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EVOLUTION EXPERIMENTALE DE LA DIVERSITE EN MILIEU HETEROGENE

Jury

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« To understand the fate of diversity in heterogeneous environments, the appropriate experiment is as follows. A base population, which may be genetically uniform or diverse and either sexual or asexual, is inoculated into an environment, which may be heterogeneous or homogeneous, and natural selection is allowed to proceed for as many generations as one's patience allows. Samples are then extracted from each of the selection lines and their fitness assayed across a range of environments, preferably the same environments used during selection. The results are then cast in a genotype-by-environment matrix, where each of the selected lines are the different levels of genotype"

Rees Kassen 2002, Journal of Evolutionary Biology

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Et voilà cette période de ma vie qui s'achève. Ce fut une période de découvertes, de joies, de petites frustrations, remplie de nouvelles expériences. J'ai eu l'occasion de rencontrer des personnes qu'avec certitude je ne vais jamais oublier et sans lesquelles je n'aurais jamais réussi à arriver au bout.

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"Dispersal scales up the biodiversity-productivity relationship in an experimental source-sink metacommunity" P. A. Venail, R. C. MacLean, C. Meynard & N. Mouquet.

In press, **Proceedings B.**

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"Evolutionary diversification in temporally fluctuating environments: effect of the grain" P. A. Venail, O. Kaltz, I. Olivieri, T. Pommier & N. Mouquet.

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Evolution expérimentale de la diversité en milieu hétérogène

Résumé

Cette thèse aborde d'un point de vue expérimental le rôle de l'hétérogénéité environnementale spatiale et temporelle comme moteur de diversification biologique. Il comporte deux grandes parties : une synthèse générale sur les principales thématiques abordées pendant ma thèse et une présentation des publications dérivées de mes propres recherches et de collaborations.

Dans la synthèse, j'introduis la biodiversité en soulignant l'importance d'étudier les conditions qui favorisent son émergence et son maintien pour mieux comprendre son organisation. Je présente ensuite le type de diversité sur lequel se concentre mon travail ainsi que l'approche spatiale que j'ai utilisée pour son étude: la metacommunauté. J'évoque l'intérêt croissant des écologistes pour inclure les échelles évolutives dans les approches modernes des communautés et je présente l'évolution expérimentale avec des microorganismes comme un outil qui permet d'intégrer ces deux approches de la diversité. Je développe ensuite certains aspects théoriques qui définissent les conditions pour la diversification en milieu hétérogène. Pour finir, je présente la relation diversité-fonctionnement comme une façon de comprendre les conséquences des variations de la biodiversité.

Dans la deuxième partie je commence par présenter sous forme d'articles scientifiques les résultats de mes propres recherches. Ces articles explorent différents aspects de l'évolution et la diversification de la bactérie *Pseudomonas fluorescens SBW25* dans un environnement hétérogène. Les deux premiers en présence d'hétérogénéité spatiale et le quatrième dans un environnement temporellement fluctuant. Au niveau des collaborations, je présente un article de revue sur le concept de spécialisation écologique et les résultats d'une expérience d'évolution visant à explorer la dynamique évolutive de la relation biodiversité-productivité avec des bactéries marines Méditerranéennes.

Discipline : Ecologie et Evolution des Communautés.

Mots-clés : Diversité, évolution expérimentale, métacommunautés, *Pseudomonas fluorescens* SBW25, spécialisation, hétérogénéité environnementale, fonctionnement des écosystèmes, dispersion, grain, adaptation locale.

Institut des Sciences de L'Evolution de Montpellier, Université Montpellier II, 2010

Experimental evolution of diversity in heterogeneous environments

Abstract

This thesis proposes an experimental approach to the study of the importance of spatial and temporal environmental heterogeneity on the emergence and maintenance of biological diversity. It is composed of two main parts: a general review of the principal subjects I have explored during my thesis and a presentation of the manuscripts derived from my own research and from different collaborations.

In the synthesis I start by introducing the biodiversity concept and I underline the importance of considering the conditions to its emergence and maintenance in the understanding of its organization. I present the kind of biological diversity I'm interested in and the approach of spatial organization I have considered: the metacommunity. I also underline the interest of current ecology research in including evolutionary processes on the modern concept of communities and I present experimental evolution with microorganisms as an interesting tool allowing the exploration of the ecological and evolutionary aspects of diversification. I also explore some of the theoretical aspects of the conditions for diversification in heterogeneous environments. Finally, I present the biodiversity-ecosystem functioning relationship as a way to understand the consequences of diversity variation.

In the second part of the thesis I start by presenting the different manuscripts derived from my own work. They explore different aspects of the evolution and diversification of the bacteria *Pseudomonas fluorescens* SBW25 in heterogeneous environments. Two of them present different aspects of diversification in spatially heterogeneity environments while the other deals with diversification in a temporally fluctuating environment. I finish by presenting two studies I have collaborated in: a review about the ecological specialization concept and an experimental approach to the dynamics of the diversity-productivity relationship using Mediterranean Sea bacteria.

Discipline: Evolution and Community Ecology.

Keywords: Diversity, experimental evolution, metacommunities, *Pseudomonas fluorescens* SBW25, specialization, environmental heterogeneity, ecosystem functioning, dispersal, grain, local adaptation.

Préambule à la synthèse générale

Je suis actuellement professeur visitant et post-doc à l'Universidad de los Andes à Bogotá (Colombie). C'est une activité qui combine recherche et enseignement, similaire à celle d'ATER du système français (Assistant Temporel d'Enseignement et de Recherche) ou au « *teaching fellow* » du système anglo-saxon.

Cette synthèse a été conçue comme une introduction aux articles. Elle se caractérise par sa structure et son langage didactiques. La thématique de la biodiversité étant très vaste, je cherche à ce que le lecteur reconnaisse le cadre contextuel sur lequel se place mon travail de thèse et qu'il perçoive ces limitations. Loin de vouloir considérer tous les mécanismes que la littérature propose pour comprendre la diversité biologique, mon travail de recherche consiste à étudier expérimentalement le **rôle de l'hétérogénéité environnementale comme moteur de diversification.** Bonne lecture!

I. SYNTHESE GENERALE

A- Biodiversité.

