kinds of deep reefs, distributed along the Northeast Atlantic margin.
2. Assessing the level of genetic connectivity among these populations
3. Assessing the putative human fingerprint on the amount of genetic diversity and/or the clonal architecture for each deep reef

To this aim, deep reefs of the Bay of Biscay, Celtic Sea and Iceland were sampled using a standardized random sampling strategy. The multilocus genotypes of both *L. pertusa* and *M. oculata* were determined using respectively 9 and 7 microsatellites markers. In addition, video surveys performed within each sampling location provided the opportunity to assess the demographic status of these corals and allowed a preliminary evaluation of compared fishing intensity among reefs.

2.1. Study sites and sampling strategy

The Bay of Biscay (BoB), Celtic and Icelandic Seas are three regions in the North-East Atlantic Ocean supporting important Cold-Water Coral populations on their sea-bottom reliefs. The continental slope of the Bay of Biscay is regularly cut by a succession of canyons connecting the continental shelf and the abyssal plain. In this area, CWC reefs are typically located between 600 and 900 meters depth, standing mostly above soft sediment. In general, reefs in Bay of Biscay are relatively sparse, with heterogeneous density, while size of colonies was variable among sites. Eight canyons were explored and sampled during the BobEco cruise (September/October 2011). During this cruise, the region of Logachev mounds on Rockall Bank, in the Celtic Sea was also visited. This area corresponds to a carbonate mound colonized by CW corals, forming reefs denser than in the BoB. In addition, the coverage of dead coral was visually important. Reefs from the South Iceland's continental shelf are shallower (250-400 meters depth), standing also above soft sediment. During the IceCTD cruise (June 2012), two of these reefs were explored and sampled in this region. The first visited location (Londsjup) in the South-East of Iceland stands along a gradient of depth. The deeper portion (400-600m) supports a very dense and healthy reef, composed by continuous coverage of large colonies of *Lophelia pertusa*. The shallower portion was less exuberant, supporting more tiny and disseminated colonies of CWC. Westward, the second location was more scarce exhibiting tiny colonies of both *L. pertusa* and *Madrepora oculata*.

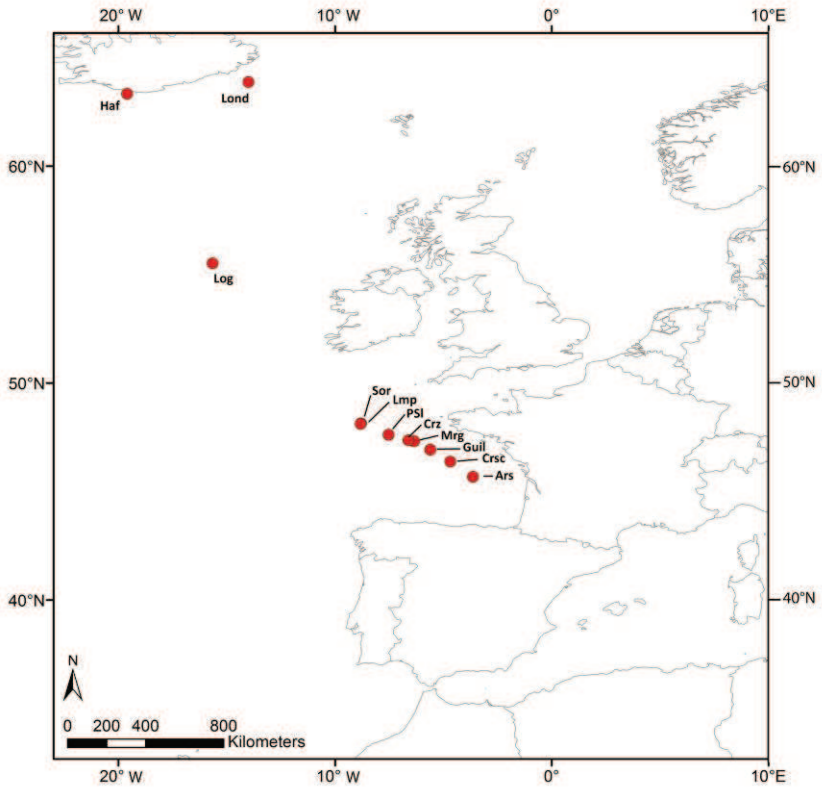

Figure 35 *Sampling locations distributed among the canyons of the Bay of Biscay, the carbonate mounds of Ireland and the icelandic continental slope. These reefs of the NE Atlantic are structured by both Lophelia pertusa and Madrepora oculata. Canyons of: Lacaze-Duthiers (LcD), Ars (Ars); le Croisic (Crsc); le Guilvinec (Guil); Morgat (Mrg); Crozon (Crz); Petite Sole (PS); Lampaul (Lmp); Sorlingues (Sor). Logachev Mound (Log) and locations of Londsjup (Lond) and Hafadsjup (Haf)*

Coral colonies were collected within eight locations supporting reefs (Fig. 35), using the Remotely Operated Vehicle Victor 6000 (Ifremer, France). Two distinct sampling strategies were applied, depending on the objectives of related dives of the ROV. For dives dedicated to genetic sampling, the methodology recommended by Arnaud-Haond *et al,* (2007) for partially clonal species was applied. A 100*200 m²-sampling quadrat was defined, and 35 GPS-coordinates were randomly generated and distributed within the quadrat. For each geographical coordinate, one to three colonies of both *L. pertusa* and *M. pertusa* were sampled. For distinct colonies collected at a single coordinate (different species and different colour morphs for the same species), the distance among them is comprised between 0.5m and 2m. The geo-reference of each sample was corrected during the ROV-dive, depending on the sampling possibilities, e.g. no landing place for the ROV due to the relief of the sea bed or dense reef or absence of colonies.

When dives were dedicated to the exploration, an opportunistic sampling of *L. pertusa* and *M. oculata* was conducted along an exploratory transect. In such cases, geographical coordinates are not available, but such samples were included for connectivity analyses (see below). In addition, videos of vertical and oblique camera were recovered.

Demographic & anthropogenic impact estimates through video analysis

Video analyses were performed for assessment of both demographic status of *L. pertusa* and *M. oculata* in each location and anthropogenic impacts as well. The demography being potentially variable within location, related analyses were performed at the level of the sampling quadrat. Using randomly generated times, 20 pictures extracted from the vertical camera were selected. Several criteria of suitability were defined (i) the altitude of the camera belongs to the interval 0.8m – 2.0m (ii) the clearness of picture is enough to allow distinction between *L. pertusa* and *M. oculata* colonies (iii) the surface of the sea-bed is perpendicular to the vertical camera's axis to avoid distortion of picture.

The surfaces are analysed using ImageJ, a freeware program (http://rsbweb.nih.gov/ij/) that enables the user to select areas and measure them using a scale. The images are calibrated using a calibration grid. Thus, the total surface seen on the image is known, for each picture. This will be used as a scale providing a quantitative measurement of coverage (e.g. in cm^2). The estimated parameters are (i) number of colonies of each species per square meter (ii) the surface of bare sediment (iii) the surface of dead corals (iv) when possible, the surface of each colony used as a proxy of its size related to the age.

Assessment of anthropogenic impact was performed using vertical camera videos along the whole exploratory transect. Each fishing items item was recorded and identified. Among them, fishery items (cables, nets, long lines, trawling traces), and items that may come from fisheries were found. The transect length (without quadrat was measured using ArcGIS®, allowing the assessment of the number of items per kilometre, for each category of items. For Logachev mounds, a different protocol was used, based on the framegrabs taken on board.

DNA extraction, microsatellite amplification and loci scoring

When samples of corals arrived on board, they were immediately processed for DNA extraction. DNA extraction of *M. oculata* samples was instantly performed on fresh tissue, using the classical CTAB methods (Doyle and Doyle, 1988). For each colony, the fresh tissue was from recovered from few polyps, and the remaining part of the colony was preserved in ethanol 96° for back-up, and stored at -80°C. For *L. pertusa* samples, this protocol did not provide a satisfying quality of extracted DNA. Thus, samples were stored in ethanol 96° at -80°C until extraction on land. For *L. pertusa*, tissue was recovered from one to four polyps and dried over-night. DNA was extracted using the Fast DNA®SPIN kit for soil according to the protocol provided by the manufacturer (MP Biomedicals, France).

For *Lophelia pertusa*, 9 microsatellite loci among the 13 proposed by Morrison etal, 2008 were chosen and amplified using the protocol published in Dahl *et al,* (2012), except for the annealing temperature tunned at 55°C in for the present study (Genbank accession numbers: EF577410, EF577411, EF577413, EF577415, EF577416, EF577417, EF577418, EF577420 and EF577423 corresponding to the labels A5, A105, C44, C61, C91, C120, C126, C142 and D3). PCR products were visualized using the ABI-3100 FNVR automated sequencer (Applied Biosystems) and scored using Geneious v5.6.4 (Biomatters®). Two independent readings were done for scoring error corrections. For *Madrepora oculata*, 10 microsatellite loci developed by Morrison *et al* (unpublished) were amplified and scored using a similar protocol. At the moment we write this thesis, the genotyping is finalized for only 7 markers.

Clonal discrimination and genotypic structure

Clonal discrimination was performed for both *M. oculata* and *L. pertusa*, on the basis of the Multilocus Genotypes (MLG) of the sampling units. First, allelic frequencies are assessed using the round-robin method (Parks and Werth, 1993), avoiding the over-estimation of the frequency of rare alleles. The probability P_{sex}, taking into account departures from the Hardy-Weinberg, was assessed. This corresponds to the probability that two identical MLGs originate from distinct reproductive events. When $P_{sex} < 0.01$, the two identical MLGs are considered to belong to the same genet.

Clonal diversity was estimated through a set of three parameters, describing richness and diversity of the clonality (Arnaud-Haond *et al*, 2007a). R is the clonal richness, estimated as the ratio of the number of discriminated *genets* with the number of sampling units:

$$R = (G-1) / (N-1)$$

with G the numbers of genets discriminated for the considered location and N the number of sampling units. When geographical coordinates were available, the distance between the replicates of the same genet was assessed. In addition, the clonal sub-range CR (Alberto *et al*, 2005; Harada *et al*, 1997), corresponding the maximum distance between two replicats of the same genet was

assessed for each location. Estimations of these parameters of clonal diversity and structure were performed with Genclone 2.1 (Arnaud-Haond and Belkhir, 2007).

Genetic diversity and spatial structure

The assessment of index relative to genetic diversity and structure is performed using a derived dataset in which a single replicate of each discriminated MLG is retained. Genetic diversity within quadrats was estimated through three indexes. The mean number of alleles per locus (Â) was standardized to the lowest number of sampling units collected in quadrat (Table 13). The locations sampled during exploratory dives were not taken into account for this standardization. The estimates of observed (H_O) and unbiased (H_E) multilocus heterozygosity (Nei, 1978) were also calculated. Departures from the Hardy-Weinberg equilibrium was tested, using a permutation procedure (1000 permutations) to generate a distribution of the overall inbreeding coefficient F_{is} and to test whether the assessed value of F_{is} is significantly different from 0.

Genetic structure among samples was estimated with θ (Weir and Cockerham, 1984), providing a value of pairwised F_{st} from a procedure of 1000 permutations. The hypothesis of Isolation by distance was assessed through a Mantel test, considering that the distribution of locations following the coastline stands along a linear axis (one dimension). In this case, Rousset (1997) recommended to plot the geographical distance between locations with the F_{st} / (1-F_{st}).

Autocorrelation analyses were also performed to estimate evolution of the pattern of clonal extension and spatial genetic structure "SGS" at the within-quadrate level. We used the kinship estimator coefficient of Ritland (F_{ij}) as a genetic relatedness statistic (Ritland, 1996). We performed regression analyses of mean F_{ij} against the Log_e of mean geographic distance, within each distance class. This allowed us to test the adequacy of IBD models in each quadrat. These analyses are first performed including all sampling units, notably the replicates. In this case, the SGS is mostly influenced by the spatial extent of clones. In a second time, spatial autocorrelation is assessed using a procedure of 1000 permutations, in order to include only one replicate from each MLG, at each permutation (randomly chosen one of the possible geographical coordinates). This second step aims to examine the dispersion through sexual propagules, at the within-quadrat scale. The slope of regression (b) allowed us to calculate the S_p-statistic (Vekemans and Hardy, 2004). This statistic corresponds to the spatial autocorrelation profile, varying from 0 (no limitation to gene dispersal at the scale of the sampling) to +∞ (theoretical case, where the structure is maximal). Its equation is the following:

$$S_p = \frac{-b}{1 - F_{(1)}}$$

with $F_{(1)}$ the kinship value for the first distance class. Autocorrelation parameter estimations were performed with GENCLONE 2.1 (Arnaud-Haond and Belkhir, 2007).

Demographic status, size of colonies and relationships with anthropogenic impacts

The mean density varies among locations, ranging from 0 to 1.4 colonies per square meter for *L. pertusa* and from 0 to 1.6 for *M. oculata*. Within the canyon of Crozon in Bay of Biscay and the location of Londsjup in Iceland, no colony was observed on the randomly selected pictures for *M. oculata* and/or *L. pertusa* (Fig. 36), reflecting the important patchiness of reefs. The random procedure of pictures used for the density assessment did not allow capturing this patchy distribution. In the Bay of Biscay and Celtic Sea, the mean size of *M. oculata* colonies appears relatively constant among canyons, ranging from 0.006 to 0.013m², while the population of Hafadsjup (Iceland) exhibited larger colonies (0.023m², but see Fig. 36). This parameter is more variable in *L. pertusa*, showing a larger variability within the Bay of Biscay.

No significant relationship was observed between these descriptors of the demographic status and the levels of fishing intensity FI assessed through video analyses), when considering the mean density (FI vs density : $R^2 = 0.16$; $p = 0.28$ and $R^2 = 0.24$; $p = 0.18$ for *L. pertusa* and *M. oculata* respectively). The same lack of relationship was observed when considering the mean size of colonies (FI vs size : $R^2 = 0.06$; $p = 0.54$) for *M. oculata*, whereas for *L. pertusa* populations, this regression is significantly positive ($R^2 = 0.82$; $p = 0.005$, yet this appears driven by the location of Morgat exhibiting both some large colonies and strong indices of fishing pressure in other parts of the reef(Fig. 39). When removing this location, the relationship did not remain significant ($R^2 = 0.09$; $p = 0.56$).

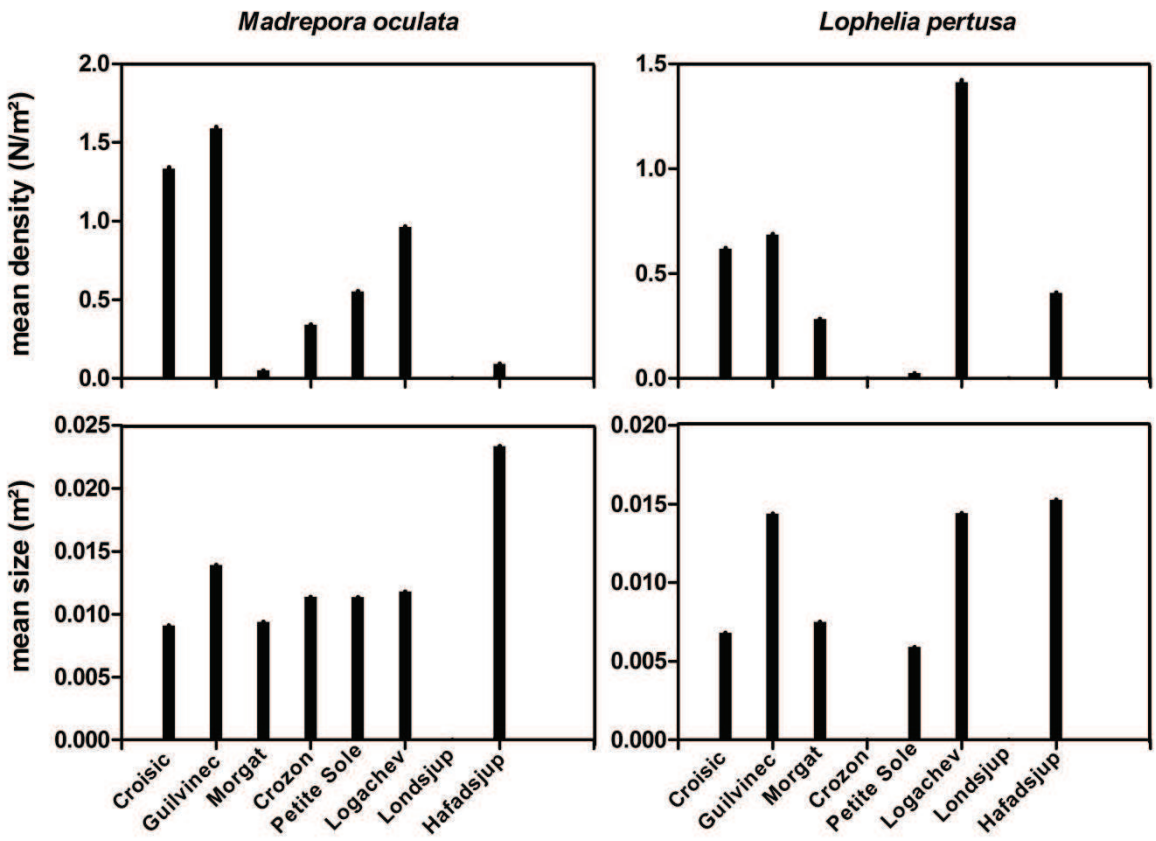

Figure 36 *Demographic status of Lophelia pertusa and Madrepora oculata populations from the sampled reefs, distributed among eight locations.*
Both densities and sizes of colonies were assessed through picture analyses. Due to the patchiness of reefs (Canyon of Crozon and Londsjup), no colony M. oculata and/or L. pertusa were found on randomly selected pictures.

Clonal diversity and spatial extent of clones

The levels of clonal richness are very high for both species, indicating low rate of clonality at the grain scale analysed. The clonal richness ranged from 0.88 to 1 for *L. pertusa* (Table 13) and from 0.80 to 1 for *M. oculata* (Table 14). Within the *L. pertusa* dataset (265 sampling units), only 13 MLGs with replicates were found. The distance between replicates could be captured by the assessment of the clonal subrange CR (Table 13) for 11 of the 13 repeated MLGs (the geographical coordinates of the two remaining were not available). For seven MLGs, the assessed distance was null, indicating that the replicates were collected on distinct colonies neighbouring each other and sharing the same GPS coordinates and their real distance is likely in the order of one meter or less (see material and methods). The distance between the replicates of the four last MLGs was of 98m (Canyon of Croisic), 17m (Canyon of Le Guilvinec), and finally 23 and 152m (quadrat 1 of the Canyon of Petite Sole).

Similarly, within the *M. oculata* dataset (283 sampling units), 16 MLGs with replicates were detected, among which 11 leads to null distance between replicates. For the 5 remaining MLGs, the distance ranged from 11m (Canyon of Croisic) to 92m (quadrat 1 of the Canyon of Petite Sole).

No clear relationship emerged from the crossing of fishing intensity and the clonal richness (FI *vs* R : $R^2 = 0.30$; $p = 0.13$ and $R^2 = 0.03$; $p = 0.63$ for *L. pertusa* and *M. oculata* respectively). While no significant correlation was observed between the fishing intensity and the CR, neither for *L. pertusa*, nor for *M. oculata* ($R^2 = 0.02$; $p = 0.75$ and $R^2 = 0.06$; $p = 0.51$ respectively), these parameters appear related for the three *L. pertusa* populations of Bay of Biscay where the CR is not null (FI *vs* CR: $R^2 = 0.99$; $p = 0.04$), indicating higher clonal fragmentation/dispersal with increased fishing pressures.

Genetic diversity and departures from Hardy-Weinberg equilibrium

For both species, a strong homogeneity of the values of allelic richness and heterozygosity was observed. Standardized allelic richness varies from 11.9 to 13.9 and from 3.5 to 5.5 alleles per locus for *L. pertusa* and *M. oculata* respectively (Table 13 and 14). Such constancy is found for observed heterozygosity too, ranging from 0.74 to 0.89 for *L. pertusa* populations, but seem slightly more variable for *M. oculata*, as this index ranged from 0.33 to 0.62 (Table 14). Regarding the locations where the sampling was performed during exploratory dives, the values of allelic richness appear low, likely due to the low number of sampling units collected in each of these locations (Tables 13 and 14). In the Bay of Biscay, the Canyon of Le Guilvinec and Morgat support *L. pertusa* populations exhibiting no significant departures from Hardy-Weinberg equilibrium (HWE). All other locations are characterized by significantly positive values of F_{is} (Table 13), comprised between 0.03 and 0.18. In the Bay of Biscay, such departures from HWE within *M. oculata* populations can reach strong levels between 0.15 and 0.19 (Canyons of Guilvinec -quadrat 2- Morgat and Petite Sole –quadrat 2-Table 14), but appears close to zero within for the Canyons of Croisic and Petite Sole (quadrat 1). For both species, highly significant F_{is}-values were recorded in the Celtic and Icelandic Seas, reaching 0.1 to 0.3 (Tables 13 and 14).

Genetic structure among and within reefs

Two contrasting patterns of genetic structures were obtained for the two species of corals. A weak genetic structure occurred among *L. pertusa* populations, F_{st}-values being in the large majority low and non-significant (Table 15) and the consequent Mantel's test revealing also a non-significant pattern of Isolation-By-Distance (Fig. 37; $R^2 = 0.00$; $p = 0.83$). A stronger genetic structure was observed among *M. oculata* populations, and suggests a regional pattern. In the Bay of Biscay, the values of pairwise-F_{st} are null or almost between quadrats from the same canyon (Canyons of Petite Sole and Le Guilvinec), but reached 0.12 between the samples of Lampaul and the quadrat 1 of Petite Sole (Table 16). The populations of Logachev and Iceland are genetically divergent and also differentiated from the populations of the Bay of Biscay. The Mantel's test confirmed this regional

pattern of the genetic structure (Fig. 38), leading to a significant relationship between geographical and genetic distances for each pairs of locations ($R^2 = 0.49$; $p < 0.0001$).

Fine-grained spatial genetic structure was rarely significant (Tables 13 and 14). Significant values of the S_p-statistic within *L. pertusa* populations were found in the quadrat 2 of the Canyon of Petite Sole (Sp = 0.089 ; p = 0.039)and within the reef of Logachev ($S_p = 0.101$; $p = 0.034$). In the *M. oculata* dataset, such significant fine grained structure was only observed within the Icelandic location of Londsjup ($S_p = 0.419$; $p = 0.008$).

Tableau 13 *Clonal and genetic diversities of Lophelia pertusa populations, assessed using 7 microsatellite markers. N, number of sampling units, corresponding to one colony; G, number of detected MLGs; R, the clonal richness; CR, the clonal subrange in meter; Â(16 the clonal richness standardized for 16 sampling units; H_e, $H_{n.b}$ and H_o, expected, non-biased and observed heterozygosity; F_{is}, inbreeding coefficient and S_p, statistic of spatial autocorrelation using the Ritland's coefficient. *, p < 0.05; **, p < 0.1 and ***, p < 0.001. Grey locations indicate canyons where the sampling was performed along exploratory transect.*

	Clonal architecture				Genetic diversity					
	N	**G**	**R**	**CR**	**Â(16)**	**He**	**Hn.b**	**Ho**	**Fis**	**Sp**
Croisic Canyon Q1	39	37	0.95	98.1	13.77	0.89 ± 0.08	0.9 ± 0.08	0.83 ± 0.11	0.075***	0.000
Guilvinec Canyon Q1	24	23	0.96	0.00	13.237	0.88 ± 0.07	0.9 ± 0.08	0.87 ± 0.07	0.036	0.000
Guilvinec Canyon Q2	16	15	0.93	17.4	12.00	0.86 ± 0.08	0.89 ± 0.08	0.89 ± 0.11	-0.004	0.164
Morgat Canyon Q1	27	24	0.88	0.00	13.47	0.87 ± 0.08	0.89 ± 0.09	0.89 ± 0.12	-0.003	0.023
Crozon Canyon Q1	13	13	1.00	0.00	*11.86*	0.84 ± 0.09	0.88 ± 0.09	0.78 ± 0.14	0.115**	0.050
Petite Sole Canyon Q1	27	26	0.96	151.9	13.61	0.87 ± 0.09	0.89 ± 0.09	0.84 ± 0.12	0.063**	0.000
Petite Sole Canyon Q2	31	29	0.93	0.00	13.86	0.89 ± 0.06	0.91 ± 0.06	0.86 ± 0.11	0.057**	**0.089**
Lampaul Canyon	*7*	7	1.00	-	*8.86*	0.83 ± 0.1	0.89 ± 0.11	0.86 ± 0.12	*0.04*	-
Sorlingues Canyon	*3*	3	1.00	-	*5.00*	0.78 ± 0.05	0.93 ± 0.05	0.86 ± 0.18	*0.1*	-
Logachev Mounds	25	25	1.00	0.00	13.27	0.88 ± 0.07	0.9 ± 0.07	0.81 ± 0.15	0.102***	**0.101**
Londsjup transect	*9*	8	0.88	-	*9.43*	0.82 ± 0.1	0.88 ± 0.11	0.77 ± 0.18	0.135**	-
Londsjup Q1	24	24	1.00	0.00	12.25	0.85 ± 0.11	0.87 ± 0.11	0.76 ± 0,15	0.129***	0.113
Hafadjup Q1	20	19	0.95	0.00	12.8	0.88 ± 0.06	0.91 ± 0.06	0.74 ± 0.20	0.183***	0.125

Tableau 14 *Clonal and genetic diversities of Madrepora oculata populations.*

*These proxies were assessed using 7 microsatellite markers, expect for the locations Londsjup Q1 and Hafadsjup Q1 for which only 6 markers were available for instance. N, number of sampling units, corresponding to one colony; G, number of detected MLGs; R, the clonal richness; CR, the clonal subrange in meter; Â(16 the clonal richness standardized for 16 sampling units; H_e, $H_{n.b}$ and H_o, expected, non-biased and observed heterozygosity; F_{is}, inbreeding coefficient and S_p, statistic of spatial autocorrelation using the Ritland's coefficient. *, p < 0.05; **, p < 0.1 and ***, p < 0.001. Grey locations indicate canyons where the sampling was performed along exploratory transect.*

	Clonal architecture				Genetic diversity					
	N	**G**	**R**	**CR**	**Â(17)**	**He**	**Hn.b**	**Ho**	**Fis**	**Sp (1000)**
Croisic Canyon Q1	35	35	1.00	0.00	4.97	0.64 ± 0.10	0.65 ± 0.10	0.62 ± 0.16	0.05	0.012 (30)
Guilvinec Canyon Q1	28	26	0.93	10.8	4.44	0.62 ± 0.17	0.63 ± 0.17	0.58 ± 0.27	0.08*	0.113 (28)
Guilvinec Canyon Q2	17	16	0.94	0.00	5.00	0.67 ± 0.14	0.69 ± 0.17	0.56 ± 0.27	0.19***	0.000 (15)
Morgat Canyon Q1	30	30	1.00	0.00	4.66	0.59 ± 0.16	0.60 ± 0.16	0.51 ± 0.25	0.15***	0.000 (28)
Crozon Canyon Q1	23	22	0.95	0.00	4.776	0.58 ± 0.22	0.59 ± 0.22	0.50 ± 0.23	0.16**	0.000 (23)
Petite Sole Canyon Q1	24	22	0.91	92.4	4.60	0.59 ± 0.20	0.60 ± 0.20	0.58 ± 0.27	0.04	0.161 (23)
Petite Sole Canyon Q2	28	27	0.96	0.00	4.96	0.63 ± 0.13	0.64 ± 0.14	0.55 ± 0.24	0.16***	0.119 (27)
Lampaul Canyon	*12*	12	1.00	-	*4.42*	0.59 ± 0.24	0.62 ± 0.25	0.58 ± 0.24	0.06	-
Logachev Mounds	27	25	0.92	0.00	5.47	0.55 ± 0.29	0.56 ± 0.29	0.47 ± 0.29	0.16***	0.022 (27)
Londsjup transect	*8*	6	0.71	-	*3.57*	0.46 ± 0.28	0.50 ± 0.30	0.33 ± 0.30	0.35***	-
Londsjup Q1	30	28	0.93	74.7	3.49	0.45 ± 0.28	0.46 ± 0.29	0.33 ± 0.32	0.29***	**0.419 (30)**
Hafadjup Q1	21	17	0.80	0.00	4.49	0.58 ± 0.24	0.60 ± 0.25	0.45 ± 0.33	0.25***	0 .000 (19)

Discussion

Demographic status

Geography, in particular the modality of the distribution of colonies within reefs, affects the assessment of the demographic status and makes comparison among regions cumbersome. Notably, the Icelandic reefs from Londsjup are formed by punctual large aggregates of extremely large colonies (reaching more than 1.5 to 2m in height) of corals separated by soft sediments. The very low, if not null, assessed densities do not reflect the real demography of these reefs, at least in comparison of zones in the Bay of Biscay where more homogeneous and slightly denser areas of incomparably smaller (up to 10 to 30 cm) colonies were observed. Fishing activity in Iceland is intense, as revealed by the numerous observations of longlines crossing the reefs, whereas no trawling traces were observed. This partly explains the occurrence the large coralline formations as the impact of longlines, although not null, is negligible compared to that of bottom trawling (Weaver *et al*, 2011). In the Bay of Biscay and Logachev Mounds, where the morphology of reefs are similar, characterized by a relatively homogeneous distribution of rather small colonies, differences in both density and size of colonies were detected. This differential demography could be due to either historical and/or ecological differences among canyons, to various levels of fishing impacts, or to a combination of these two former hypotheses. The lack of significant relationship between the demographic descriptors and index of fishing activities prevent to favor one of these explanations. The fishing intensity was assessed by counting fishing items along exploratory transects in each canyon, reflecting also a mean intensity at the scale of the canyon. However, this intensity is very heterogeneously distributed, fishing activities being concentrated in the north flank and the head of canyons (Bourillet, pers comm). The *proxy* used may be subject to a large variance and not fully reflect the fishing intensity affecting the sampled reefs. At the moment we write this thesis we are still awaiting a much better *proxy* based VMS data that would allow a more reliable quantification of the intensity of trawling in different zones, yet authorization to use those otherwise public data was asked to the “Direction des pêches maritimes et de l'aquaculture” which has not yet delivered this authorization.

Features of clonality in the populations of cold-water corals

Low level of clonality and its predominant local incidence, revealed by the proximity of most colonies derived from the same genets and sharing the same MLG, are in agreement with a previous fine grained study of Northern reefs (Dahl *et al*, 2012) *of L. pertusa. These results* led the authors (Dahl *et al*, 2012) to conclude that ‘*This suggests that Lophelia does not develop asexual larvae and that coral fragments are not transported over long distances*’. However, clonal richness values were higher in the present work than in previous studies. In both cases, differences reflect sampling strategy. First, the clonal richness (with R comprised between 0.8 and 1 for both species) obtained here is much

higher, than the values reported by Le Goff-Vitry *et al*, (2004) for European *L. pertusa* reefs collected in similar spatial areas (R lower than 0.1 for several locations sampled by trawling). Our results may largely be explained by the fact that samples were collected and conditioned individually, preventing the fragmentation of colonies during sampling and their incidental duplication in the dataset artificially lowering R estimates. Second, high value of clonal richness assessed for both *L. pertusa* and *M. oculata* indicate that sexual reproduction is largely dominant, and an only weak proportion of colonies originate from clonal dispersal. The lower values reported in Northern Sweden by Dahl *et al,* (2012) is likely due to the different grain size used for sampling. Higher local density in their small scale study favored the systematic sampling of adjacent colonies that, as they reported and we observed are the most likely to be clonemate. For both species, only 5% of collected colonies were indeed issued from an event of fragmentation/recruitment of the same initial colony. Thus, the random sampling strategy retained here allow us to we confirm that the Swedish case is not specific to the reef studied. The phenomenon of clonal extension remains rare and local as the two thirds of replicated MLGs occurred when adjacent colonies were collected at the same sampling points. Occasionally, replicates of MLGs are separated by large distances, between tens to hundreds meters, as indicating by the values of clonal subrange (Tables 13 and 14). The more likely explanation for such values of clonal extension implies a mechanical action affecting the reefs (Dahl *et al*, 2012). Despite the lack of significance of the relationship between fishing intensity index and the CR, first elements may suggest that this clonal dispersal represents a human fingerprint within CWC reefs (Fig. 40). Indeed in the same way as in this previous study, long distances among fragments were mostly observed in trawled areas.

Besides, detecting replicated MLGs when using a low sampling (compared to population) density may alert on the level of replicates needed in a given population to be sampled with a low density strategy. This leads us to suspect that clonality may still have a local influence on population dynamics, particularly in impacted areas.

Genetic diversity and structure at large and fine grained spatial scales

Both *L. pertusa* and *M. oculata* populations exhibit very homogeneous levels of genetic diversity. Comparisons with previous studies about *L. pertusa* genetics remain tricky, as besides sampling strategy, the number or nature of microsatellites differs with the present work. However, a similar consistency of allelic richness values was formerly observed in the northeast Atlantic (Le Goff-Vitry *et al*, 2004) and Scandinavian fjords (Dahl *et al*, 2012). The total panmictic snapshot obtained for *L. pertusa* (Table 15 and Fig. 37) suggests both high dispersal potential and strong levels of present day or recent connectivity at the scale of the whole Northeast Atlantic, in contrast with the pioneer work of Atlantic (2004)Le Goff-Vitry *et al,* (2004). Such discrepancies are likely due to the same problem of blind sampling previously detailed. Jointly considering the lack of regional and inter-canyon genetic structure, the homogeneity of genetic diversity values and the global absence of fined grained SGS, the dynamics of *L. pertusa* seems to fit a rather large dispersal with high turn-over and a lack of competition for space in most reefs, maintaining high levels of clonal richness.

Yet, significant fine-grained SGS was detected in the quadrat 2 of the Canyon of Petite Sole where a saturated density (Fig. 41) of colonies, indicating a total absence of fishing activity, was revealed by ROV-diving observations (unfortunately, the demographic status of this particular reef could not be assessed due to video failure during ROV dive) and in Logachev Mounds beneficiating of a protected status for fishing activities (no fishing items were recorded within this site). These strong patterns of spatial autocorrelation, occurring in the two more healthy reefs explored during the BobEco cruise (see Material and Methods), indicates different dynamics of recruitment and space occupation in long term undisturbed reefs. It can be underlined as well that the healthy Icelandic reefs also exhibited nearly significant SGS ($p=0.06$ for Londsjup). In addition, this suggests that, despite the very low growth rates of colonies, the equilibrium between dispersal and clonal growth in *L. pertusa* populations can be reached in absence of fishing pressure.