« …biological diversity or biodiversity is the variety of life, and refers collectively to variation at all levels of biological organization. »

Gaston et Spicer 2003, Biodiversity: an Introduction. 2nd edition

1. Qu'est-ce que la biodiversité?

La biodiversité est une diversité de diversités. *L'Evaluation des Ecosystèmes pour le Millénaire* (2005) la définit comme toute la variabilité du monde vivant. Elle intègre les différences entre les individus appartenant à une même population, entre les populations à l'intérieur d'une espèce, entre les espèces d'une communauté, entre les communautés au sein d'un écosystème, et enfin, entre les écosystèmes.

Si bien la plupart des disciplines de la biologie se sont intéressées à la biodiversité, il est encore difficile de quantifier avec précision la totalité de formes du vivant habitant un hectare de forêt tropicale ou encore moins leur nombre total sur Terre. A présent, près se 1.75 millions d'espèces ont été décrites et on estime l'existence de plus de 14 millions (Tilman 2000).

Les différentes approches de la biodiversité ont souvent exploré différentes échelles spatio-temporelles de son organisation. La biologie évolutive par exemple s'est principalement intéressée aux conditions d'apparition de nouveaux variants génétiques au sein d'une population (Hartl 2000) et à l'émergence de nouvelles espèces (Coyne et Orr 2004) sur des longues périodes de temps. Les perspectives écologiques au contraire sont intéressées par la distribution des organismes à différentes échelles spatiales (Whittaker 1972) mais sur des courtes échelles temporelles. L'approche écologique de la diversité explore les mécanismes responsables de la

coexistence des espèces au sein des communautés (Morin 1999) en utilisant souvent la richesse taxonomique comme mesure de diversité (Magurran 2003). D'autres indices de biodiversité tels que la diversité fonctionnelle (*FD*, Petchey et Gaston 2002) ou la diversité phylogénétique (*PD*, Cadotte et al. 2009) sont aujourd'hui disponibles, mais pas un seul d'entre eux n'est capable de résumer les nombreuses facettes qui caractérisent la biodiversité (Purvis et Hector 2000). Dans mon travail de thèse je me suis surtout intéressé à la diversité génotypique au sein de populations bactériennes.

2. Pourquoi la biodiversité?

Comme nous venons de voir, la biodiversité intègre des notions qui proviennent de diverses disciplines de la biologie. Pourtant, il est évident que la biodiversité n'est plus une préoccupation exclusive des biologistes. Les 20 dernières années se sont caractérisées par une préoccupation croissante sur le déclin de la biodiversité, en grande partie comme résultat d'activités anthropiques (Hannah et Bowles 1995, Vitousek et al. 1997). *Conservation International* estime qu'une nouvelle espèce est déclarée éteinte toutes les 20 minutes (Conservation International 2010). Pourtant, non seulement de nombreuses espèces disparaissent tous les jours sous nos yeux mais aussi des communautés et des écosystèmes entiers sont en train d'être exploités, modifiés et dégradés afin de compenser les besoins d'une population humaine croissante (Sala et al. 2000, Evaluation des Ecosystèmes pour le Millénaire 2005). Il est aujourd'hui important de comprendre la relation que la biodiversité mène avec la société humaine (Mikkelson et al. 2007) et avec les biens et les services que les écosystèmes offrent (Hooper et al. 2005). C'est justement le rôle de l'Homme comme direct responsable du processus de destruction de la biodiversité qui différencie cette actuelle extinction massive des cinq précédentes qui ont marqué la longue histoire de la vie sur notre planète (Leakey 1999). Témoignant du fort intérêt que non seulement les biologistes mais également les politiciens et les médias donnent à cette

problématique et afin d'alerter l'opinion publique sur l'état et les conséquences de son déclin, 2010 a été déclarée l'année mondiale de la biodiversité par l'Organisation des Nations Unies (www.un.org/fr/).

Mais plus que le rythme actuel de disparition d'espèces ou la vitesse de dégradation des écosystèmes ce qui préoccupe d'avantage ce sont les conséquences de cette destruction (Chapin et al. 2000). Par exemple, la diversité génétique au sein d'une population (variations entre individus) joue un rôle capital car elle lui donne la capacité de répondre face à des modifications environnementales et de s'adapter (Barton et Partridge 2000, Forest et al. 2007, Barrett et Schluter 2008, Jump et al. 2008). L'érosion de ce capital adaptatif met en péril l'avenir des populations naturelles. Beaucoup d'études ce sont centrés autour des effets de la biodiversité sur le fonctionnement des écosystèmes (Loreau et al 2001, Hooper et al. 2005, Srivastava et Vellend 2005). De nombreuses approches théoriques, des expériences de terrain ainsi qu'en laboratoire ont été développées pour tenter de comprendre la relation entre diversité et fonctionnement des écosystèmes (Voir partie **II. E**). Un certain consensus existe aujourd'hui sur l'importance de maintenir une forte diversité pour garantir un meilleur fonctionnement des écosystèmes (Hooper et al. 2005) mais certaines incertitudes persistent, empêchant d'utiliser cet effet positif de la biodiversité sur le fonctionnement des écosystèmes comme seul argument pour sa protection (Srivastava et Vellend 2005). Déchiffrer l'importance de la biodiversité nécessite non seulement une meilleure compréhension de sa relation avec le fonctionnement des écosystèmes mais aussi des mécanismes qui déterminent son émergence et son maintien (Tilman 2000).

3. Comment apparait-elle?

a. Au sein d'une population.

La mutation est la principale source de variabilité génétique au sein d'une population. Elle se définit comme une modification aléatoire dans le génome qui peut représenter un simple changement dans un seul nucléotide (mutations ponctuelles) jusqu'à la duplication d'un caryotype entier. Le taux de mutation ou fréquence d'apparition de nouveaux variants génétiques dépend entre autres de l'organisme en question et de la région du génome considérée. Par exemple chez les bactéries le taux de mutation avoisine 1/300 par génome et par réplication alors que chez les eucaryotes il peut varier de 0.1 à 100 par génome et par génération (Drake et al. 1998). Ces taux sont à priori très faibles mais suffisants pour assurer une importante variabilité génétique dans les grandes populations ou chez des espèces avec des grands génomes. Il semblerait que 0.1% de la variance génétique dans un caractère quantitatif est introduite par mutation à chaque génération (Barbault et Sastrapradja dans Global Biodiversity Assessment 1996). Cependant, en plus des mutations, d'autres mécanismes sont aussi responsables de l'émergence de variabilité génétique à l'intérieur des populations, parmi lesquels la dérive génétique, la dispersion ou flux génique et la recombinaison. Ces mécanismes agissent souvent en synergie pour expliquer et pour générer de la variabilité. Dans la plupart des expériences réalisées pendant ma thèse, la source ultime de variabilité génotypique est la mutation.

b. Au sein d'une communauté.