Tableau 15 *Pairwise Fst-matrix using the ϑ estimator, after 1000 permutations, among Lophelia pertusa populations. Nine microsatellite markers were included in this analyses *, p < 0.05; **, p < 0.1 and ***, p < 0.001.*

	Croisic_Q1	Guilvinec Q1	Guilvinec Q2	Morgat Q1	Crozon Q1	Petite Sole Q1	Petite Sole Q2	Lampaul_Tr	Sorlingues_Tr	Logachev Q1	Londsjup_Tr	Londsjup Q1	Hafadjup Q1
Croisic_Q1	-	0.002	0.003	0.000	0.010*	0.000	0.000	0.007	0.000	0.005	0.000	0.005	0.004
Guilvinec Q1		-	0.008	0.004	0.020**	0.002	0.000	0.007	0.015	0.010*	0.000	0.003	0.001
Guilvinec Q2			-	0.005	0.024**	0.005	0.005	0.002	0.000	0.015**	0.001	0.010	0.012*
Morgat Q1				-	0.013*	0.000	0.001	0.004	0.006	0.012*	0.000	0.007	0.007
Crozon Q1					-	0.007	0.015**	0.022	0.014	0.013*	0.009	0.014*	0.019*
Petite Sole Q1						-	0.000	0.005	0.000	0.008*	0.000	0.002	0.002
Petite Sole Q2							-	0.000	0.004	0.009*	0.000	0.007	0.005
Lampaul_Tr								-	0.000	0.014	0.000	0.009*	0.011
Sorlingues_Tr									-	0.000	0.000	0.003	0.000
Logachev Q1										-	0.000	0.013**	0.010*
Londsjup_Tr											-	0.000	0.000
Londsjup Q1												-	0.008
Hafadjup Q1													-

Tableau 16 *Pairwise Fst-matrix using the ϑ estimator, after 1000 permutations, among Madrepora oculata populations. Six microsatellite markers were included in this analyses *, p < 0.05; **, p < 0.1 and ***, p < 0.001.*

	Croisic_Q1	Guilvinec Q1	Guilvinec Q2	Morgat Q1	Crozon Q1	Petite Sole Q1	Petite Sole Q2	Lampaul_Tr	Logachev Q1	Londsjup_Tr	Londsjup Q1	Hafadjup Q1
Croisic_Q1	-	0.031*	0.048**	0.056***	0.100***	0.039**	0.032**	0.117***	0.111***	0.113***	0.262***	0.201***
Guilvinec Q1		-	0.000	0.019*	0.039**	0.011	0.008	0.050**	0.067***	0.065**	0.235***	0.178***
Guilvinec Q2			-	0.047**	0.037**	0.050**	0.026*	0.034*	0.046**	0.045	0.182***	0.123***
Morgat Q1				-	0.079***	0.019*	0.053***	0.097***	0.111***	0.152***	0.307***	0.246***
Crozon Q1					-	0.104***	0.089***	0.002	0.037**	0.093**	0.201***	0.139***
Petite Sole Q1						-	0.004	0.123***	0.120***	0.125**	0.313***	0.251***
Petite Sole Q2							-	0.093***	0.108***	0.095**	0.265***	0.206***
Lampaul_Tr								-	0.051**	0.113***	0.228***	0.156***
Logachev Q1									-	0.028	0.086***	0.056**
Londsjup_Tr										-	0.094**	0.078**
Londsjup Q1											-	0.013
Hafadjup Q1												-

The level of connectivity among *M. oculata* population was much less important with all three regions significantly differentiated (Table 16) and a significant pattern of Isolation By Distance in line with a lower recent or contemporary dispersal in comparison with the *L. pertusa*. Indices of genetic differentiation were even observed within the Bay of Biscay, suggesting that the genetic exchanges are reduced even at the regional scales among locations. No spatial autocorrelation was detected, except in Iceland (Table 14), but positive S_p-values were found in the quadrats of the Canyon of Petite Sole, as for *L. pertusa.* This suggests an influence of environmental stability on the demography of both species in this small area. Considering results reported for the distribution of ITS polymorphism in both species at the previous chapter, these elements may further support a scenario of a persistence of *M. oculata* in several Atlantic refugees during the last glaciation, or at least a more ancient recolonization. Such scenario would explain a regional structure possibly reflecting equilibrium between migration and drift. This is contrasting with the panmixia in *L. pertusa* that, rather than more efficient dispersal, may be the footprint of a more recent common and large pool of founders from identical or similar refugee having massively recolonized the Bay of Biscay.

For both species, the level of genetic variability does not seem related with the index of fishing intensity, potentially due to a lack of impact in the areas studied, or to both the large degree of genetic connectivity (at least for *L. pertusa*) and the lack of statistical power of the FI-index we could access here.

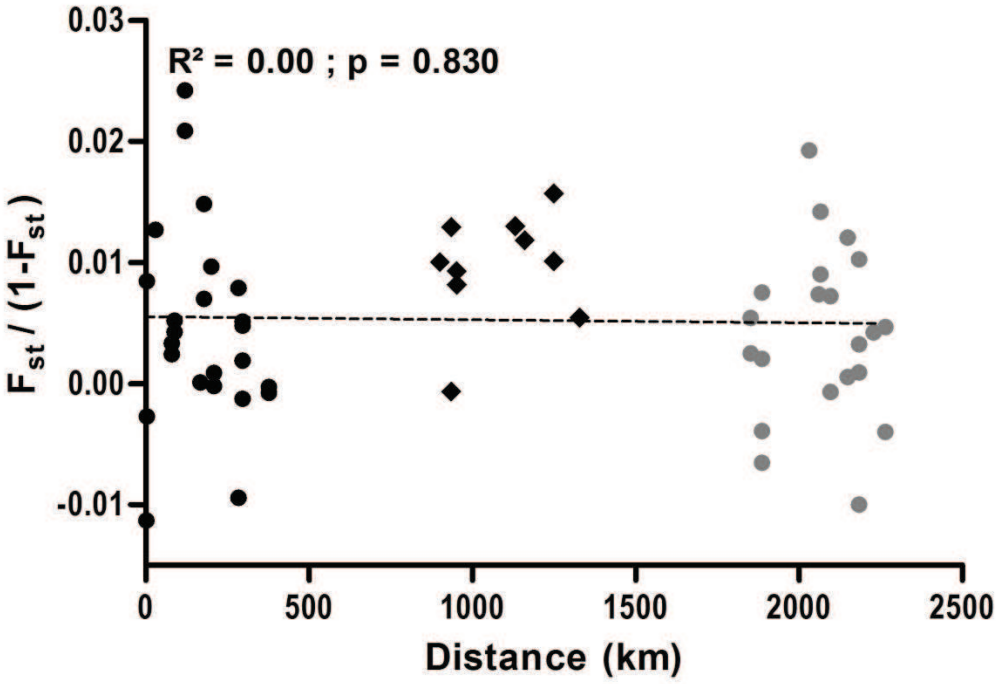

Figure 37 *Isolation By Distance for Lophelia pertusa.*
Due to the relatively linear distribution of reefs along the European margin, the 1D-model was used, as recommended by Rousset (1997). The correlation between geographical and genetic distances is not significant.

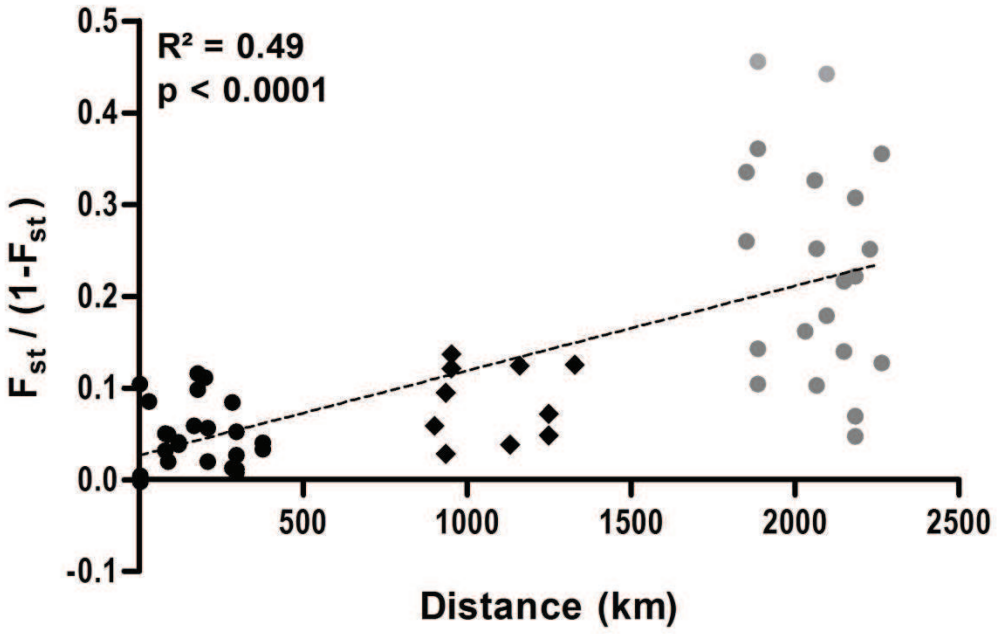

Figure 38 *Isolation By Distance for Madrepora oculata.*
The 1D-model was also used here.The correlation between geographical and genetic distances is significant.

Conclusion

This study represented the first fully georeferenced assessment of clonal features in cold-water corals populations, allowed by a standardized random sampling procedure. The populations of *Lophelia pertusa* and *Madrepora oculata* are predominantly sexual, exhibiting high values of clonal richness and limited influence of clonality, essentially at the very local scale (≈ 1m) and likely through fragmentation. Rare events of dispersal through fragmentation were observed in trawled area as in the local study of Dahl *et al,* (2012), implying a mechanical action. Their detection despite a low density of sampling, suggest this phenomenon is not anecdotic and might constitute of factor of resilience for reefs damaged mechanically, if such disturbance would remain casual and not lead to the complete abrasion of the reef. The possibly high dispersal ability of *L. pertusa* revealed here on large scale in contrast with preliminary studies represents another putative factor of resilience, favoring the odd of recolonization for fully erased reefs. While existence of fishing impacts is well known, their fine quantification remains today partial due to impaired data accessibility for scientific studies. Significant fine-grained spatial genetic structure typical from stable partially clonal populations, was found in two of the most healthy reefs visited during the cruises BobEco and IceCTD. This demonstrates that such equilibrium pattern can arise in CWC, but requires trawling lack of disturbance obviously not compatible with trawling.

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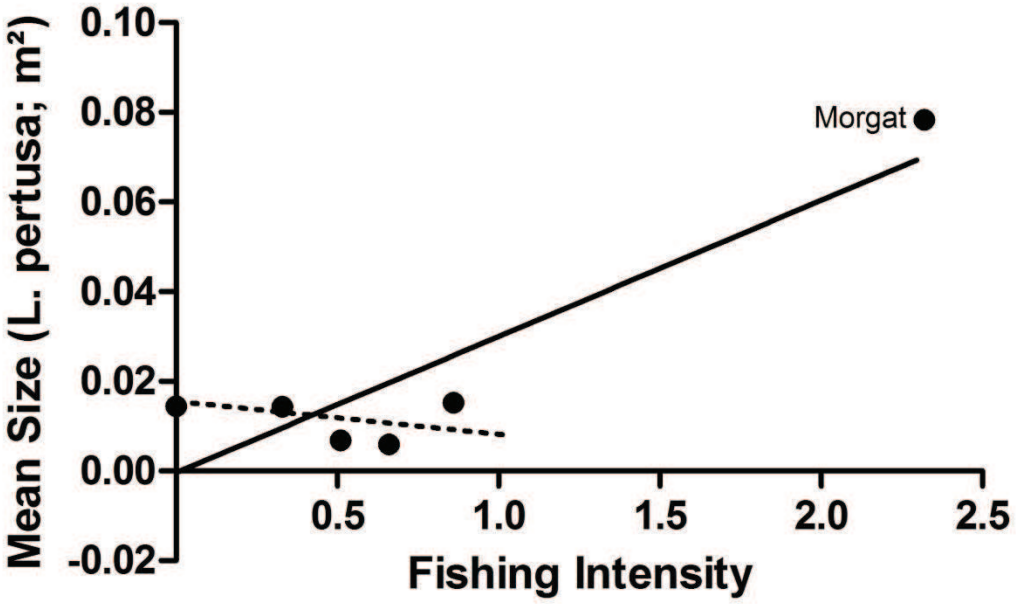

Figure 39 *Linear regression between the fishing intensity estimates and the mean size of L. pertusa colonies.*
The global regression is significant (full line, $R^2 = 0.82$; $p = 0.005$)) but becomes non-significant when removing the outlier (dashed line, $R^2 = 0.09$; $p = 0.56$).

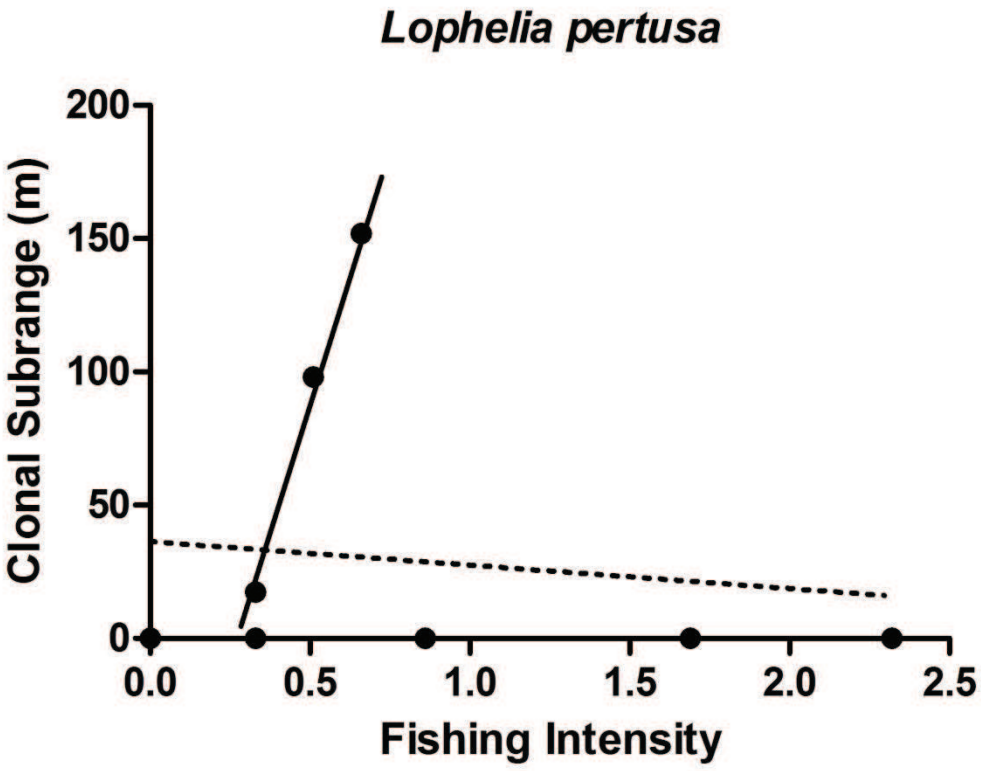

Figure 40 *Linear regression between the fishing intensity and the clonal subrange of L. pertusa populations.*
When including all locations, no significant correlation was observed (R^2 = 0.02 ; p = 0.75; dashed line). In the Bay of Biscay, the shape of the clouds of points suggests a relation between these variable, when the CR is not null (R^2 = 0.99 ; p = 0.04; full line).

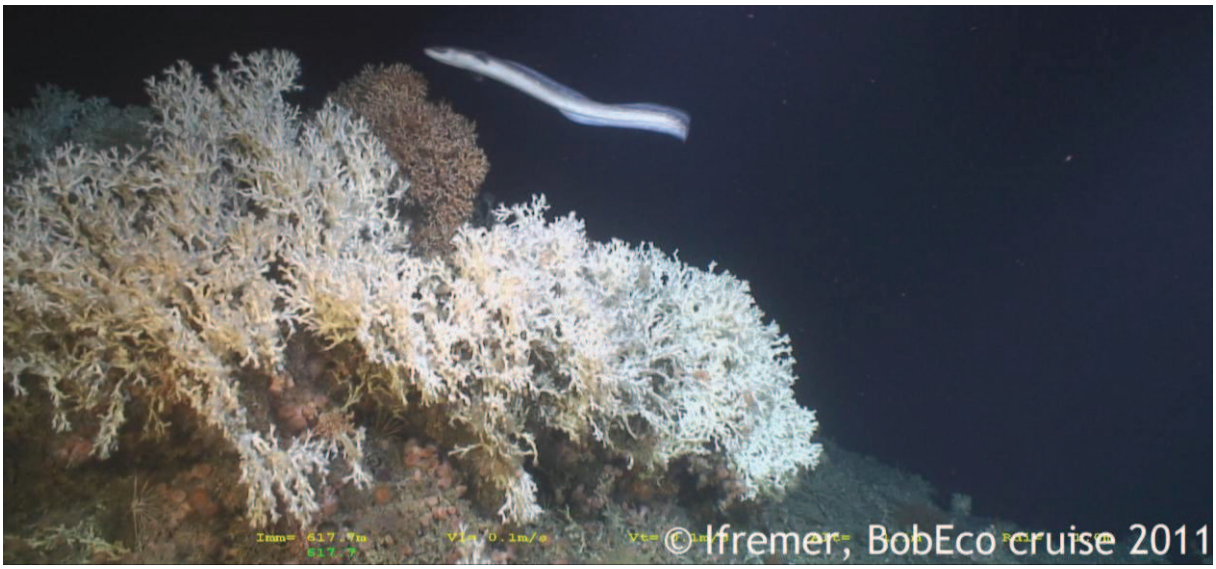

Figure 41 *The Canyon the Petite Sole supports one of the more healthy reefs in the Bay of Biscay, with large colonies of both Lophelia pertusa and Madrepora oculata.*
Considering the slow growth rate of the cold-water corals, this attests of the absence of trawling activities during the last decades.

SYNTHESE ET OUVERTURE

Un des buts de ce travail est de fournir quelques éléments de connaissances et de réflexion pour la conservation et la gestion d'écosystèmes marins clés, à savoir les herbiers de phanérogames et les récifs de coraux profonds.

La conservation des écosystèmes passe par plusieurs étapes. La première consiste à laisser la possibilité aux conditions physico-chimiques de fluctuer avec une influence anthropique minimale. Nous savons d'ores et déjà que cette influence ne pourra pas être nulle, le changement global annoncé ayant un impact probable à large échelle sur l'évolution de nombre de paramètres physico-chimiques. Au niveau des organismes et des communautés, la conservation se traduit par la préservation des paramètres assurant la pérennité de ces communautés, et des populations qui les composent.