La différenciation entre espèces (spéciation) commence souvent par l'apparition de différences entre individus au sein d'une même espèce (Coyne et Orr 2004). L'hypothèse écologique de la spéciation (Schluter 2001) propose que les différences entres espèces sont basées sur l'accumulation progressive

de différences génétiques entre sub-populations d'une même espèce (pas forcement en allopatrie) résultant de l'adaptation à des conditions environnementales différentes. Sous l'effet de la sélection divergente et après des périodes suffisamment longues, cette différenciation peut résulter dans l'isolement reproductif et l'apparition de nouvelles espèces. Cependant, l'hypothèse écologique de Schluter (2001) n'est qu'une façon parmi d'autres de générer des nouvelles espèces. La spéciation est un processus complexe et il est au-delà des objectifs de cette thèse d'explorer toutes les hypothèses de spéciation aujourd'hui disponibles (voir Coyne et Orr 2004, Schluter 2001).

Lorsque la spéciation se produit de manière rapide et aboutit à une différenciation phénotypique et écologique à partir d'un ancêtre commun, elle reçoit le nom de radiation adaptative (Schluter 2000). Des araignées *Tetragnathes* d'Hawaii (16 espèces, Gillespie 2004), des épinoches des grands lacs africains (Schluter 1993), les lézards du genre *Anolis* des Antilles (plus de 140 espèces, Losos 1994) et les pinsons de l'archipel de Galápagos (15 espèces, Grant 1981) sont parmi les exemples de radiations adaptatives naturels les plus connus. Entre autres, ces études ont révélé des fortes différences dans les taux de spéciation à l'intérieur de chaque groupe ainsi que l'existence de fortes convergences écologiques dans des radiations adaptatives indépendantes (Schluter 2000). C'est le cas par exemple des lézards *Anolis* des Antilles majeures (Losos et al. 1998) et des araignées *Tetragnathes* de l'archipel d'Hawaii (Gillespie 2004). Dans ces deux cas, les radiations successives qui ont marqué la mise en place de la diversité sur cet archipel se caractérisent par l'émergence, sur chaque île, d'espèces très similaires d'un point de vue morphologique et écologique (écomorphes).

Récemment, l'utilisation de microorganismes (bactéries, virus, levures) a permis d'explorer en détail le processus de diversification (Lenski et Travisano 1994, Travisano et Rainey 2000, Maclean 2005, Buckling et al 2009). Dans ces systèmes, les nouveaux mutants écologiquement distincts peuvent être considérés comme analogues de nouvelles espèces écologiques (en absence de transmission horizontale de matériel génétique, Elena et al.

2005), permettant d'explorer à la fois les conditions pour l'apparition et maintien de nouveaux variant génétiques et de nouvelles espèces.

Pendant ma thèse, je me suis intéressé au rôle joué par l'hétérogénéité environnementale dans le processus de diversification de la bactérie *Pseudomonas fluorescens* SBW25 qui est devenue un modèle d'étude des conditions d'émergence de la diversité ces 20 dernières années.

4. Comment se maintient-elle?

a. Au sein d'une population.

Alors que la mutation, la recombinaison et l'immigration génèrent constamment de la diversité, la sélection et la dérive génétique sont généralement responsables de son érosion. Un équilibre dynamique entre mutation, flux génique, dérive et sélection sert à maintenir de la variabilité génétique au sein d'une population (Gillespie et Turelli 1989, Lenormand 2002). La théorie sur l'équilibre entre mutation et sélection étant très vaste (Turelli et Barton 1990), ici, je ne ferai qu'évoquer ces principaux éléments.

Pour des loci individuels, la fréquence d'équilibre dépend de la valeur sélective des mutants et du taux de mutation (Hartl et Clark 1997). Plus le taux de mutation et la valeur sélective des mutants sont faibles, plus difficile il sera de maintenir du polymorphisme. Pour des traits quantitatifs (contrôlés par plusieurs locus, QTL), les prédictions sont moins claires. La plupart des QTL présentent un fort polymorphisme mais il est difficile de savoir si cela provient de quelques loci avec grandes variations génétiques ou de beaucoup de loci avec des petites variations (Coyne et Lande 1985).

En plus des mutations, l'effet érosif de la sélection sur la diversité génétique peut également être contrasté par la dispersion (flux génique). Dans le cas de populations spatialement structurées (métapopulations), la sélection peut favoriser l'adaptation de deux populations d'une même espèce à des

conditions environnementales différentes (Levins 1968) et favoriser la diversification à l'échelle de la métapopulation (Hanski 1999). Trop de dispersion peut s'avérer nocif pour la diversité en aboutissant à une homogénéisation de la métapopulation. Plus loin j'explore plus en détail le rôle de la dispersion dans le processus adaptatif en milieu spatialement structuré.

Il est important de souligner que la sélection peut aussi aider au maintien du polymorphisme. Cela est possible quand la valeur sélective d'un génotype dépend de sa fréquence dans une population (Ayala 1974, Weeks et Hoffmann 2008, section **E.4.c** page 54).

b. Au sein d'une communauté.

A présent, la littérature propose plus de 100 mécanismes pour expliquer la coexistence des espèces (Chesson 2000, Wright 2002). Le principe d'exclusion compétitive (Gause 1934) propose que le nombre d'espèces qui coexistent dans une communauté ne puisse dépasser le nombre de ressources disponibles. A terme, l'espèce mieux adaptée va exclure toutes les autres. Ce principe stipule donc que pour coexister deux espèces ne peuvent partager la même niche écologique (Hutchinson 1957) ; c'est-à-dire ne peuvent partager les mêmes ressources et les mêmes conditions environnementales. Cependant, certains mécanismes sont capables d'expliquer la coexistence d'espèces en présence d'une seule ressource (Chesson 2000), par exemple avec une partition des niches dans le temps en présence d'une variabilité temporelle dans les conditions du milieu (Hutchinson 1961, Chesson 1994, Abrams 2004).

Palmer (1994) classe les mécanismes de coexistence en six grandes catégories : (1) ceux qui permettent aux espèces rares d'être favorisées, (2) ceux qui réduisent les opportunités de compétition, (3) ceux qui créent une diversité transitoire (4) ceux qui augmentent les ressources disponibles, (5) l'immigration et finalement (6) l'hétérogénéité environnementale. Des

approches empiriques récentes ont révélé que ces différents mécanismes sont souvent complémentaires et synergiques et expliquent la surprenante diversité des écosystèmes les plus diverses tels que les forêts tropicales (Wright 2002) et les récifs de coraux (Van Woesik 2000). En résumé, c'est surtout en « échappant » à la compétition que les espèces arrivent à coexister. Cependant, il existe une théorie alternative à celle de la différenciation écologique (niche) pour expliquer la diversité des systèmes biologiques.

La *théorie neutre* de la biodiversité (Hubbell 2001, Bell 2001) s'inspire de la théorie de la biogéographie insulaire de McArthur et Wilson (1963) car elle assume une équivalence écologique des espèces. Elle suggère que la théorie de la niche à elle seul ne peu expliquer l'étonnante diversité qu'on observe dans des systèmes très diversifiés tels que les forêts tropicales. Selon cette théorie, la distribution des espèces, leurs abondances et leur coexistence sont basées sur des processus historiques (spéciation, extinction) et régionaux (dispersion). La théorie neutre de la diversité propose que la stochasticité du processus de dispersion propre aux espèces détermine en grande mesure la structure et composition spécifique des communautés naturelles.

Dans mon travail je privilégie une approche de la diversité comme résultat de la compétition en m'intéressant aux mécanismes de coexistence qui favorisent une différenciation des niches. Je m'intéressé plus précisément à l'hétérogénéité environnementale (variation spatiale ou temporelle des conditions environnementales biotiques ou abiotiques rencontrées par les organismes) comme mécanisme de maintien de la diversité par différenciation écologique des espèces. Je considère également le rôle de la dispersion dans l'émergence et le maintien de la diversité génotypique en milieu structuré (section **E. 3. c** page 49).

B- Des populations bactériennes aux métacommunautés.

"Competition between haploid clones is conceptually no different from competition between species".

Dean 2005, Genetics

1. *Quelles espèces?*

Dans des domaines de la biologie tels que la systématique ou l'écologie des communautés l'unité fondamentale de travail est l'espèce. Cependant, les limites entre espèces varient selon la discipline en question. Le concept biologique d'espèce qui est le plus utilisé a été donnée par Ernst Mayr à la moitié de XXème siècle est se base sur la possibilité d'interfécondité des individus d'une même espèce (Mayr 1963). Cette définition bien sur perd toute sa validité pour des organismes asexués tels que les bactéries (de Queiroz 2005). D'autres concepts d'espèce sont pourtant disponibles : espèce phylogénétique, espèce morphologique ou espèce écologique/fonctionnelle, entre autres. Une différentiation des espèces sur ces attributs fonctionnels et non sur leurs relations phylogénétiques s'est développée depuis deux décennies (Lavorel et al. 1997). Les membres d'une même espèce écologique partagent une même niche écologique (Hutchinson 1957, Schluter 2001). En travaillant avec des organismes haploïdes et asexués c'est justement cette dernière approche que j'utiliserai, basé sur une caractérisation purement phénotypique et écologique pour différencier les espèces fonctionnelles. Cette classification pose le problème de définir les limites entre les espèces. Lorsque sous forme de besoins trophiques distincts, deux génotypes occupent des niches potentielles différentes il est préférable de parler d'écotypes.

2. *Quel type de diversité?*

La littérature propose plusieurs mesures de diversité biologique. Au niveau génétique on parle de nombres d'allèles, de fréquences alléliques, de proportion de loci polymorphes, de taux d'hétérozygotie, d'indices de fixation (Fis, Fst et GSt) et de distance génétique entre autres (Hartl 2000). Pour des traits quantitatifs (traits contrôlés généralement par plusieurs gènes) on privilégie l'utilisation du polymorphisme phénotypique comme mesure de diversité (Falconer 1981). La génétique quantitative elle utilise surtout la technique de partition de la variance phénotypique totale au sein d'une population (Falconer et Mackay 1996). Elle permet de calculer entre autres l'héritabilité d'un caractère phénotypique, qui est la part de la variance phénotypique d'un trait qui s'explique par des facteurs génétiques. C'est cette méthode de partition de la variance que j'ai utilisé pour mesurer la diversité à l'intérieur de mes population/communautés expérimentales (voir section **E.5.b** page 56)

En écologie c'est le nombre d'espèces présentes dans un lieu donné (*richesse spécifique*) qui est le plus utilisé comme mesure de diversité. D'autres indices qui tiennent en compte la fréquence de chaque espèce sont aussi très répandus (par exemple les *indices de Shannon ou de Simpson*, Magurran 1988). Cependant, comme mentionné précédemment, on peut également mesurer le nombre de rôles fonctionnels joués par un groupe d'organismes (Lavorel et al. 1997). On parle alors de *diversité fonctionnelle* (Tilman et al. 1997*)*. Plusieurs définitions de diversité fonctionnelle sont aujourd'hui disponibles et l'utilisation de ce terme pour parler de la diversité de rôles écologiques dans une communauté est devenue de plus en plus fréquente dans la littérature écologique (Petchey et Gaston 2006). Elle est devenue cruciale dans le débat sur la relation entre biodiversité et fonctionnement des écosystèmes (Hector et al. 2000, Wardle et al. 2000,

Chapin et al. 2000, Diaz et Cabido 2001). De plus, des récentes études ont révélé l'importance de considérer la diversité fonctionnelle et non la diversité taxonomique pour comprendre la relation entre diversité et des propriétés écosystémiques telles que la productivité et/ou la biomasse (Tilman et al. 1997; Loreau et al 2001) et pour mieux prédire les conséquences de son déclin (Chapin et al. 2000, Loreau et al. 2002).