L'exploration des relations entre génétique et démographie, au sein de populations naturelles d'espèces structurantes, fournit le cadre le plus à même de dégager des éléments d'information sur l'influence de la diversité intra-spécifique (clonale et génétique) sur la dynamique, la résistance et la résilience des populations, et en conséquence des communautés et écosystèmes qu'elles supportent. Ceci permet d'intégrer les aspects dynamiques des populations, les relations de connectivités génétiques et/ou démographiques dans le temps et l'espace, et les interactions entre connectivité, diversité génétique et démographie. Cette complexité ne peut être capturée par la voie expérimentale.

La pérennité d'une population correspond à sa capacité à maintenir sa démographie sur le court et long terme. Elle dépend donc de :

- Sa capacité à répondre aux perturbations, à un temps « t ». Ceci résulte d'une combinaison de la résistance et la résilience, sur le court terme (à l'échelle spatiale locale), et correspond respectivement à la capacité moyenne des individus à supporter une perturbation et à produire localement de nouveaux « modules » -par croissance clonale- ou nouveaux individus-par reproduction et auto-recrutement. Cette capacité est-elle la résultante des capacités individuelles, ou est-ce une propriété émergente de la population ?
- Sa capacité à revenir à un niveau démographique d'équilibre après réduction démographique, sur une échelle spatio-temporelle intermédiaire. Ceci dépend donc des capacités de résistance/résilience intégrées sur une échelle de temps plus vaste, et du degré d'interconnexion entre populations dans un système de méta-populations. Cette connectivité est le facteur fondamental de la recolonisation, lorsque la réduction démographique est extrême. Un bel exemple d'extinction-recolonisation a été observé sur un herbier de zostère marine échantillonné lors de cette thèse.
- Sa capacité à s'adapter, sur le moyen et long terme, c'est-à-dire sa capacité à produire des génotypes nouveaux, possédant une fitness élevée dans les conditions environnementales futures. Une population doit alors être en mesure de conserver une variabilité génétique nécessaire et suffisante à l'émergence de tels génotypes. Pour les organismes clonaux et/ou partiellement clonaux, la pérennité d'une population peut également dépendre de la présence de « super-clones », aussi appelé « General Purpose Genotypes » (Baker, 1974;

Lynch, 1984), caractérisés par une plasticité phénotypique forte leur permettant de surpasser les variations environnementales. Cette catégorie de clones est le résultat de la sélection génotypique, une forme de sélection caractéristique des espèces clonales. Ceci est rendue possible par le fait que le génotype est une entité durable chez de tels organismes et est de ce fait soumis à sélection au même titre que les allèles dans les populations d'organismes non clonaux (Ayala, 1998).

Pour répondre au mieux à cette problématique, il est nécessaire d'explorer le réseau de relations entre diversité génétique / démographie et connectivité, dans le cadre de populations naturelles. La clonalité est un trait d'histoire de vie qui influence profondément ces relations, et qui a constitué, de fait, le fil rouge de ce travail.

LES CONSEQUENCES DE LA CLONALITE SUR LA DYNAMIQUE ET LA TRAJECTOIRE EVOLUTIVE DES POPULATIONS

Quelle est l'origine de la diversité des clones et de leur architecture, au sein des populations partiellement clonales ?

L'apparition de nouveaux clones au sein des herbiers est la résultante d'évènements de reproduction sexuée, suivi du recrutement de la propagule sexuelle (larve ou graine), ou mais dans une moindre mesure de l'implantation d'un fragment clonal dérivant. Le suivi temporel des herbiers de Zostère marine (Becheler *et al*, 2013) a révélé une augmentation du nombre de lignées clonales pour un certain herbier. D'autres ont, au contraire, manifesté une réduction de cette richesse clonale. Ces évolutions différentielles révèlent l'action concertée de la dispersion et de la compétition à l'espace.

La compétition dispersion VS croissance

Dans un système où la régulation démographique est densité-dépendante, le succès de l'installation d'une propagule dépend, en particulier, de l'intensité de la compétition à l'espace qu'elle rencontre. Ce phénomène se manifeste, au sein des herbiers, à travers la structure génétique à faible échelle spatiale, soit au sein d'un même patch de plantes, soit entre deux patchs distants de quelques dizaines de mètres (Becheler *et al*, 2010).

L'existence de telles structures génétiques (F_{st} entre quadrats proches ou autocorrélation spatiale) n'est pas attendue sur le plan théorique, car elle se manifeste à des échelles spatiales de toute évidence inférieures aux capacités de dispersion de ces phanérogames. Les patrons significatifs d'autocorrélation spatiale, qui ont été observés au sein de certains patchs de zostères marines, correspondent à de l'isolement par la distance à faible échelle spatiale et traduisent une structure génétique mosaïque de l'herbier. Chez les plantes terrestres, un tel schéma est lié à des capacités de dispersion très limitées (Vekemans and Hardy, 2004). En milieu marin, ce ne sont pas ces capacités qui sont en cause dans la limite au flux de gènes à cette échelle, la dispersion physique des gamètes et/ou des graines excédant largement cette échelle spatiale. Le phénomène probablement à l'origine de la structure génétique à fine échelle spatiale serait la dispersion collective de graines, formant un

pool génétique relativement apparenté, et recrutant en nuage (Selkoe *et al*, 2006). En tous les cas, la structure inter-nuage doit excéder la structure intra-nuage. Les recrutements de ces différents nuages de graines génèrent des îlots génétiques au sein des herbiers, possédant une richesse clonale initiale maximale (chaque graine possède un génotype différent). Avec la mise en place de la croissance des clones, les premiers arrivés et/ou les plus compétitifs excluent les lignées les moins performantes. Ainsi, l'émergence d'une architecture clonale se traduit par une diminution de nombre de clones formant ces patchs, sous l'effet d'une régulation densité-dépendante.

A l'échelle inter-quadrat, la dispersion physique a bien lieu, mais la migration efficace (i.e la succession transport/recrutement/reproduction dans l'herbier d'arrivée) semble limitée par cette compétition à l'espace. Les clones déjà implantés exercent, via la croissance clonale, une pression sur les propagules immigrantes qui peut conduire à leur exclusion. Ainsi, la compétition à l'espace représente un facteur potentiel de limitation de la migration efficace, et initie la mise en place d'une divergence entre les différents patchs d'herbiers, par une sélection différentielle des clones dominants. Ce mécanisme expliquerait la structure inter-quadrat.

Ainsi, à densité maximale de ramets (i.e. les modules des clones, correspondant soit aux « shoots » chez les zostères, soit aux colonies de coraux), le nombre de clones présents dans une population dépend de l'interaction entre dispersion et croissance. Quel est alors le rôle des perturbations dans un système où la régulation démographique est densité-dépendante ?

Le rôle des perturbations

Une définition pratique, pour le cas présent, de « perturbation » est donnée par Eriksson (1993). Il s'agit selon lui d'un évènement biotique ou abiotique libérant un espace dans le continuum de plantes. Ceci ouvre une fenêtre d'opportunité pour un évènement de dispersion. Il s'agit d'un premier effet des perturbations. La déstructuration de l'architecture clonale préalable, se traduisant par une diminution de l'intensité de la compétition inter-clone, constitue une seconde conséquence. Les perturbations modulent l'équilibre dispersion/croissance clonale, favorisant le succès du recrutement des propagules immigrantes (Reusch, 2006). Elles maximisent la diversité clonale.

Si les perturbations considérées ont une action locale, créant un trou continu favorable au recrutement d'un nuage de propagules, elles peuvent favoriser la structure mosaïque de la population.

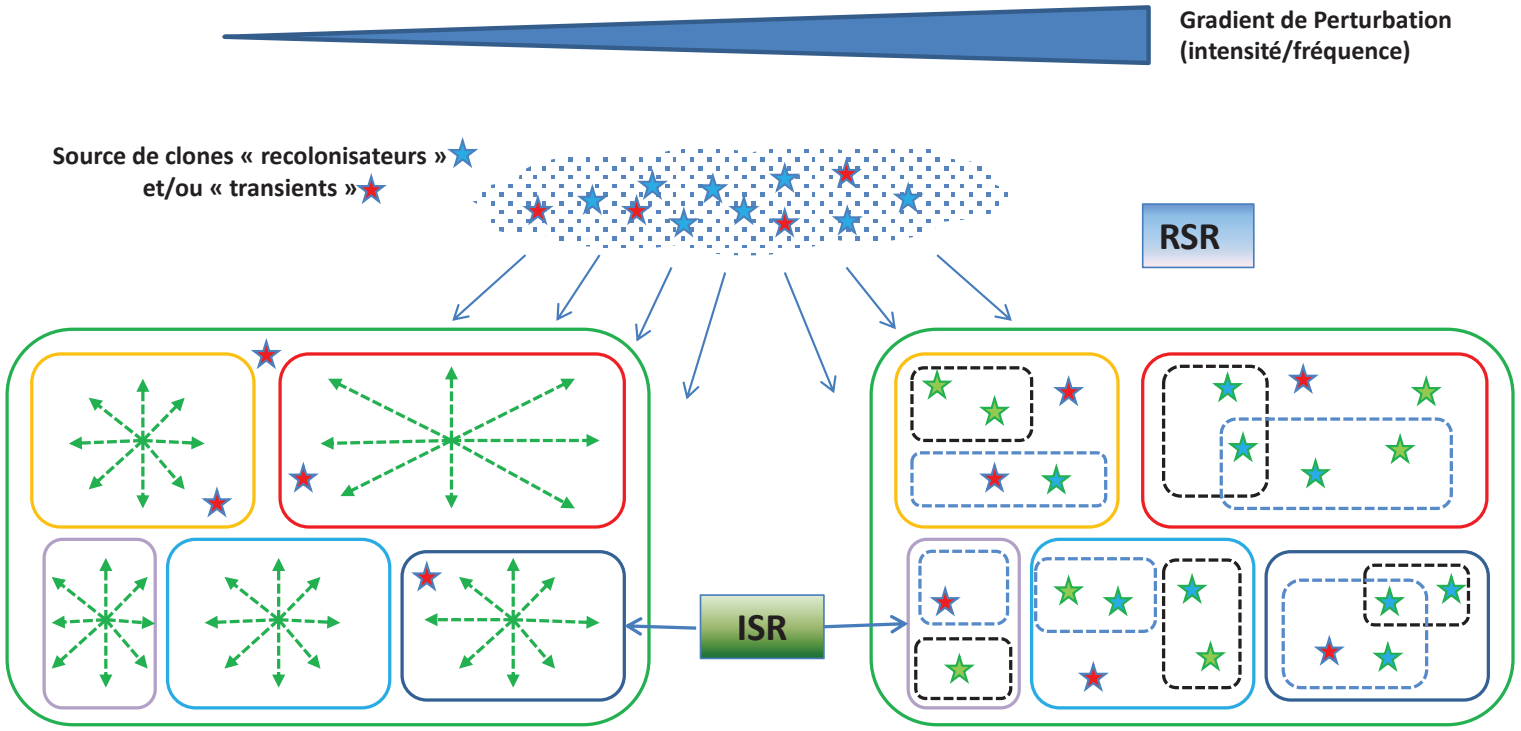

Figure 42 *Equilibre entre dispersion et croissance clonale, dans la compétition à l'espace.*
Les perturbations modulent cet équilibre en ouvrant des fenêtres d'opportunité pour le recrutement des clones « transients ». ISR : recrutement initial de propagules. RSR : recrutement répété. M-structure : structure génétique à l'échelle intra-population (autocorrélation spatiale)

Ainsi, la structure génétique spatiale à fine échelle et l'architecture clonale sont le résultat de l'équilibre entre dispersion et compétition inter-clones, dont le rapport de force est modulé par les perturbations (fréquence et intensité).

En lieu et place d'une longue explication, préférons citer le passage suivant de l'article de Sand-Jensen (2007, How to write consistently boring scientific literature), et proposons le schéma bilan (Fig. 42) :

Scientific papers and books can be made impressively dull by including few and only bad illustration in an otherwise good text. Because illustrations, which are fundamentally engaging and beautiful, can often portray very complex ideas in forms that are easy to visualize but impossible to explain in thousands of words, boring science writing should not use them.

La dualité core/transient

Cette dualité est suggérée par le suivi temporel, qui a révélé des observations apparemment contradictoires:

- L'existence d'une structure temporelle forte, excédant même la structure génétique spatiale.
- L'existence de lignées clonales communes aux échantillonnages de 2009 et 2012. Ces clones sont donc pérennes, et sont caractérisés par leur dominance spatiale.

La définition de la dualité « core » *versus* « transient » provient de cette apparente contradiction. L'idée provient de l'étude des analyses de réseaux réalisées lors des interprétations des résultats du suivi temporel (Becheler *et al*, 2013). Les réseaux de génotypes, construits pour chaque site, révélaient une proportion variable de clones satellites (Fig. 43). Cette apparente contradiction a conduit à émettre l'hypothèse de l'existence d'une seconde catégorie de clones, appelés « transients » dans l'article publié sur ce travail (Becheler *et al*, 2013). Ces clones « transients » représenteraient des lignées à durée de vie éphémère, sujettes à un turn-over probablement annuel. Cette catégorie supposée de clones serait responsable de la structure temporelle.

Les clones appelés « transients » désignent les individus génétiques éphémères dans les populations de zostères, issus de la dispersion. Ils seraient soumis à un fort turn-over, sur une base probablement annuelle, qui explique la structure temporelle. Ils sont caractérisés par des petites tailles (i.e. faibles nombre de réplicats). Ils coexistent avec les lignées clonales pérennes, durablement établies dans la population. Ces lignées ont une durée de vie plus ou moins importante, leur ayant permis d'acquérir une taille conséquente ; ils sont le résultat de la croissance végétative. Ce « core » de clones dominants explique la structure entre patchs de phanérogames.

Cette hypothèse de dualité amène à se questionner sur les rôles respectifs de ces catégories de clones, tant dans le maintien démographique que de la trajectoire évolutive des populations. Par exemple, la compétition entre clones, sélectionnant les lignées les plus compétitives, pourrait

potentiellement conduire à des effets fondateurs en l'absence de goulot d'étranglement démographique.

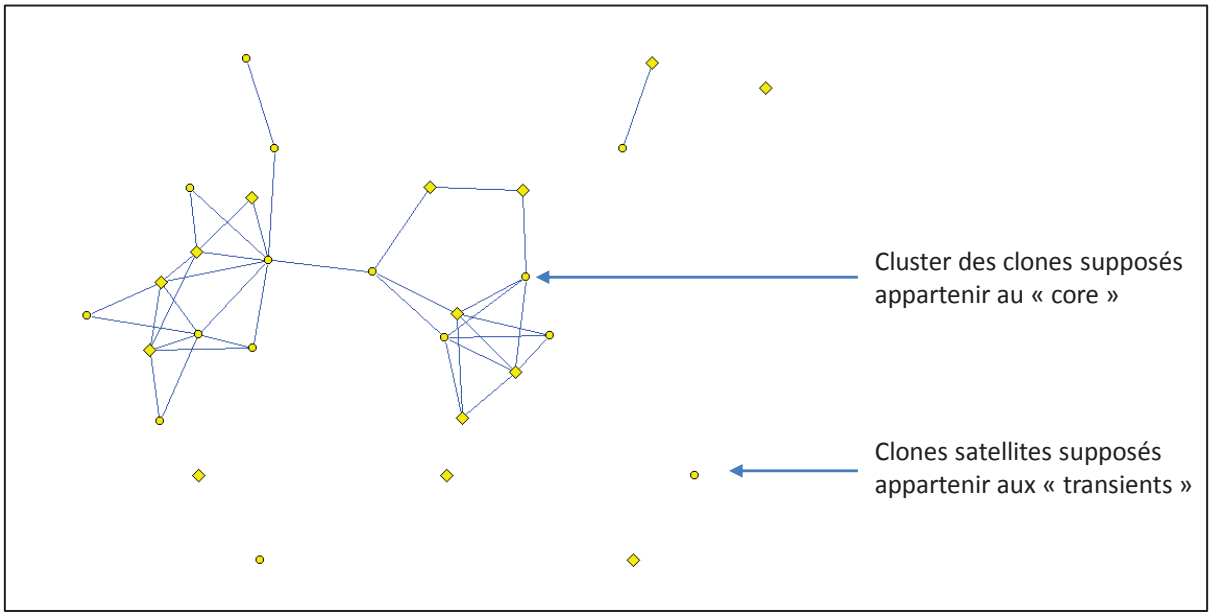

Figure 43 *Réseau de genets de l'herbier de Molène.*
Les nœuds représentent les genets, ou lignées clonales, et les liens représentent la distance génétique, en nombre d'allèles partagés. L'idée d'une dichotomie entre « core » et « transient » provient de l'étude de ce type de réseaux où l'on observe des genets appartenant à un cluster bien défini (initialement supposé représenter le core) ainsi que des genets satellites (initialement supposés représenter les clones transients). L'approfondissement de cette idée n'a pas validé cette idée initiale. Il semblerait même que l'inverse soit plus probable. Les genets satellites représenteraient les clones persistants, divergeant du pool d'allèles d'où proviennent les « transients ».