Malgré son importance, il existe encore une controverse sur la façon correcte de la mesurer (Petchey et Gaston 2006). Dans ce travail de thèse j'explore une mesure de diversité fonctionnelle dérivée de la technique de partition de la variance phénotypique, l'Inconsistency (voir section **E. 5. C** page 59)

3. Génétique des populations et écologie des communautés : mêmes questions, mêmes réponses?

Comme nous venons de voir, alors que pendant des décennies les généticiens des populations se sont intéressés au problème de la génération et du maintien des variants génétiques au sein d'une population (voir plus haut), les écologistes des communautés se sont eux concentrés sur les différentes façons d'échapper au principe d'exclusion compétitive pour expliquer la coexistence des espèces (Hutchinson 1961, Chesson 2000, Wright 2002). C'est donc à des échelles et avec des unités de travail différentes que ces deux disciplines se sont intéressées à la biodiversité ; diversité spécifique pour l'une et diversité génétique pour l'autre. Des études récentes ont démontré des fortes similitudes dans leurs approches et même des possibles corrélations (Vellend et Geger 2005). Des processus parallèles semblent donc agir aux différentes échelles. Celui qui semble faire l'unanimité de son importance dans l'émergence et le maintien de la biodiversité, que ce soit génétique,

taxonomique ou fonctionnelle est la sélection en environnement hétérogène. L'hétérogénéité environnementale peut être définie comme une variation spatiale ou temporelle des conditions environnementales (biotiques ou/et abiotiques) rencontrées par les organismes. Cette variation peut être biotique comme par exemple par la présence de différents prédateurs ou abiotique comme par exemple par la présence de différentes températures.

Dans un tel scénario d'hétérogénéité environnementale, les pressions de sélection auxquelles sont soumis les organismes dans chaque condition environnementale sont différentes et les populations d'une même espèce peuvent s'adapter à ces conditions environnementales contrastées. Quand la sélection opère dans chaque environnement, les individus s'adaptent localement pouvant aboutir à la spécialisation écologique et à l'émergence d'interactions génotype x environnement (Gillespie et Turelli 1989, Bell 1990, Kassen et Bell 2000). L'émergence de ce type d'interactions garantit le maintien d'un polymorphisme/coexistence stable (Felsenstein 1976, Gillespie et Turelli 1989, Bell 1990). Je montrerai plus loin que l'apparition d'interactions GxE dépend de la présence de compromis évolutifs ou « tradeoffs » (Egas et al. 2004, Kneitel et Chase 2004). Généticiens des populations et les écologistes se sont accordés sur l'importance de l'hétérogénéité environnementale pour expliquer le maintien de la biodiversité (Amarasekare 2003, Kneitel et Miller 2003, mais voir aussi Hubbell 2001). Dans ce travail j'aborderai dans un premier temps l'influence de l'hétérogénéité spatiale dans l'émergence et le maintien de la diversité puis j'étudierai le rôle de l'hétérogénéité temporelle.

4. Une approche spatiale de la diversité : métapopulations et métacommunautés.

L'univers dans lequel interagissent les différentes entités biologiques (individus, populations, espèces, communautés) se caractérise par sa structure fragmentée et par les multiples conditions environnementales qu'il propose (Mouquet et al. dans Holyoak et al. 2005). Dans l'approche régionale de la biodiversité, les populations et les communautés ne sont plus considérés comme indépendantes les unes des autres mais plutôt insérées dans une matrice spatiale d'interactions et de flux d'individus. Par conséquent, la dispersion est un facteur clef pour comprendre la dynamique et la persistance des populations (Harrison 1991, Hanski 1999) et des communautés (Holyoak et al. 2005). Les approches en métapopulations et métacommunautés présentent des similitudes dans leur façon d'aborder la structure spatiale des populations et des communautés.

a. Les métapopulations.

Une métapopulation est définie comme un groupe de populations locales d'une même espèce occupant des patches connectés par la dispersion (Hanski et Gilpin 1997). Dans sa version originale (Levins 1969) cette théorie considère que seule une fraction des patches est occupée à un moment donnée et que la dynamique globale se caractérise par l'extinction et la recolonisation par dispersion des patches vides (Hanski 1999). Une métapopulation ne pourra persister que si le taux de colonisation des patches vides est supérieur à leur taux d'extinction (Levins 1969). Cette version de la théorie des métapopulations à été durement critiqué en raison de son manque de « réalisme » qui se traduisait par un manque d'exemples naturels pour son application (Harrison 1991). Depuis, la théorie des métapopulations a suivi plusieurs « mises à jour ». Hanski (1999) les organisa en trois modèles « de

base » à partir desquels les autres peuvent être dérivés : (1) le modèle classique de Levins, (2) le modèle de deux populations et (3) le modèle en « lattice ». Tous ces modèles ont fait des apports importants à la théorie moderne des métapopulations.

D'autres approches parallèles à celle de la métapopulation ont aussi souligné le rôle de la dispersion et du composant régional dans le maintien des populations fragmentées : « l'effet de masse » (Shmida et Wilson 1985, Loreau et Mouquet 1999) et les « sources-puits » (Pulliam 1988, Mouquet et Loreau 2002). Ces mécanismes proposent le maintien par dispersion d'individus dans des habitats défavorables dans lesquels ils présentent un taux de croissance négatif. Leur maintien dépend d'un apport constant de migrants à partir d'habitats plus favorables ou d'un pool régional.

La théorie des métapopulations propose des scénarios de compétition interspécifiques qui aident à définir les conditions pour la coexistence des espèces (Hanski 1999). En effet, les interactions interspécifiques peuvent influencer les taux de dispersion, d'extinction et de colonisation et par conséquent modifier les dynamiques des différentes espèces. L'approche spatiale des systèmes biologique proposée par les métapopulations augmente les échelles d'interaction des organismes. Par exemple, la présence d'une espèce dans un patch peut prévenir l'arrivée et l'invasion d'une deuxième et cette espèce peut par contre coloniser plus facilement des patches vides.

b. Les métacommunautés.

La théorie de la biogéographie insulaire (MacArthur et Wilson 1967) considèrerait déjà la migration comme un facteur clef dans la compréhension de la biodiversité. Elle propose une vision régionale de la diversité dans laquelle le nombre d'espèces dans une communauté résulte d'un équilibre entre immigration et extinction. Dans sa plus simple interprétation, cette théorie postule que plus une « ile » est grande et proche du continent, plus elle peut contenir d'espèces comme conséquence 'une plus forte immigration et un

risque d'extinction mineur. Cependant, cette théorie ne considère pas directement le problème de la coexistence des espèces et n'est pas en mesure d'expliquer certains patrons de la biodiversité (Connor et MaCoy 1979).

Avec l'apparition du concept de metacommunauté (Wilson 1992, Leibold et al. 2004), la structure spatiale de l'environnement (et par conséquent le dispersion) ont acquis toute son importance dans la compréhension des dynamiques spatiales des communautés (Holyoak et al. 2005). Les processus locaux et régionaux de coexistence ont enfin été intégrés dans un même cadre conceptuel. Une métacommunauté est définie comme un groupe de communautés locales spatialement ségréguées dans des patches différents et connectées par la dispersion (Wilson 1992). Comme pour les métapopulations, différentes approches des métacommunautés ont été proposées (Leibold et al. 2004, Holyoak et al. 2005) : (1) une perspective de « *patch dynamic* », (2) le « *species sorting* », (3) le « *mass effects* » et (4) la perspective neutre.

La perspective « *patch dynamic* » ressemble beaucoup au modèle original des métapopulations de Levins (voir plus haut). Cette perspective considère des patches identiques dont seule une fraction sera occupée à un moment donné comme résultat d'un équilibre entre extinction et colonisation. La diversité locale des patches dépend donc du résultat des interactions locales des espèces et aussi du niveau de dispersion. La perspective « *species sorting* » assume une hétérogénéité environnementale. La variation dans la qualité des patchs, une ségrégation spatiale des niches ainsi que la dispersion déterminent la composition locale des espèces. On s'attend à ce que les espèces occupent les patches dans lesquels elles sont les mieux adaptées. Une dispersion limitée favoriserait l'adaptation des espèces à leur milieu (Mouquet et Loreau 2003). La perspective « *mass effect* » décrit une situation dans laquelle les espèces seraient maintenues dans des habitats défavorables grâce à un apport contant d'individus par dispersion. Plus le niveau de dispersion est fort, plus la fréquence d'organismes localement maladaptés sera importante. Au même temps cette perspective prévoit une diversité locale

maximale pour des niveaux de dispersion intermédiaires. La perspective neutre de la diversité (Hubbel 2001) suppose une équivalence écologique des espèces et ce serait surtout la dispersion qui définit la diversité des systèmes biologiques. Elle prédit donc une relation positive entre dispersion et diversité.

C. Evolution dans les métacommunautés.

"Ecological systems are dynamic on evolutionary scales of time and space. Thus, processes on all temporal and spatial scales are connected and contribute to shaping ecological systems at all levels."

Ricklefs 2007, The American Naturalist

Aujourd'hui, les écologistes sont en train non seulement de connecter les processus locaux de compétition avec des processus régionaux pour comprendre l'organisation des systèmes écologiques (Ricklefs 1987, Leibold et al. 2004) mais sont aussi en train d'inclure de plus larges échelles temporelles dans leur approche de la biodiversité (Webb et al. 2002, Wiens et Donoghue 2004). Cette approche considère donc les communautés comme des systèmes ouverts et temporellement dynamiques (Ricklefs 2007, Fussmann et al. 2007). Des mécanismes tels que la spéciation et l'extinction sont à présent pris en compte pour expliquer les patrons de diversité que nous observons (Webb et al. 2002, Ricklefs 2004) ainsi que pour mieux comprendre l'assemblage des communautés (Ackerly 2003). On parle d'une « nouvelle fusion entre écologie et évolution » (Westoby 2006). Les analyses phylogénétiques sont ainsi devenues un outil important pour la compréhension de l'influence des processus évolutifs dans la structure des systèmes biologiques (Webb et al. 2002, Ricklefs 2007, Cadotte et al. 2010).

Récemment, Urban et collaborateurs (2008) ont présenté l'écologie évolutive des métacommunautés comme l'étude de l'influence de la variation des traits génétiquement contrôlés à l'intérieur des espèces sur les interactions interspécifiques dans une matrice de communautés locales connectées par la dispersion. L'évolution étudie le changement dans les fréquences des différents variant génétiques à l'intérieur des populations et des espèces, alors que la dynamique des communautés s'intéresse aux

changements d'abondance des différentes espèces. Historiquement, l'étude de la variation des traits à l'intérieur des espèces est du domaine exclusif de la génétique des populations alors que ce sont les écologistes qui étudient les interactions interspécifiques. Cependant, les connections entre les processus à l'intérieur de ces deux échelles d'organisation ainsi que leurs possibles interactions sont de plus en plus évidentes (Vellend et Geber 2005). Les différents relations entre diversité génétique et diversité spécifique peuvent être classifiées en trois catégories: (1) lorsque les deux sont affectées de forme parallèle par les caractéristiques de l'environnement (aire, hétérogénéité…), (2) lorsque le niveau de diversité génétique au sein d'une population détermine sa performance et sa viabilité (niveau d'adaptation) et par conséquent détermine l'environnement des autres espèces, celle-ci peut avoir un effet direct sur la diversité spécifique, (3) lorsque le nombre d'espèces dans une communauté affecte les pressions de sélection des populations. Leur étude propose une synthèse d'études théoriques et expérimentales qui illustrent l'étroite relation entre diversité génétique et diversité spécifique.

L'apparition du concept d'écologie évolutive des métacommunautés montre bien comment la façon de penser l'organisation du vivant est en train de changer. Une synthèse entre l'écologie des communautés et évolution est en train de se produire (Johnson et Stinchcombe 2007, Cavender-Bares et al. 2009). Un exemple assez frappant de l'action conjointe des processus dits « écologiques » avec des processus dits « évolutifs » dans l'organisation des systèmes biologiques est celui de la dispersion. Une grande partie de mon travail de thèse se concentre justement sur le rôle de la dispersion dans le processus de diversification et d'adaptation des métacommunautés. Le rôle de la dispersion dans le processus adaptatif des métacommunautés est double. Elle agit dans le processus adaptatif à l'intérieur des populations ainsi que dans l'assemblage des communautés. Dans les deux cas on s'attend à un effet bénéfique pour des niveaux de dispersion intermédiaires (Mouquet et al. 2002, Urban 2006). Elle apporte des nouveaux variants qui vont accélérer le processus adaptatif et favorisent le « species sorting » (Urban et al. 2008). Par

exemple, Perron et collaborateurs (2007) ont montré que l'arrivée de migrants favorise l'adaptation de la bactérie pathogène *Pseudomonas aeruginosa* à des antibiotiques. Cependant, beaucoup de dispersion homogénéise les populations et les communautés au même temps qu'elle entraîne des maladaptations (Ronce et Kirkpatrick 2001). Malgré quelques différences, la dispersion présente donc des effets analogues sur l'adaptation et l'assemblage des communautés. Dans mon travail de thèse l'utilisation des clones bactériens comme des analogues des espèces d'un point de vue fonctionnel me permet d'adresser le double effet de la dispersion que je viens d'évoquer.

D- Evolution expérimentale.