Mise en relief des travaux expérimentaux croisant diversité clonale et réponse démographique aux perturbations

Les biais de l'approche expérimentale

Les travaux pionniers dans l'exploration des relations entre diversité intra-spécifique et stabilité démographique (Crutsinger *et al*, 2006; Ehlers *et al*, 2008; Hughes and Stachowicz, 2004; Hughes and Stachowicz, 2009; Reusch *et al*, 2005) ont montré une relation entre la diversité clonale et stabilité des populations expérimentales face au broutage massif des plantes (Hughes and Stachowicz, 2004) ou choc de température (Ehlers *et al*, 2008; Reusch *et al*, 2005). La portée des conclusions en termes d'influence de la diversité clonale sur la résistance et la résilience des populations peut être relativisée pour deux types de raisons. Tout d'abord, l'approche expérimentale est limitée par son caractère simplificateur des systèmes. Elles ne permettent pas de capturer la complexité du fonctionnement d'une prairie sous-marine naturelle (Arnaud-Haond *et al*, 2010). L'extrapolation de ces résultats au fonctionnement des prairies naturelles n'est donc pas immédiate. De plus, les

faibles échelles temporelles considérées ramènent les populations expérimentales (construites soit au sein de prairies naturelles, par un assemblage de clones présélectionnés, soit en mésocosme) à des systèmes fermés. L'influence potentielle de la connectivité ne peut être appréhendée.

Outre ces limites inhérentes à l'expérimentation, ce sont les modalités de construction et d'analyses des assemblages expérimentaux qui sont discutables. Afin de répliquer les assemblages, ce sont les clones de grandes tailles qui ont été utilisés (Hughes and Stachowicz, 2004; Reusch *et al*, 2005). Ces expériences ne tiennent alors compte que du « core », et excluent les clones « transients ». De plus, cette sélection de grands clones peut largement influencer l'issue de l'expérience. Notamment, les prairies de Posidonie constituées de grands clones semblent être plus résistantes aux perturbations (Arnaud-Haond *et al*, 2010). Pourtant, elles sont caractérisées par une faible diversité clonale –étant donné la large dominance spatiale de certaines lignées- et une faible diversité allélique. Ce résultat contradictoire peut s'expliquer par les fitness élevées des lignées de grande taille (Diaz-Almela *et al*, 2007). Un effet d'échantillonnage de ces grands clones pourrait expliquer la survie accrue des assemblages les plus divers. Toutefois un autre effet peut interférer avec les résultats de ces expériences : celui de la diversité génétique *sensu stricto* (ie diversité génomique, ou richesse allélique), un paramètre ignoré dans ces différentes expériences.

La diversité allélique, une variable cachée dans les approches expérimentales (Massa *et al*, accepté)

En parallèle des travaux présents, Sonia Massa (Université de Faro, Portugal) a également réalisé une thèse sur la problématique des relations entre diversité intra-spécifique et stabilité des populations. Le montage des plans expérimentaux prévoyait de manipuler conjointement à la fois la diversité allélique et la diversité clonale, chez *Zostera noltii*. Ceci avait pour but de dissocier leurs possibles effets respectifs, dans la réponse démographique des assemblages soumis à une perturbation (en l'occurrence, un bloom d'algues). Le premier résultat de ces travaux (Massa *et al*, accepted) révèle l'impossibilité de dissocier richesses clonale et allélique, pour des faibles nombres de clones (typiquement le nombre de clones utilisé par les montages expérimentaux). Cette corrélation entre richesse allélique et richesse clonale implique qu'il est impossible de manipuler le nombre de clones et le nombre d'allèles séparément. Dès lors, identifier la variable la plus impliquée dans la réponse démographique devient également impossible. Toutefois, cette corrélation ne permet pas de considérer que la richesse clonale serait un bon proxy de la richesse génétique. En effet, si cette corrélation est forte aux niveaux de diversités clonales manipulés (moins de 12 genets), elle tend à disparaitre quand une prairie porteuse d'un beaucoup plus grand nombre de lignées est analysée.

Dans ce travail, les analyses basées sur des régressions multiples ont néanmoins tenté de dissocier les effets de ces deux variables. Les résultats indiquent une probable influence prédominante et positive de la richesse allélique, sur la résistance des populations. La diversité clonale, quant à elle, n'a montré aucune relation significative, à une exception près où son effet semblait négatif.

Pourquoi la diversité clonale n'est-elle pas liée à la stabilité démographique des populations naturelles dans ce travail?

D'une part, il est bien entendu possible que le non-rejet de l'hypothèse nulle (une absence de relation entre diversité génotypique et stabilité) reflète sa véracité. Il est difficile de discuter d'autres hypothèses sur les coraux dont le taux de clonalité apparaît relativement faible, car cela réduit son impact possible sur la réponse démographique des populations. Pour la Zostère marine, nous avons vu que la richesse clonale est davantage liée aux conséquences des perturbations et de l'acquisition de dominance spatiale des lignées clonales du « core ». Si les populations naturelles correspondaient à des systèmes fermés, sans aucune interconnexion avec les populations voisines, c'est même une relation inverse entre diversité clonale et stabilité démographique qui serait attendue, sur le long terme. Ceci résulte de l'effet de la stabilité sur l'établissement de la dominance clonale d'une part, et de la meilleure résistance des clones sélectionnés dans le temps d'autre part (voir aussi Arnaud-Haond *et al*, 2010).

Le nombre de clones, formant une population naturelle, est généralement bien supérieur à la quantité de clones qu'il est possible de manipuler expérimentalement. Ceci place donc la question des relations entre diversité intraspécifique et stabilité démographique à une échelle où nombre d'allèles et nombre de clones ne sont virtuellement plus corrélés (Becheler *et al*, 2013; Becheler *et al*, 2010). La richesse allélique représente donc une variable cachée derrière la diversité clonale dans les approches expérimentales qui est susceptible de conserver un effet positif à l'échelle des assemblages naturels que constituent les prairies.

A TRAVERS QUELS MECANISMES LA DIVERSITE GENETIQUE AU SENS STRICT PEUT-ELLE FAVORISER LA STABILITE DEMOGRAPHIQUE ?

Les diversités génétique et spécifique favorisent la stabilité des populations et des communautés via des mécanismes parallèles

Considérés conjointement, les travaux de Sonia Massa (voir l'article cité plus haut, Massa *et al*. soumis) et le chapitre 2 de ce manuscrit suggèrent que l'effet de la diversité intra-spécifique (allèles & génotypes) sur la stabilité démographique est, en réalité, lié à la diversité génétique au sens strict.

Il a été récemment montré que la diversité phylogénétique représentait un meilleur déterminant de la stabilité des communautés, que la diversité spécifique ou la diversité fonctionnelle (Cadotte, 2013; Cadotte *et al*, 2008; Cadotte *et al*, 2009; Cadotte *et al*, 2010; Cadotte *et al*, 2012).

La diversité fonctionnelle, telle qu'elle était classiquement utilisée, consiste à créer des groupes d'espèces sur la base de traits phénotypiques mesurables. Cadotte et collaborateurs interprètent leurs résultats de la façon suivante : la diversité phénotypique est une mesure indirecte, mais fine, de la diversité fonctionnelle. Prenons pour exemple la famille des fabacées (anciennement appelées légumineuses, une famille qui a la particularité d'être en symbiose avec les bactéries leur permettant de fixer l'azote atmosphérique), fréquemment utilisée par les expériences « diversité/stabilité » en champs. L'ensemble des espèces de cette famille était assigné à un seul groupe fonctionnel.

Pourtant, un groupe de 4 espèces de fabacées appartenant toutes au genre monophylétique *Trifolium,* ne contient pas la même quantité de diversité fonctionnelle qu'un groupe contenant 4 espèces plus profondément divergentes. Considérer ces deux groupes hypothétiques comme appartenant au même groupe fonctionnel conduit à passer à côté d'une partie de l'information.

Ainsi, cette approche phylogénétique intègre bien plus finement les divergences fonctionnelles. De plus, elles montrent que les mécanismes sous-jacents ont les allèles pour origine. Elle permet donc de faire un pont entre les théories diversité-stabilité à l'échelle des communautés (diversité spécifique ou fonctionnelle) et leur application à l'échelle sub-spécifique (richesse allélique), et de proposer des mécanismes similaires.

Hypothèse d'assurance (McCann, 2000) : La capacité d'une population/communauté à absorber des perturbations est dépendant du nombre de variants génétiques/espèces possédant un fort rôle stabilisant, ainsi que de la capacité des variants génétiques /espèces dans la population/communauté à répondre différemment aux perturbations. Une diversité croissante augmente la probabilité que de tel(le)s variants génétiques /espèces existent dans l'écosystème.

Redondance fonctionnelle : on formule l'hypothèse que l'on peut assigner des variants génétiques /espèces à des groupes fonctionnels. La disparition dans une population/communauté d'un(e) variant génétique/espèce a un effet moindre, s'il existe d'autres variants génétiques /espèces assurant la même fonction au sein de l'écosystème.

Complémentarité fonctionnelle : l'utilisation optimale de la ressource de l'écosystème repose sur son partage dans le temps et l'espace. Ceci implique l'existence d'une *différentiation de niche*. Chaque variant génétique/espèce possède une niche écologique propre.

Idiosyncrasie : il n'y a pas de relation claire entre diversité et stabilité. Chaque population/communauté possède un fonctionnement propre, dépendant moins du nombre de variants génétiques/espèces qui la compose, que de leur qualité. A l'échelle des gènes, c'est typiquement le schéma obtenus par l'hybridation de deux pools issus d'adaptation locale divergente et dont la résultante est plus diverse mais moins performante (*outbreeding depression*) car les complexes de gènes sont déstructurés.

Ces mécanismes théoriques reposent sur l'hypothèse classique de la génétique des populations, prévoyant que la diversité estimée aux marqueurs est un bon proxy de la diversité globale du génome, et ses variants génétiques.

La dispersion est le processus par lequel les individus migrants, et les allèles dont ils sont porteurs, sont déplacés d'une population de naissance vers un nouveau site d'installation. C'est un facteur central de la génétique des populations, de la dynamique des populations et de l'écologie des communautés (Broquet and Petit, 2009), qui détermine le degré d'interdépendance des populations.

L'interconnexion génétique *versus* démographique

A partir de l'utilisation de marqueurs génétiques, l'étude de la structure permet d'inférer, dans une certaine mesure, le flux de gènes (aux biais théoriques près). Cela renseigne-t-il sur le flux démographique ?

Cas des coraux profonds

La structure génétique entre les populations de *Madrepora oculata* semble révéler un schéma régional. Bien que les résultats obtenus soient encore provisoires, l'intégration de trois marqueurs microsatellites étant prévus prochainement, une certaine cohérence est observée entre les résultats issus de l'étude « microsatellites » et de l'étude de séquences ITS. Golfe de Gascogne, Mer Celtique et Islande abritent des populations dont le degré de dépendance inter-région est faible. Cette information permet aux gestionnaires de dessiner les contours des zones à considérer pour la gestion de ces populations. En effet, l'existence de ce type de structure permet d'identifier les ensembles de sous-populations interdépendantes sur le plan génétique, et possiblement sur le plan démographique. Brièvement, on peut considérer que la conservation des populations du Golfe de Gascogne ne dépend pas, ou très peu, des plans de gestion adoptés en Islande.

L'absence quasi-totale de structure génétique, observée sur le jeu de données microsatellites de *Lophelia pertusa,* rend la question de la gestion plus problématique. Cette faible structure peut révéler une dispersion forte entre les sites et les régions et suggérer qu'un récif fortement impacté peut bénéficier d'un « effet secours » lié à l'immigration depuis d'autres récifs, pour le maintien de sa démographie. Toutefois, une telle observation peut aussi cacher une histoire commune récente (typiquement dans le cas de *L. pertusa* une recolonisation massive ?) suivie de l'absence d'échanges depuis un temps trop faible et dans des populations de taille trop importante pour avoir eu le temps de laisser une signature de la dérive génétique sur le génome des populations étudiées.

Le prérequis à l'estimation de flux (géniques ou démographiques) est la définition des contours des populations. Or, avec des valeurs de F_{st} nulles ou tendant vers zéro, il est impossible de poser ces contours. Les récifs du nord-est Atlantique sont génétiquement interdépendants, formant une sorte de grande métapopulation. Cette information seule ne nous renseigne pas sur le flux démographique. Mais quelques observations plaident pour un flux contemporain. Premièrement, les larves *L. pertusa* ont une durée de vie larvaire, correspondant à leur phase dispersante, estimée à une vingtaine de jours (en aquarium, M. Dahl, communication personnelle) qui leur confère, en

théorie, un bon potentiel de dispersion (mais voir Riginos, 2012). Dans le Golfe de Gascogne où les courants balayant le talus continental sont forts, les colonies de coraux sont présentes dans chacun des sites étudiés, quel que soit l'état de santé apparent de la zone.

Cas des Zostères marines

La définition des contours populationnels est également problématique pour les phanérogames marines étudiées ici. En revanche, les problèmes méthodologiques ne découlent pas de l'absence de structure, mais au contraire de son omniprésence aux différentes échelles spatiales et dans le temps, caractérisant le schéma de *genetic patchiness*. Dès lors, la délimitation des ensembles interdépendants est, dans ce cas également, ardue sur la seule base de l'étude de la structure génétique.

La dualité « core » *versus* « transient » fournit quelques pistes de réflexion sur le degré de dépendance démographique d'un herbier vis-à-vis de ses voisins. Le compartiment des clones « transients » illustre l'influence de la connectivité sur le maintien démographique. Il démontre que la dynamique d'un patch d'herbier ne dépend pas uniquement de facteurs intrinsèques (morts de ramets et production par auto-recrutement et/ou croissance végétative), mais également de l'apport de ces transients, via des évènements de dispersion. Leur importance démographique est révélée pour des éléments. Tout d'abords, leur proportion dans un herbier est significative, puisque ce type de clones a été échantillonné en nombre. Ensuite, entre 2009 et 2012, les augmentations substantielles de la richesse clonale R observées dans certains herbiers suggèrent une influence du degré d'interconnexion avec les patchs ou herbiers voisins sur la démographie, via le flux de clones transients.

Les freins à l'estimation de la dispersion

Les deux types de dispersion (individus VS gènes) ne sont pas totalement interdépendants. La connectivité démographique peut, par exemple, ne pas se traduire par une connectivité génétique. En effet, rien ne garantit que les migrants, en particulier les clones « transients », se reproduisent dans la population d'arrivée. A l'inverse, une disruption du flux de gènes peut être suffisamment forte pour être détectée par des marqueurs génétiques et révèle dans ce cas un nombre de migrants de toute évidence trop faible pour assurer une connectivité démographique significative.

Les flux réel de migrants (Nm) et flux de gènes (N_em), avec N taille de la population, N_e taille efficace de la population et m le taux de migration, sont donc deux flux distincts. Or, c'est le taux de migrants, c'est-à-dire m, qui intéresse les gestionnaires, souhaitant avoir une idée de l'effet secours des migrants capables d'intégrer une population impactée à partir d'une population source.

Le généticien des populations est capable d'appliquer une méthode indirecte pour estimer N_em. A ce sujet, Waples & Gaggioti (2006) écrivent "*genetic methods have an inherent difficulty in evaluating the concept of population under the ecological paradigm; demographic independence depends on m, whereas the magnitude of genetic differentiation scales with the product Nm*". Ce produit est

souvent inféré abusivement à partir de l'indice de structure (F_{st}) qui en fait renseigne sur N_em. De plus les hypothèses sur lesquelles reposent les inférences sont souvent irréalistes (Waples and Gaggiotti, 2006; Whitlock and McCauley, 1999), et les erreurs d'estimations sont probablement de plusieurs ordres de grandeur. Enfin, il faut être capable de dissocier les facteurs du produit N_em, chose rarement possible, excepté dans certains cas particuliers (lorsque des séries démographiques sont disponibles, par exemple, ou lorsque les traits d'histoire de vie des espèces et les caractéristiques des populations permettent, dans des cas extrêmement rares, de faire des inférences directes de type analyses de parenté).

Pour conclure, le cadre théorique de la génétique des populations ne permet pas, en l'état, d'estimer de façon systématique et directement traductible en mesures de gestion, les flux réels de migrants, et les contours des populations sont parfois compliqués à définir.

COMPRENDRE LE DIFFERENTIEL DE FITNESS ENTRE LES LIGNEES CLONALES D'UNE POPULATION

Un avantage évolutif potentiel de la clonalité réside dans la capacité des génotypes performants à perdurer (Otto and Lenormand, 2002). L'existence de très anciennes lignées clonales chez *Posidonia oceanica* (Arnaud-Haond *et al*, 2012) est une illustration de la durée de vie potentiellement illimitée des individus clonaux, et du phénomène de General Purpose Genotype (Baker, 1974; Lynch, 1984). La lignée clonale représente donc une entité évolutive à part entière (Ayala, 1998), ce qui a amené Pan & Price (2001) à définir le concept de sélection génotypique, pour les espèces partiellement clonales.

Le différentiel de fitness existant dans au sein des populations est un paramètre susceptible d'influencer profondément les rapports de compétition entre lignées clonales. Une compréhension approfondie de la dynamique de ces populations passe notamment par l'étude de la variabilité intra-populationnel des traits de fitness. Un trait phénotypique reconnu comme bon *proxy* de la fitness des organismes partiellement clonaux est la taille des lignées clonales, estimée soit par le nombre de réplicats (Prugnolle *et al*, 2004), soit par l'étendue spatiale des clones pour des organismes sessiles (Pan and Price, 2001). Un cadre théorique qui pourrait apporter des éléments de réponses est la génétique quantitative, une branche de la génétique qui s'attache à l'étude des traits phénotypiques continus.

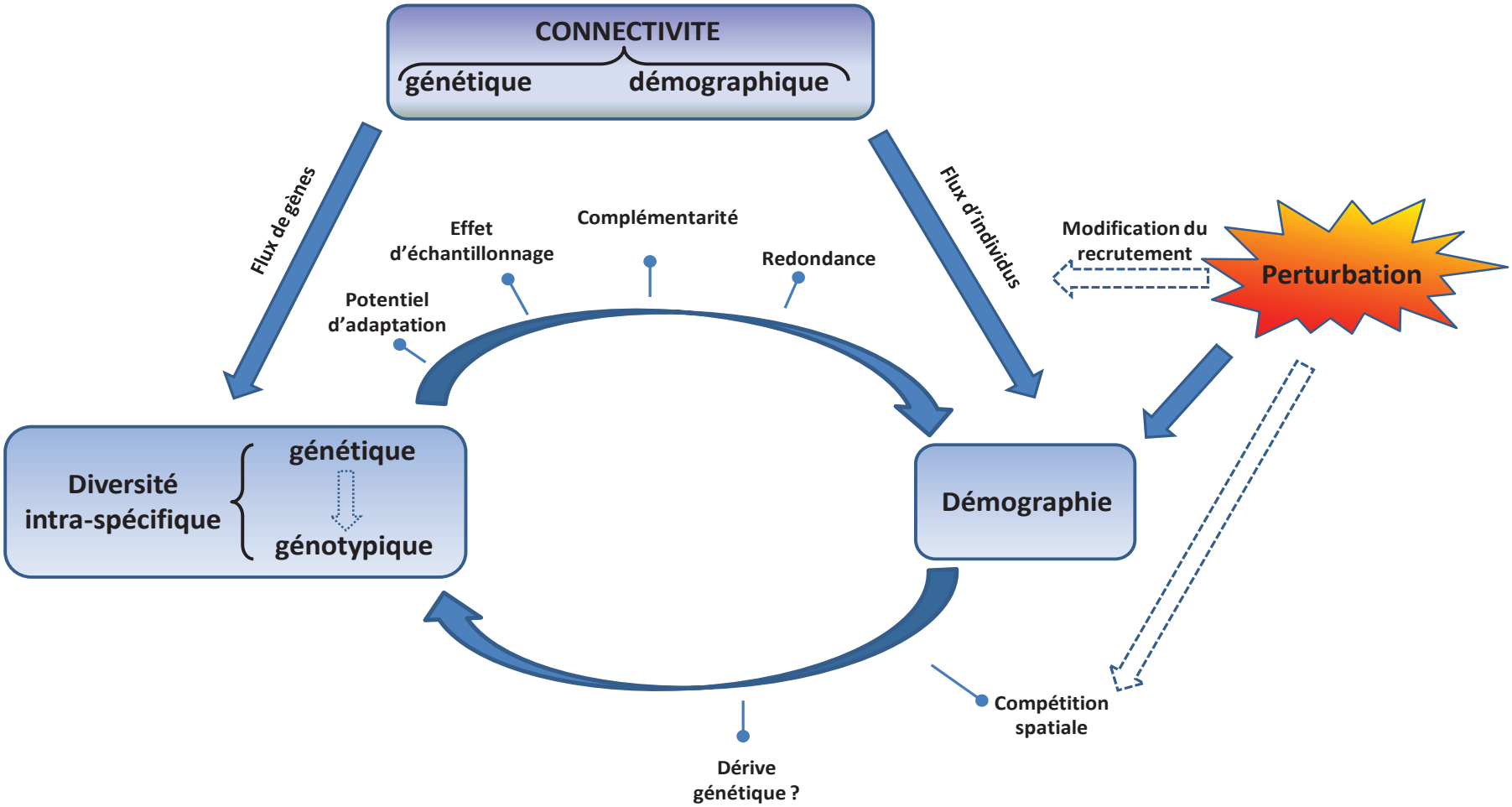

Figure 44 *Bilan des interactions entre connectivité, génétique et démographie. Les flèches pleines représentent les influences directes. Les flèches en pointillé illustrent les influences indirectes.*

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ANNEXES

ANNEXE 1 METHODOLOGIE POUR L'ETUDE DE LA CLONALITE EN GENETIQUE DES POPULATIONS

Pourquoi une méthodologie adaptée est-elle nécessaire ?

La clonalité est un mode de reproduction sans recombinaison, conduisant à la production de descendants génétiquement identiques aux parents –aux mutations somatiques près. L'ensemble [parents-descendants] constitue un individu génétique, également appelé genet. Chaque sous-unité du genet, correspondant à un individu morphologique, est appelé ramet.

Les espèces partiellement clonales sont capables d'alterner reproduction sexuée (avec recombinaison) et reproduction clonale. Une population partiellement clonale est composée d'une somme de genets, au même titre qu'une population sexuée, dérivant d'œufs distincts. Le généticien des populations s'intéresse à l'unité évolutive, c'est-à-dire à l'ensemble des individus génétiques interagissant pour la reproduction. Il lui est donc nécessaire de distinguer les genets entre eux. Or, la production de ramets par chaque genet, rend cette distinction impossible sur le terrain (voir les illustrations ci-dessous).

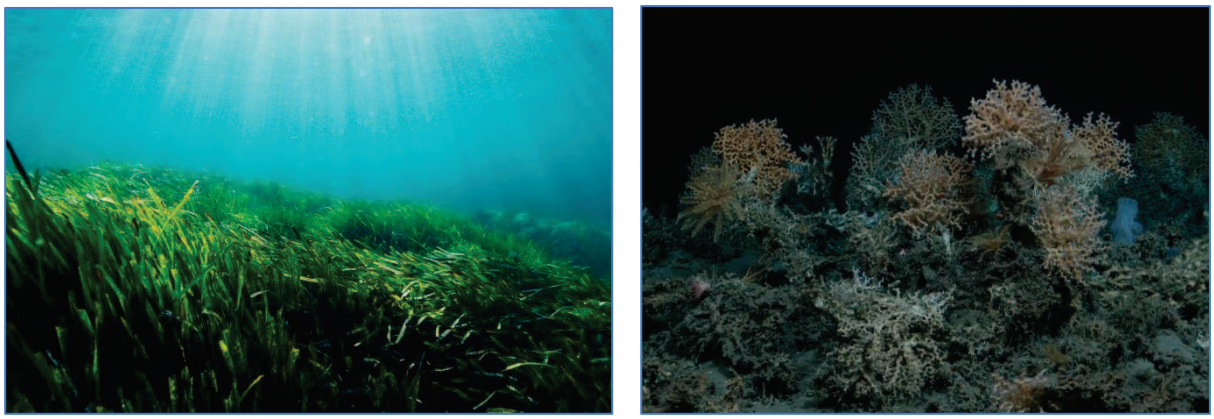

Figure 45 *Illustration de l'impossibilité de distinguer les lignées clonales sur le terrain. Suivre les connexions du rhizome est techniquement irréalisable, d'autant que des portions de rhizomes peuvent dégénérer, déconnectant ainsi les ramets appartenant au même genet. De même, les colonies de coraux d'eaux froides peuvent être bien distinguables, mais une fraction d'entre elles peut provenir de la fragmentation d'une colonie initiale.Image de Posidonie :www.parcsnationaux.fr. Image de coraux pronfonds : photothèque © Ifremer.*

Il faut alors utiliser l'outil moléculaire pour discriminer les clones sur la base de leurs génotypes.

De plus, les genets sont potentiellement différents en termes de taille. Certains peuvent être largement dominants, d'autres, au contraire, sont sous-représentés. Deux extrêmes peuvent être définis. Dans le cas d'une clonalité pure, une population peut être constituée par un seul génotype, répliqué N fois. La diversité génotypique est nulle. Au contraire, si chaque ramet correspond à un genet distinct, la clonalité est nulle. La diversité génotypique est alors maximale. La distribution des ramets au sein des genets est une information primordiale pour l'étude de telles populations. **Les descripteurs mathématiques de la biodiversité, développés initialement pour la description des communautés d'espèces, peuvent être adaptés aux populations partiellement clonales.**

Enfin, la clonalité donne également la capacité aux individus génétiques de se multiplier dans l'espace, et d'occuper une portion plus ou moins grande de cet espace 2D ou 3D. Ainsi, la structure génétique spatiale au sein des populations est indissociable de la distribution spatiale des clones (Arnaud-Haond *et al*, 2007), rendant nécessaire **l'étude des composantes spatiales de la clonalité.**

Les grandes lignes de cette méthodologie sont explicitées ci-dessous. L'objectif n'est pas de rentrer dans les détails, qui sont disponibles dans l'article d'Arnaud-Haond *et al*, (2007).

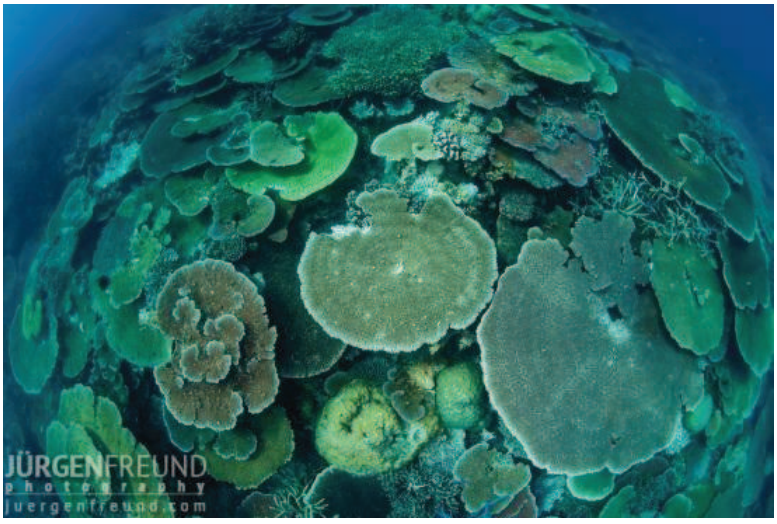

Figure 46 *Colonies tabulaires de coraux tropicaux, de tailles diverses. Comment décrire cette distribution de tailles ?*

Quels types de marqueurs moléculaires utiliser ?

La résolution requise pour le jeu de marqueurs moléculaires doit être individuelle. Il est donc nécessaire d'utiliser un jeu, dont la combinaison « nombre de marqueurs (i.e le nombre de locus étudiés) » ET « variabilité des marqueurs (i.e. polymorphisme allélique) » puisse constituer une sorte

de code-barres génétique de l'individu. La bijection entre code-barres moléculaire et genet est le but recherché.

Classiquement, les grandes familles de marqueurs utilisés par les études de populations clonales sont les allozymes, les AFLPs, les RAPDs et les microsatellites (qui ont été utilisés dans le cadre de cette thèse).

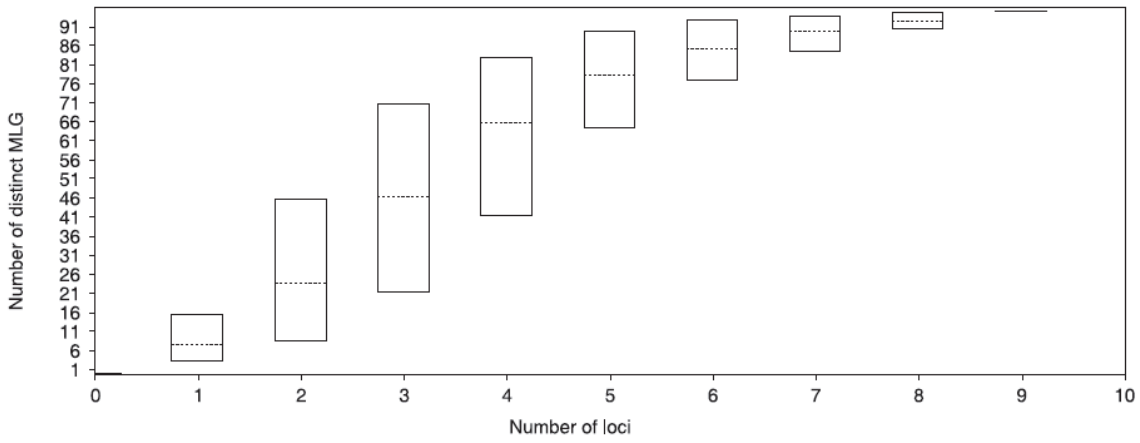

Figure 47 *Diagramme, provenant de la revue de Arnaud-Haond et al. (2007) décrivant la résolution génotypique des microsatellites sur un échantillon de 220 ramets de Cymodocea nodosa, une plante marine.*
Ces ramets ont été génotypés sur la base de 9 marqueurs microsatellites, et analysés pour toutes les combinaisons possibles de K locus, K étant compris entre 1 et 9. Les extrémités des boites représentent les nombres minimum et maximum des génotypes identifiés, la ligne centrale le nombre moyen. Les auteurs estiment, dans le cas présent, qu'un jeu de 7 marqueurs offre une résolution satisfaisante, puisque que le nombre de génotypes identifiés atteint un palier. Ce nombre est variable suivant le niveau de polymorphisme des marqueurs choisis.

Quelle stratégie d'échantillonnage adopter ?

La stratégie d'échantillonnage repose principalement sur 4 paramètres :

-la taille de l'échantillon, c'est-à-dire le nombre de ramets à prélever

-taille et forme de l'aire d'échantillonnage (transect, cercle, quadrat de dimension à définir) ainsi que sa réplication éventuelle (voir Becheler *et al*, 2010, inclus dans ce manuscrit).

- régime d'échantillonnage (aléatoire strict, hasard ou intervalles régulier)

-le respect d'une distance minimale entre deux unités d'échantillonnage, afin de limiter la collection de ramets appartenant au même clone.

Ces critères influencent profondément les descripteurs de la distribution clonale, ainsi que la perception de la structure spatiale.

La stratégie choisie dans ce travail est la suivante :

- Taille d'échantillonnage : 30 ramets
- Forme de l'aire d'échantillonnage : quadrat rectangulaire, dont les dimensions L et l sont fonction de la connaissance a priori des modalités d'élongation clonale de l'espèce étudiée
- Réplication : Oui, quand elle est possible (voir au cas par cas, par la suite). Notamment, les contraintes de temps et de logistiques, lors des échantillonnages de coraux profonds, n'ont pas permis de répliquer les quadrats.
- Régime d'échantillonnage : aléatoire strict. Des coordonnées XY sont générées au préalable. Cette disposition permet notamment, de limiter les biais lors de l'échantillonnage.
- Distance minimale entre deux unités d'échantillonnage : Non. Utilisant des coordonnées aléatoires et ne respectant pas de distance minimum entre deux unités d'échantillonnage, tous les ramets de la population d'étude ont la même probabilité d'être échantillonnés. Ceci est idéal pour l'étude de la diversité clonale. De plus, ceci permet d'obtenir des distances entre deux unités d'échantillonnage comprises entre 0 et la racine carrée de $[L^2 + l^2]$, correspondant à la diagonale du quadrat. Ces distances sont distribuées de façon continue dans cet intervalle. Ceci constitue un pré-requis pour l'étude de la structure génétique spatiale.

  NB : la standardisation de cette stratégie d'échantillonnage est absolument nécessaire si l'on souhaite comparer les valeurs de diversité intraspécifique des populations entre elles.

Le principe de la discrimination clonale

Une fois les génotypes multi-locus de chaque unité d'échantillonnage établis, les données sont organisées tel que présenté sur le tableau 17 :

Tableau 17 *Exemple de tableau de génotypes*

	Locus 1		Locus 2		Locus 3		Locus 4	
UE 1	100	102	145	145	180	185	206	210
UE 2	100	102	145	145	180	185	206	210
UE 3	100	102	145	**147**	180	185	206	210
UE 4	104	108	149	151	180	188	210	214

Les unités d'échantillonnage (UE) 1 et 2 partagent le même génotype multilocus. UE 3 est distinct des deux précédentes pour seulement un allèle du locus 2. L'UE 4, quant à elle présente un génotype bien différent.

Les questions que l'on doit se poser sont les suivantes :

- Si deux génotypes multi-locus, noté MLG par la suite, sont identiques (UE 1 et UE 2), s'agit-il d'un même clone ? est-il possible qu'un même MLG provienne indépendamment de différents évènements de reproduction sexuée ?
- Deux MLGs distincts appartiennent-ils nécessairement à deux clones distincts ? Par exemple, UE 1 et UE 3 possédent des génotypes différents. Mais l'unique locus qui les différencie ne provient-il pas d'une mutation somatique ou d'une erreur humaine lors du génotypage ?

Répondre à ces questions revient à vérifier la bijection entre le MLG (le code barre de l'UE) et le clone (ou genet) comme évoqué plus haut.

Pour cela, une démarche en deux étapes est recommandée par Arnaud-Haond etal 2007, chacune évaluant la probabilité liée aux deux questions posées ci-dessus.

Peut-on raisonnablement supposer que tous les réplicats d'un même MLG appartiennent à un même genet ?

A partir d'une estimation des fréquences alléliques dans un échantillon (un quadrat d'herbier ou de récif de corail, dans le travail présent), il est possible de calculer Psex, la probabilité que deux Unités d'échantillonnage partageant le même MLG proviennent des 2 évènements de reproduction sexuée distincts, et ne soient donc pas des clones (hypothèse nulle). En reprenant le tableau de génotypes ci-dessus, P_{sex} permet d'estimer la probabilité que les UE 1 et 2.

Si P_{sex} est inférieure au seuil que l'on se fixe (0.05 ou 0.01), on rejette l'hypothèse nulle. On peut supposer que les 2 MLGs identiques appartiennent au même clone/genet, avec seulement 1 ou 5 chances sur 100 de se tromper.

Peut-on raisonnablement penser que des MLGs distincts appartiennent à des genets distincts ?

Cette question se pose surtout lorsque deux UE présentent des MLGs très proches, distincts à un seul locus (UE 1 et 2 et UE 3, par exemple). Cette petite différence peut être due au fait qu'il s'agit de lignées clonales distinctes. Mais ceci peut aussi s'expliquer par une mutation somatique ou une erreur de génotypage ; les deux MLGs légèrement distincts appartiendraient donc à une même lignée clonale.

Pour répondre à cette question, la méthode préconisée est la suivante. On retire le locus qui distingue les MLGs considérés, sur l'ensemble de l'échantillon (le quadrat d'herbier ou de récif). Les deux MLGs distincts pour ce locus deviennent alors des MLGs identiques (si on retire le locus 2, les UE 1, 2 et 3 portent alors le même génotype). A partir des mêmes fréquences alléliques que celles estimées pour l'étape précédente, P_{sex} est estimée à nouveau.