"Experimental evolution has transformed evolutionary biology from a historical science, in which unseen processes are inferred from evolutionary end points, to one in which evolution can be studied in real time."

Buckling et al. 2009, Nature

1. Présentation générale.

Les écologistes ont souvent critiqué l'utilisation de microcosmes pour étudier le fonctionnement des communautés considérant que ces systèmes ne pouvaient expliquer les processus agissant sur les systèmes naturels (Carpenter 1996). Les études en biologie évolutive par contre ont depuis longtemps utilisé les microcosmes pour étudier le processus adaptatif (Dallinger 1878, Bell 2008). L'évolution expérimentale consiste à utiliser des organismes vivants pour étudier en temps réel le processus adaptatif. L'étude de l'adaptation des organismes soumis à des conditions contrôlées d'un laboratoire commença il y a plus de 130 ans avec les travaux de William Dallinger (1878). Pendant 7 ans (avant que son matériel n'explose!) il a réussi à soumettre ses populations expérimentales à un gradient croissant de température. Il a montré que les populations évoluées survivaient efficacement à des températures extrêmes mais cette adaptation s'accompagna d'une perte de la capacité de croître à la température optimale de croissance de départ. Pourtant, ces travaux ne faisaient que décrire un patron sans expliquer les processus sous-jacents.

Pendant les années 90, les expériences en évolution expérimentale se sont beaucoup développées. Toutes sortes d'organismes sont aujourd'hui utilisés en évolution expérimentale (virus, bactéries, levures, nématodes, insectes,

plantes). Les travaux avec *Drosophila* sp. (Burke et Rose 2009), *Saccharomyces cerevisiae* (Zeyl 2006), *Daphnia pulex* (Scheiner et Yampolsky 1998) *Chlamydomonas reinhardtii* (Bell 1997) entre autres ont beaucoup contribué à la compréhension du processus adaptatif. Chez les bactéries ce sont surtout *Escherichia coli* (par exemple dans le laboratoire de Richard Lenski) et *Pseudomonas fluorescens SBW25* (par exemple dans les laboratoires de Paul Rainey et Angus Buckling) que depuis 20 ans sont utilisées en évolution expérimentale. Ces travaux ont permis entre autres de mieux connaître la dynamique adaptative, de souligner l'importance du hasard dans le processus adaptatif et de connaitre le déterminisme génétique des adaptations (Jessup et al. 2004, Buckling et al. 2009).

Certaines études avec *P. fluorescens SBW25* se sont déjà intéressées au rôle de la structure spatiale du milieu dans l'émergence et le maintien de la diversité. L'expérience de Paul Rainey et Michael Travisano (1998) en est un excellent exemple. Ces auteurs ont déterminé expérimentalement le rôle primordial que joue l'hétérogénéité spatiale dans la création d'opportunités écologiques et la mise en place d'une radiation adaptative *in vitro* (Figure 1).

En quelques jours, une population initialement pure se diversifie écologiquement et morphologiquement en plusieurs morphotypes. Ceci n'est possible que dans un milieu hétérogène, permettant la mise en place d'un gradient vertical en oxygène. L'homogénéisation par simple agitation des tubes de culture détruit ce gradient, limite les opportunités écologiques et maintient les autres variants morphologiques à basse densité. Une fois la radiation effectuée, la destruction du gradient environnemental (par homogénéisation) entraine aussi la disparition des variants.

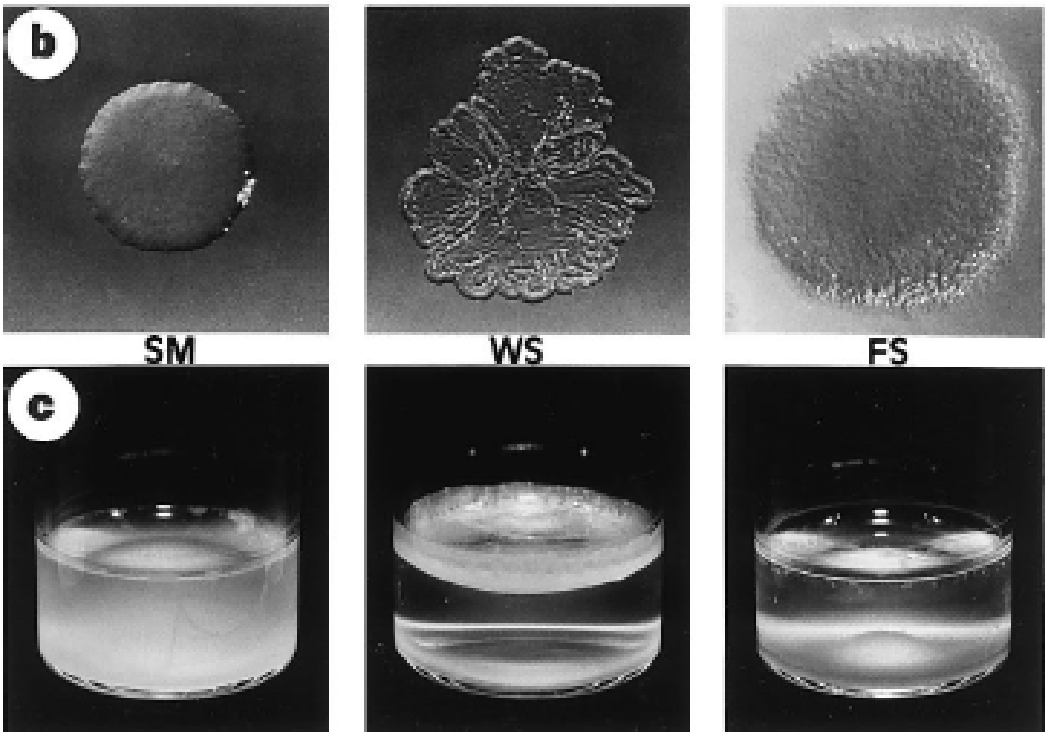


Figure 1 : Résultat expérimental de la radiation adaptative de *Pseudomonas fluorescens* SBW25 en présence d'un environnement spatialement hétérogène. Au moins trois morphotypes différents émergent qui se caractérisent par leur morphologie coloniale b) et leur spécialisation écologique c). Photo extraite de Rainey et Travisano 1998.