Si P_{sex} est inférieure au seuil fixé, on considère alors que UE 1, 2 et UE 3 appartiennent au même clone (il y a bien eu une mutation somatique ou une erreur de génotypage). L'ensemble UE1 UE2 et UE 3 forment alors un MLL (Multi-Locus Lineage, au sens utilisé par Arnaud-Haond *et al*, 2007), un ensemble de MLGs dérivant d'une même cellule oeuf. En revanche, si P_{sex} est supérieure au seuil, UE 1 et 2 et UE 3 sont considérés comme des lignées clonales effectivement distinctes.

Une fois cette bijection entre MLG et clone testée de façon probabiliste, il devient alors possible d'étudier la composition clonale des populations.

Richesse et diversité clonale

Pour une communauté d'espèces, la richesse représente le nombre total d'espèces composant cette communauté. Pour une population partiellement clonale, la richesse clonale R reflète le nombre de clones présents dans la population.

$R = (G - 1) / (N - 1)$

Avec G le nombre de lignées clonales discriminées dans l'échantillon, et N le nombre d'unités d'échantillonnage prélevées. Si toutes nos UE sont appartiennent à une seule et même lignée clonale, R vaut 0. Ceci peut arriver pour certaines populations extrêmement clonales. Si l'ensemble des UE échantillonnées appartiennent à des lignées clonales distinctes, la richesse clonale est alors maximale (R = 1). Notons que R est très variable suivant l'effort d'échantillonnage. Aussi, si les méthodes d'échantillonnages ne sont pas rigoureusement standards, il devient difficile voire impossible, de comparer différentes valeurs de richesse clonale.

Cependant, la richesse clonale ne traduit pas directement l'existence de lignées clonales dominantes, i.e présentant de nombreux réplicats dans la population. Les indices de diversité, classiquement utilisés en écologie des communautés, telles que les indices de Simpson et de Shannon, peuvent être extrapolés aux populations clonales. Un dernier indice, qui est également utilisé dans ce travail, est calculé à partir de la distribution des ramets au sein des genets. Cette distribution suit une loi de Pareto, une loi de puissance de paramètres (m ;β) qui a pour expression $P(X > x) = (x / m)^{-\beta}$. C'est précisément cette loi qui théorise le principe des 80-20, traduisant que 80% des richesses d'un pays sont détenues par 20% de la population. Pour des organismes clonaux, on peut observer qu'une grande majorité de clones sont sous-représentés, tandis qu'un petit nombre d'entre eux possèdent un grand nombre de réplicats. La figure 48 illustre cette distribution déséquilibrée.

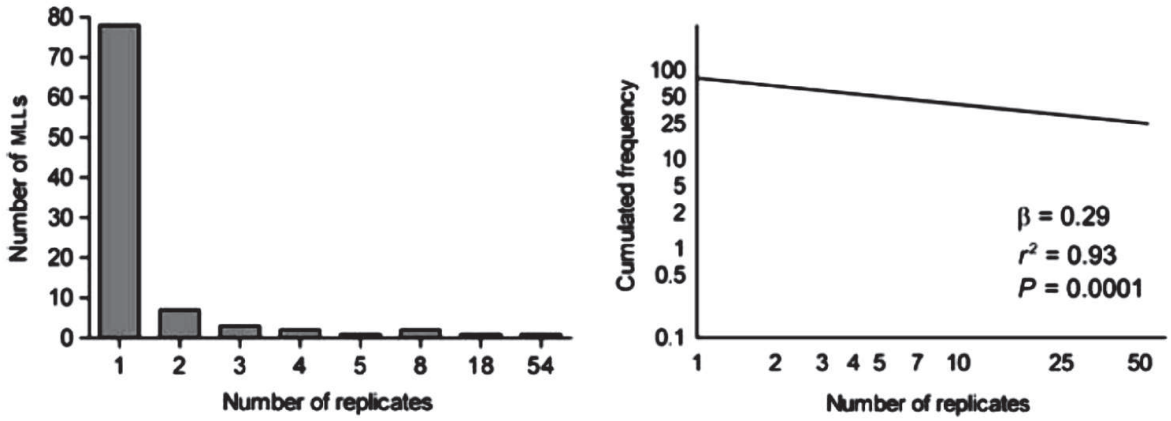

Figure 48 *Distribution des réplicats entre les MLLs chez Cymodocea nodosa dans la baie d'Alifax (Alberto et al, 2005), dont l'allure est typique d'une loi de puissance (à gauche). A droite, une transformation log-log de l'inverse de la distribution cumulée permet de linéariser, et de calculer le paramètre β, correspondant alors à la valeur absolue de la pente. Cette figure provient de Arnaud-Haond et al, 2007.*

Plus la distribution des ramets au sein de genets est déséquilibrée, plus la valeur de β est faible.

Composantes spatiales

La stratégie d'échantillonnage mise en œuvre permet d'accéder aux composantes spatiales de la clonalité. Ceci permet d'une part de faire des analyses d'autocorrélation spatiale, ainsi que des estimations de taille de genets.

L'autocorrélation spatiale permet d'estimer l'échelle spatiale pour laquelle la clonalité influence la distribution de la variabilité génétique. Pour cela, le coefficient d'appartement (Loiselle *et al*, 1995, Ritland 1996) entre paires d'individus appartenant à différentes classes de distance est représenté en fonction de la distance géographique. Il s'agit de tester le modèle d'isolement par la distance, à l'échelle intra-populationnelle.

Une dernière métrique est également utilisée, appelé *clonal subrange.* Il s'agit de la plus grande distance séparant deux réplicats du même genet. C'est une approximation minimale de la taille du plus grand genet dans le jeu de donné considéré.

ANNEXE 2 LES TRAITS DE VIE ADULTES ET LARVAIRES SONT DES DETERMINANTS DE L'AIRE DE DISTRIBUTION DES POISSONS DE RECIF

Cette annexe est constituée d'un article de biogéographie de Luiz *et al*. (2013), issu d'un travail auquel j'ai été associé. L'idée originale, de Michel Kulbicki (IRD), était de rechercher des prédicteurs de l'aire distribution de poissons de récif, autres que la durée de vie larvaire, classiquement utilisée en tant que proxy du potentiel de dispersion. J'ai eu l'occasion de réaliser mon stage de Master sur cette problématique, sous l'encadrement de M. Kulbicki. La première étape consistait en la création d'une base de données de durées de vie larvaire de poissons récifaux (Pacifique Central, Pacifique Est et Océan Indien) aussi exhaustive que possible, en compilant les données par espèce disponibles dans la littérature. Pour enrichir cette base, ce paramètre a également été estimé à partir de coupes d'otolithes de jeunes poissons, pêchés suite à leur récent recrutement. Le second volet de ce stage consistait en l'exploration des relations entre durée de vie larvaire (un proxy du potentiel de dispersion), traits de vie et surface de l'aire de répartition.

Osmar Luiz a repris les résultats préliminaires et la base de données, qu'il a enrichie en incluant des données de l'Atlantique tropicale. Sa ré-analyse des données a conduit aux résultats publiés dans l'article ci-dessous.

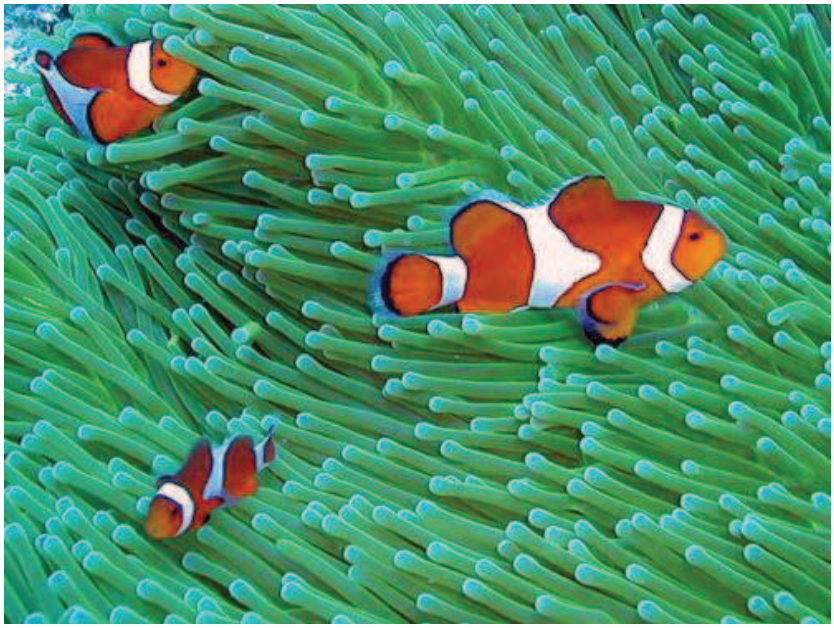

Figure 49 *Poissons clown sur leur anémone*

Adult and larval traits as determinants of geographic range size among tropical reef fishes

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Most marine organisms disperse via ocean currents as larvae, so it is often assumed that larval-stage duration is the primary determinant of geographic range size. However, empirical tests of this relationship have yielded mixed results, and alternative hypotheses have rarely been considered. Here we assess the relative influence of adult and larval-traits on geographic range size using a global dataset encompassing 590 species of tropical reef fishes in 47 families, the largest compilation of such data to date for any marine group. We analyze this database using linear mixed-effect models to control for phylogeny and geographical limits on range size. Our analysis indicates that three adult traits likely to affect the capacity of new colonizers to survive and establish reproductive populations (body size, schooling behavior, and nocturnal activity) are equal or better predictors of geographic range size than pelagic larval duration. We conclude that adult life-history traits that affect the postdispersal persistence of new populations are primary determinants of successful range extension and, consequently, of geographic range size among tropical reef fishes.

macroecology | marine dispersal | colonization

Geographic range size is a fundamental biogeographic variable that, among other effects (1, 2), strongly influences a species susceptibility to extinction (3, 4). Because most marine organisms disperse as larval propagules transported by ocean currents, it is often assumed that the duration of the larval stage is the fundamental determinant of their dispersal ability, and hence their range size (5, 6). Tropical reef fishes have geographic ranges that vary greatly in size, from a few square kilometers around tiny isolated islands to entire ocean basins (7–9). Given that pelagic larval duration (PLD) also varies greatly among such fishes, from only a few days to many months, the effects of PLD on dispersal potential became an early focus of investigation on general determinants of range size among those fishes and other near-shore marine species (10–12). However, although it has become evident that PLD is unlikely to be a primary determinant of geographic range size (13–16), alternative hypotheses have only recently begun to be considered (9).

To expand its geographic range, a species must successfully colonize new areas following the dispersal of its propagules (17). Consequently, attributes other than pelagic dispersal capacity may largely determine how widely reef fishes are distributed geographically (9). Here we assess the relative importance of seven adult and larval traits in influencing geographic range sizes of tropical reef fishes at the global scale. We do so using data from 590 species of tropical reef fishes in 47 families, the largest compilation of such data currently available for any marine group (Dataset S1). Traits directly linked to larval dispersal potential include PLD and spawning mode. Adult traits include maximum body size, schooling behavior, nocturnal activity, use of multiple habitat types, and adult depth range. The adult-biology traits chosen are not directly related to larval dispersal potential, but may influence the propensity for range expansion by affecting the establishment and persistence of new populations, as suggested by a recent study on Atlantic reef fishes (9). For example, schooling (18, 19) and nocturnal activity (20) reduce predation risk and thereby increase the chance of post-settlement survival. Broad habitat use and depth range indicate ecological generality, which is thought to influence establishment success in new environments (21). Finally, body size is linked to both predation risk and ecological generality (22).

Evaluation of these hypotheses is challenging because species traits are phylogenetically nonindependent (23) and unevenly distributed among families. Previous studies of dispersal–range-size relationships have controlled for effects of phylogeny, and limits on range-size imposed by ocean-basin size, by separately analyzing subsets of data (7, 16). However, this approach reduces statistical power (23, 24) and the ability to assess the generality of the effects of different factors. Our analysis controls for the nonindependence of shared traits among related species by using linear mixed-effects modeling (LMM) treating family and genus as nested random effects (9, 23). Our analysis includes species from three different regions that vary greatly in maximum (longitudinal or latitudinal) extent: the Indo-Central Pacific (ICP; ~22,000 km), the tropical Atlantic (TA; ~12,000 km), and the tropical eastern Pacific (TEP; ~5,000 km). To control for this variation, we include region and its interactions with other variables as fixed effects in our models. Modeling the data in this way, we are unique in being able to assess the relative importance of various adult and larval traits as determinants of range size among tropical reef-fish, as a group, at the global scale.

Significance

Marine organisms disperse mostly by ocean currents as larval propagules. Therefore, it is commonly thought that the duration of the larval stage is the fundamental determinant of geographic range size. Using a global compilation of reef fish traits, we test an alternative hypothesis: adult traits associated with population establishment and persistence in novel areas are better predictors of geographic range size than larval traits. We conclude that colonization success is as primary determinant of successful range extension and of geographic range size among tropical reef fishes.

Author contributions: O.J.L. and J.S.M. designed research; O.J.L., D.R.R., and J.S.M. performed research; O.J.L., A.P.A., D.R.R., S.R.F., M.K., L.V., R.B., and J.S.M. analyzed data; and O.J.L., A.P.A., D.R.R., S.R.F., M.K., L.V., R.B., and J.S.M. wrote the paper.

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Results and Discussion

Our analysis shows that region is the most significant predictor of geographic range size (Table 1). This result could be because of constraints of regional extent on range size within a region, or to differences in the underlying structure of the range-size frequency distribution within each region. To assess the importance of regional extent, we repeated the analysis after expressing the range size of each species as the ratio between the range size of species and the extent of the region. When range sizes were standardized in this way, all effects of species traits were maintained, and the region effect declined from the most important to the least important predictor (Table 1).

Our analysis also shows that three of five adult life-history traits—body size, schooling behavior, and nocturnal activity—are significant predictors of geographic range size (Figs. 1 and 2) for the global dataset (Table 2). Although PLD is also a significant predictor of range size at the global scale, only the largest of the three regions, the ICP, showed such a correlation (Table 2 and Table S1), confirming the finding of a previous analysis (7). The ICP correlation is largely driven by the few transpacific species that cross the world's largest oceanic barrier, the 4,000+-km-wide Eastern Pacific Barrier (EPB) that separates the ICP from the TEP (7). After the removal of those trans-Pacific species, the effect of PLD dropped from the second-most influential factor, globally, to the least-important trait (Table 1). A recent analysis of global ocean circulation patterns and habitat distributions (16) indicates that larvae of most tropical-reef species have PLDs sufficient to reach most habitat patches. Our results are consistent with that analysis: they show that the PLD effect is evident only at the largest spatial scale and when habitat patches are most isolated.

Our analysis provides further evidence for the view that range extensions are strongly influenced by adult life-history traits (9), factors likely to affect the capacity of new colonizers to survive and establish reproductive populations. All three of the positive adult-biology correlates of range size we identified—maximum body size, schooling behavior, and nocturnal activity—may enhance the probability of population establishment after propagule arrival.

Predation is one of the main processes influencing the structure and species composition of ecological communities (25), especially assemblages of coral reef fishes (26). If these factors limit the number of locally coexisting species (27, 28), predators may inhibit nonnative species establishment (29), and thereby constrain the geographic ranges of prey (30). Piscivorous predators are ubiquitous in coral reefs. Mortality is disproportionally higher among new recruits (31, 32), although juveniles and adults are not immune to predation, as evidenced by morphological, chemical and behavioral antipredatory mechanisms they use (26, 33). Among reef fishes, predation may be particularly important for species at early-stage colonization if predators tend to target rarer species (27, 34). Immigrant species may have better chances of survivorship and establishment if they can rely on specific antipredator mechanisms.

Reduction in predation risk is considered one of the main benefits of schooling in fish (35), and per capita mortality rates in schools of reef fishes decrease as school size increases (36). Predators are less successful at singling out individual prey from large schools because of the "confusion effect" (34). Survivorship may increase in both single-species schools and mixed-species schools not only through the confusion effect but also through more effective foraging by social observation, better vigilance for predators, and greater economy of time budgeting (35). Mixed-species schools are common among recruiting juveniles of reef fishes, sometimes involving species with different diets, which reduces the competitive costs while retaining the benefits of social behavior (19). Schooling may also increase the chance of finding mates among a scarce cohort of colonizers, improve food detection, and enhance access to resources protected by territorial competitors (37, 38). Thus, schooling fishes may have greater relative potential for population establishment and persistence after reaching new areas.

Recently, direct evidence has emerged that diel activity patterns of reef fishes are influenced by ongoing predation intensity: day-active nocturnal fish were much more common at a predator-depleted atoll in the Central Pacific than at a neighboring atoll with a large population of predators (20). This finding supports the view that nocturnal activity allows prey to avoid interactions with day-active predators. Differences in the morphology and behavior of nocturnal and diurnal reef fishes also are consistent with reduced predation intensity at night (39). Nocturnal planktivores are relatively deep bodied and robust, in contrast to their more streamlined diurnal counterparts, and there is a general reduction of schooling at night (39). Whatever the ultimate reasons for the development of nocturnal activity among reef fishes, nocturnal species may be exposed to a smaller subset of predators than day-active fishes (26, 39).

Table 1. Significant variables ranked according to their independent effects

All species (n = 590)					Trans-Pacific species removed (n = 564)				
Variable	df	F value	P value	IE (%)	Variable	df	F value	P value	IE (%)
Geographic range size									
Region	2	117.83	<0.001	53.3	Region	2	104.80	<0.001	66.9
PLD	1	54.72	<0.001	17.3	Body size	1	31.44	<0.001	9.6
Body size	1	69.86	<0.001	15.5	Nocturnal	1	16.69	<0.001	8.4
Nocturnal	1	18.22	<0.001	7.8	Schooling	1	15.45	<0.001	8.0
Schooling	1	12.63	<0.001	6.0	PLD	1	12.23	<0.001	6.9
Ratio between the species range size and region size									
Body size	1	111.70	<0.001	37.6	Body size	1	90.06	<0.001	36.0
PLD	1	23.28	<0.001	24.8	Nocturnal	1	38.13	<0.001	25.9
Nocturnal	1	33.17	<0.001	19.4	Schooling	1	12.94	<0.001	16.2
Schooling	1	11.47	<0.001	13.9	PLD	1	11.62	0.001	13.3
Region	2	2.09	0.123	4.2	Region	2	3.48	0.031	8.3

The independent effects (IE) value corresponds to the percentage of the explained variance accounted for by each explanatory variable as calculated using hierarchical partitioning. In Geographic range size, ranking is according to IE on geographic range size. In the ratio between the species range size and region size ranking is according to IE on the ratio between the species range size and region size. Degrees of freedom (df), test statistics (F value), and probabilities (P value) are listed for each coefficient in each model.

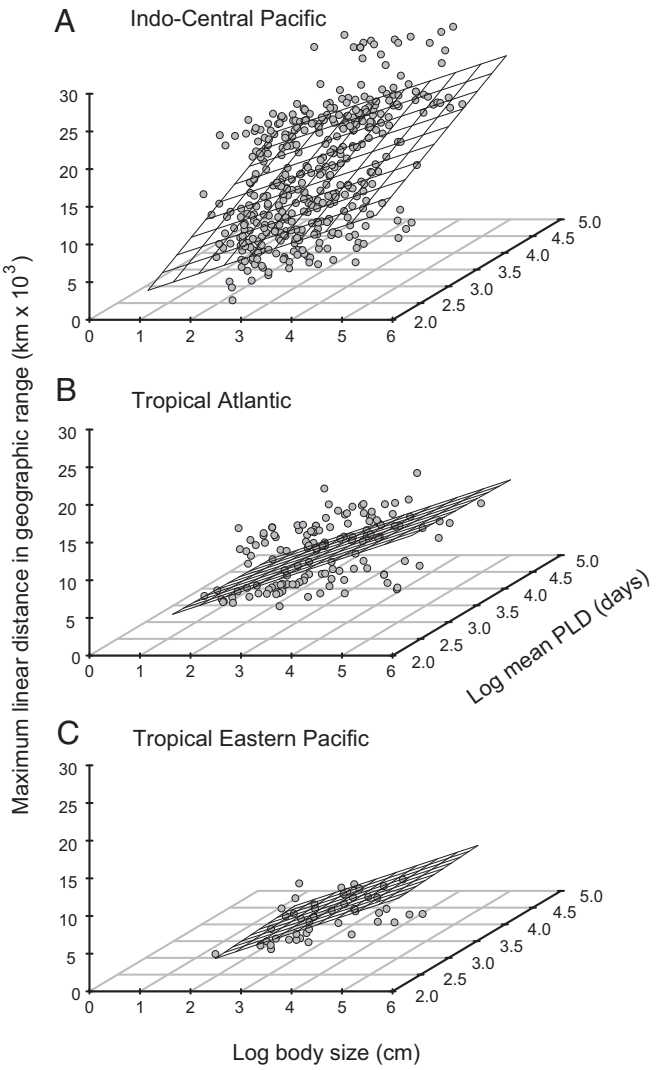

Fig. 1. Relationships among geographic range size, body size and mean PLD. (*A*) Indo-Central Pacific, (*B*) tropical Atlantic, (*C*) tropical eastern Pacific. The regression plane is the prediction from the LMM. Points represent the observed data.

Positive relationships between body size and range size have been documented for diverse taxonomic groups of both terrestrial and marine organisms (2). Larger fish species are generally less susceptible to predation than smaller species (22, 31). Mortality rate of reef fishes are high immediately following settlement of the larval stage, regardless of adult size (26, 31–33). However, juveniles of large species tend to grow faster than those of smaller species (31, 40, 41), allowing them to quickly reach a size refuge (31). Furthermore, large species can generally use a broader range of food types, and are more tolerant of environmental variability than small species (22, 42). Finally, in general, body size is positively correlated with longevity among marine fishes (43). This relationship may influence species establishment and persistence at new range outposts by reducing the probability of local extinction between sporadic long-distance recruitment (44) (i.e., the "storage effect").

Broad habitat use and depth range, both assumed to indicate ecological generality, were not significant correlates of geographic range size in our analysis. Analyses of ecological generality as a predictor of establishment success, mainly among birds, have produced mixed outcomes (45). In a previous study of the likelihood of reef fish crossing dispersal barriers in the Atlantic Ocean (9), broad habitat use correlated with crossing a coastal barrier within which adults of generalist species likely can survive, but not with crossing the Atlantic Ocean, where only pelagic propagules can succeed. Therefore, habitat generality may be important for only a specific type of barrier, which in turn may account for its lack of global relevance as a predictor of range size. Depth range and spawning mode had no statistical effect in either this study or the study on dispersal barriers in the Atlantic (9).

There are differences in the geographic distribution of reef habitat in the three ocean regions that might be expected to produce differences in factors affecting range size. The ICP consists of a vast network of islands and continental coastlines separated by less than 900 km (46), except for the ~2,000 km isolation of peripheral islands like Hawaii and Easter Island. The TEP and TA are dominated by relatively continuous continental coastlines, with patches of nonreef habitats separating biogeographically distinct provinces within each region (9, 47) and a few isolated oceanic islands separated by ocean gaps up to ~1,000 km. The TA is the only region with a large central ocean gap, the 3,500+-km-wide Atlantic. Despite these interregional differences in habitat geography, the same three adult traits (schooling, nocturnal activity, and large size) influence range size in all three ocean regions (Figs. 1 and 2), emphasizing the importance of these traits and colonization ability globally.

We conclude that adult life-history traits that enhance the probability of population establishment and persistence are important determinants of the potential for successful range extension by tropical reef fishes in general. The implication of our results is that factors affecting species persistence in new range outposts are more important for determining the size of geographic ranges than larval dispersal potential (17). The exception relates to the relatively uncommon crossings of the world's largest oceanic barrier, the EPB (48), where the PLD does have a prominent role. Our analysis has implications not only for biogeographic analyses but also for understanding the effects of climate change. Predictions of poleward range shifts by marine

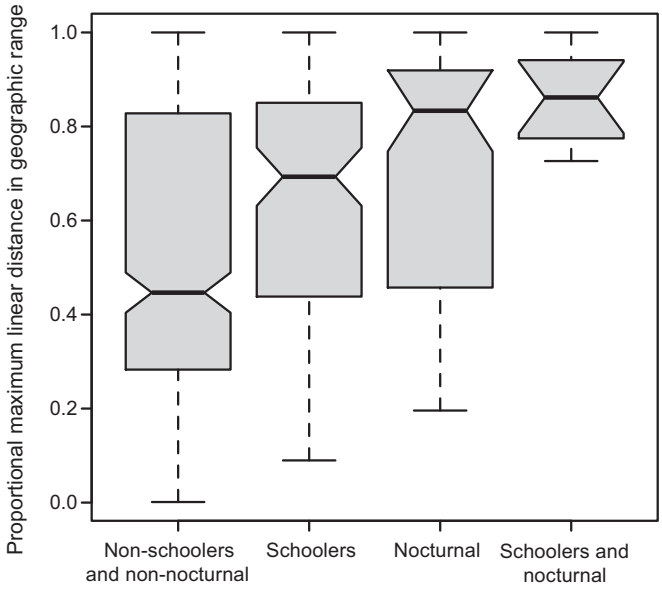

Fig. 2. Effects of schooling behavior and nocturnal activity on the ratio between the range size of species and the extent of the region. Dashed vertical lines, gray bars, and black horizontal lines represent, respectively, data range, interquartile range, and median. Nonoverlapping notches among the gray bars signify a difference at the 95% confidence level.

Table 2. Parameters of the final predictive LMM

Variable	Estimate	SE	*t* value	*P* value
Intercept	987.76	4425.75	0.223	0.823
Body size	**2210.34**	335.41	6.589	<0.001
Schooling behavior	**2030.28**	609.71	3.329	0.001
Nocturnal activity	**2503.27**	881.27	2.840	0.004
PLD: Tropical Atlantic	−247.54	1295.63	−0.191	0.848
PLD: Indo-Central Pacific	**4935.42**	1434.00	3.441	<0.001
PLD: Tropical Eastern Pacific	501.14	2406.30	0.208	0.835
Region: Indo-Central Pacific	**-10483.26**	4915.94	−2.132	0.033
Region: Tropical Eastern Pacific	−5102.71	8397.86	−0.607	0.543

Parameters estimated in a LMM with MLD of geographical range size (in kilometers) as the response variable for larval and adult traits including an interaction between PLD and biogeographic region, and genus nested within family as a random variable. Estimate, coefficient estimate of explanatory variables; SE, test statistic (*t* value) and probability (*P* value). *P* values are significant ($P < 0.05$) for coefficients if in bold. Reference levels for this regression were set as "Tropical Atlantic" for region and as "no" for both schooling behavior and nocturnal activity.

species in response to ocean warming have been based on their thermo-physiological tolerances (49). However, our results indicate other life-history traits that may constrain range extension and thereby influence extinction risks (4).

Materials and Methods

Species Traits. We selected life-history traits that are thought to potentially influence range sizes (2, 9), and for which data are readily available for all species for which we also have PLD data. Body size is correlated with many other biological attributes of species, including range size (2). Schooling behavior and nocturnal activity are mechanisms that may diminish predation risk (18–20), and thereby enhance range expansion by assisting colonization. Environmental generalist species might be expected to have greater establishment success than specialists in new and different environments (21). Here, such generalists included species that have larger depth ranges and those that use other habitats in addition to structural reefs (soft bottoms, seagrass/macroalgae beds, mangroves, and estuaries).

PLD is linked to the time larvae are susceptible to pelagic dispersal. Spawning mode (production of pelagic or benthic eggs), besides acting as a proxy for PLD (9), influences the stage of development at which larvae enter the pelagic zone. The eggs of pelagic spawners are immediately subjected to transport by currents, whereas the eggs of benthic spawners are deposited on the substrate and the larvae enter the pelagic realm only after developing for some time in the egg before hatching. Existing data on tropical reef fish PLD were compiled from the primary literature for 446 species (see references in Dataset S1). Additional data on PLDs were obtained for 227 species, from which 144 are uniquely reported, by aging settlers through analyses of daily growth increments and settlement marks in otoliths (50). Because of a lack of sufficient data in one or more regions we did not include one other factor that we assessed in our study of range expansion across large barriers in the Atlantic Ocean (9): flotsam-rafting behavior by postlarval stages. Data on spawning mode, maximum total length (our metric for body size), depth range, schooling behavior, multihabitat use, and nocturnal activity were obtained from the primary literature and the global fish data aggregator FishBase (www.fishbase.org). We considered schooling species to be those that regularly form polarized, cohesive groups of 20 or more individuals. Diurnal and nocturnal were defined by the main period of day that each species actively forages.

Geographic Range. For comparability with previous studies we used the linear distance between the farthest two range endpoints—maximum linear distance (MLD) in kilometers—as a metric of geographic range size (7, 16). Those studies found that the MLD of range size is strongly correlated with the combined maximum latitudinal and longitudinal extent of ranges, and they considered MLD to represent an adequate descriptor of a species' geographic extent (7). Data on species range limits were obtained from guidebooks (51–55) and from the Ocean Biogeographic Information System (OBIS), a global aggregator of geo-referenced collection records (www.iobis.org). Data from both FishBase and OBIS were screened by us and complemented from our own records (56) (Dataset S1). Endpoint geographic coordinates were determined to the nearest degree using Google Earth (earth.google.com). The MLD was measured using the function "geodist" in the R package "gmt" (57). Species were grouped in terms of their residence in three well-defined biogeographic regions, the physical dimensions of which delineate the maximum attainable range size in each region: the Indo-central Pacific region (from the Red Sea to Easter Island), the tropical Atlantic (from the northwest Gulf of Mexico to the southern Gulf of Guinea), and the tropical eastern Pacific (from the northern Gulf of California to northern Peru). Primarily tropical species that extend their ranges to temperate zones had their full range considered in the analysis. Although it is in the Pacific, together with the ICP, the TEP is a well-recognized biogeographic entity that has a substantial fauna with a high level of endemism (~75%) (47). Only TEP endemics were included in the TEP group in our analyses, which avoided possible confounding effects of recent trans-Pacific crossers not having realized their full range potential in that region. The few trans-Pacific species that occur in both the ICP and TEP were included as members of the ICP fauna because most of the range of each is in the ICP and most appear to have migrated from there to the TEP (48). We did not consider the western and eastern sides of the TA as separate regions because there are insufficient data on the PLDs of species endemic to the tropical eastern Atlantic, which has a substantially lower rate of endemism than the tropical western Atlantic (58).

Statistical Analysis. The LMM was fitted using the function "lme" from the package "nlme" (59) in R. The response variable was the MLD between two points along the geographic range boundary. PLD, spawning mode, maximum body size, schooling behavior, nocturnal activity, depth range, multihabitat use, and region were included as fixed variables, and genus and family were included as nested random variables. Interactions between region and each fixed factor were considered in the full model. For model selection, we followed the procedure recommended by Zuur et al. (60) of a backward stepwise removal of nonsignificant fixed-effect terms ($P > 0.05$) from the full model based on log-likelihood ratio tests (Table S1). Partitioning of variance to determine the relative importance (percent of explained variance; independent effects in Table 1) accounted for by each explanatory variable in the model was calculated by hierarchical partitioning using the R package "hier.part" (61).

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Résumé

L'influence de la diversité génétique sur la stabilité démographique des populations constitue un paradigme de l'écologie évolutive. Au sein des populations naturelles, l'étude de cette relation est complexifiée par l'influence réciproque de la stabilité sur la diversité, et leur degré d'interconnexion. Ces interrelations ont été explorées chez la plante marine *Zostera marina* et les coraux d'eau froide *Lophelia pertusa* et *Madrepora oculata*, des espèces partiellement clonales. Ce trait d'histoire de vie, influençant profondément la dynamique démographique et la trajectoire évolutive des espèces, a constitué le fil d'Ariane de ce travail.

L'échantillonnage dans l'espace (échelle régionale) et le temps (un pas de trois ans) d'herbiers de Zostère a permis de mieux comprendre la dynamique clonale de ces plantes. L'architecture et la diversité clonale apparaissent comme la résultante de l'équilibre entre dispersion/recrutement de nuages de graines dispersées collectivement, et la compétition pour l'espace entre clones. Les perturbations affectent localement l'équilibre de l'herbier. Cette dynamique originale rend impossible l'identification des contours populationnels. En revanche, nos résultats semblent indiquer que la diversité génétique au sens strict (hétérozygotie et nombre d'allèles) des herbiers de Zostères constitue un facteur de stabilité démographique, *via* sa potentielle influence sur les capacités de résistance aux perturbations saisonnières.

Les coraux d'eau froide, quant à eux, présentent des patrons biogéographiques en accord avec l'hypothèse d'une extinction dans le Golfe de Gascogne, lors des derniers épisodes glaciaires. Les marques visibles des activités de pêche posent la question des capacités de résilience de ces écosystèmes, qui dépendent entre autres du potentiel de dispersion de ces espèces. L'absence de structure génétique observée chez *L. pertusa* suggère, au moins pour cette espèce, un fort degré d'interconnexion entre les récifs, tandis que *M. oculata* montre davantage de structure régionale. La sensibilité de ces espèces aux variations climatiques et à la pression des activités anthropiques souligne la nécessité d'études approfondies, pour leur conservation.

Les résultats obtenus pendant cette thèse permettent de mieux comprendre la dynamique populationnelle des herbiers et récifs profonds, le taux de clonalité et la connectivité des populations. Ces informations sont essentielles pour avancer vers une meilleure compréhension de la dynamique et la résistance de ces espèces structurantes, et sont donc primordiales pour la conservation de ces écosystèmes clé.

Abstract

The influence of genetic diversity on the demographic stability of populations constitutes a paradigm in evolutionary ecology. The complexity of this relationship within natural populations is enhanced by the reciprocal effect of stability on diversity, and the degree of interconnection among populations. This interaction was explored within the seagrass *Zostera marina* and the cold-water corals *Lophelia pertusa* and *Madrepora oculata*, three partially clonal species. This life history trait, deeply influencing the population dynamics and evolutionary trajectory of species, constituted the underlying theme of this work.

The sampling in space (regional scale) and time (a three-years step) of eelgrass meadows allowed us to better understand the clonal dynamics of these plants. The clonal architecture and diversity may result from the equilibrium between dispersal/recruitment of collectively dispersed clouds of seeds, and the competition for space among clones. Perturbations locally affect the equilibrium of meadows. This original dynamic makes impossible the identification of population contours. Yet, our results suggest that the genetic diversity *sensu stricto* (heterozygosity and number of alleles) represents a factor of demographic stability, through its putative influence on resistance capacity for seasonal disturbances.

Cold-water corals show biogeographic pattern in line with the hypothesis of glacial extinction, within the Bay of Biscay. The noticeable footprints of fishing activities question the capacity of resilience of these ecosystems, depending on dispersal potential of the structuring species, which showed low levels of clonality. The lack of genetic structure observed for *L. pertusa* suggest, at least for this species, a high degree of interconnection among reefs at large scale, while *M. oculata* revealed a stronger regional structure. Sensitivity of these two species to climatic variations and the pressures of human activities highlight the need of thorough studies for their conservation.

Results obtained during this thesis allow a better understanding of the populations dynamics of both seagrass and deep reefs and their levels of clonality and connectivity. This information constitutes the first step toward a better understanding of dynamics and resistance of these structuring species, and is also primordial for the conservation of their key ecosystems.