Plus tard, Maclean et Bell (2002) ont fait évoluer un clone unique de *P. fluorescens SBW25* pendant plus de mille générations dans un environnement localement homogène mais spatialement structuré composé de 95 sources de carbone différentes. Ils ont ensuite mesuré les performances des populations évoluées dans l'ensemble des 95 milieux de sélection afin de quantifier la largeur de leurs niches. Dans des telles conditions, la théorie sur d'évolution de la niche en milieu hétérogène prédit une réduction de sa largeur comme résultat de la spécialisation écologique (Ackerman et Doebeli 2004). Dans leur expérience, ils ont mis en évidence une augmentation du nombre moyen de sources de carbone exploitées. Ils attribuent ce résultat au manque de coûts associés avec l'adaptation à un milieu donné. Malgré cet élargissement général de la niche, les génotypes évolués présentaient une grande variabilité dans leurs performances dans les différents milieux. Malgré l'absence de coûts,

l'émergence de cette diversité résulta d'une adaptation à différentes conditions environnementales (sélection naturelle divergente). Cette expérience illustre l'importance des expériences en évolution pour une meilleure compréhension du processus adaptatif car elles peuvent révéler des résultats qui s'écartent des simples attendus théoriques.

2. *Avantages et inconvénients*.

A mi chemin entre les études *théoriques* et la réalité des systèmes naturels, l'évolution expérimentale avec des microorganismes présente plusieurs avantages pour étudier les mécanismes écologiques et les processus adaptatifs (Gillespie et Emerson 2007). Tout d'abord la taille des populations (jusqu'à plusieurs milliards par millilitre) et leur temps de génération (plusieurs générations par jour) favorisent leur évolution rapide. Egalement la possibilité de suspendre le processus adaptatif en arrêtant leur métabolisme par congélation permet de reconstruire toute l'histoire évolutive d'une lignée. Leur taille réduite et leur facile manipulation permet de répliquer les traitements et donc soumettre plusieurs fois les populations aux mêmes pressions de sélection. Egalement, l'expérimentateur contrôle les conditions environnementales sans intervenir dans le processus naturel de sélection (Srivastava et al. 2004). Finalement, la génétique de plupart de ses microorganismes est aujourd'hui assez bien connue ce qui favorise la compréhension du déterminisme génétique des adaptations.

Cependant, l'évolution expérimentale présente également des inconvénients (Buckling et al. 2009). On souligne souvent son manque de réalisme face aux pressions de sélection que subissent les populations en milieu naturel. Dans la plupart des expériences en évolution on contrôle l'environnement abiotique, on se concentre sur un seul niveau trophique ou on se limite à étudier le processus de coévolution de seulement deux espèces

(Lenski 1991 dans Ginzburg 1991, Elena et Lenski 2003). Egalement, il est parfois déconseillé d'inférer des processus macro évolutifs à partir des processus micro évolutifs mis en évidence par l'évolution expérimentale. Ce qui se passe pendant quelques semaines à l'intérieur d'un microcosme peut difficilement s'extrapoler aux événements survenus dans une forêt tropicale au cours des derniers milliers d'années. Enfin, certains microorganismes utilisés tels que les bactéries sont très simples et ne tiennent pas en compte d'autres processus importants de génération de variabilité tels que la recombinaison et le sexe.

3. Valeur sélective.

L'étude du processus adaptatif implique une caractérisation quantitative des changements dans la valeur sélective ou réponse à la sélection (Lenski et al. 1991). Il existe toutefois plusieurs façons de mesurer la valeur sélective des populations soumises aux diverses pressions de sélection. Parmi les plus utilisées sont des tests de compétition entre souches et des mesures directes ou indirectes des paramètres de croissance (taux de croissance maximal, capacité de charge, croissance par unité de temps ; Lenski 2004). Ces différentes techniques présentent des avantages et des inconvénients et leur utilisation dépend des objectifs recherchés. Des techniques telles que la cytométrie en flux et la PCR en temps réel sont aussi préconisées par leur sensibilité mais nécessitent d'un protocole assez complexe (Poitras et Houde 2002). Les tests de compétition nous renseignent sur les capacités compétitives d'une souche relatives à une souche marquée (coloration ou aspect des colonies) afin de connaître si le traitement subit par les bactéries modifie leur valeur sélective. Le marquage ne doit apporter aucune différence sur la valeur sélective de la souche par rapport à son homologue non marqué (sélectivement neutre). Le comptage de colonies par étalement sur milieu

sélectif de culture est aussi une technique très répandue (Slonczewski et Foster 2008). Elle consiste à estimer le nombre d'unités formatrices de colonies par unité de volume à partir de la dilution d'un échantillon homogène de culture bactérienne. Il s'agit cependant de techniques coûteuses en temps et matériel. Etablir des paramètres de croissance par étalement est une technique très précise mais nécessite en plus d'une grande quantité de matériel, une extraction d'un échantillon de culture ce qui entraîne dans certains cas une perturbation considérable de la population (vortex, homogénéisation, pipetage).

Une technique simple, peu coûteuse en temps et matériel (à condition de déjà disposer d'un spectrophotomètre) est celle de lecture de la densité optique ou absorbance du milieu de culture (Slonczewski et Foster 2008). Généralement la densité optique d'une suspension cellulaire est mesurée à une longueur d'onde comprise entre 420 et 650 nm. La longueur d'onde utilisée doit être ajustée au type de matériel utilisé car différentes bactéries peuvent avoir des longueurs d'onde d'absorbance maximale différentes (Madigan et al. 2008). Dans le cas de *Pseudomonas fluorescens* SBW25 une longueur d'onde de 660 nm est préconisée car elle corrèle bien avec la densité cellulaire (MacLean et al. 2004, **Figure 2**). Lorsque l'on fait passer un rayon lumineux à travers le milieu de culture, une partie de ce dernier sera absorbé par les bactéries en suspension. Plus la densité cellulaire est importante, plus le faisceau lumineux sera absorbé. S'agissant d'une technique indirecte d'estimation de densité cellulaire, la réalisation d'une courbe de calibration s'impose avant de généraliser l'utilisation de cette technique (**Figure 2**). J'ai constaté pourtant une forte dépendance du milieu de culture et de la souche bactérienne utilisée sur l'allure de la courbe d'étalonnement (données non publiées). Une autre limitation de cette technique de quantification des performances bactériennes est son manque de sensibilité au deçà d'une certaine densité cellulaire. Dans l'exemple de la figure 2, la relation linéaire entre densité optique et le log des mesures directes de densité cellulaire démarre au dessus de 10^7 cellules par millilitre.

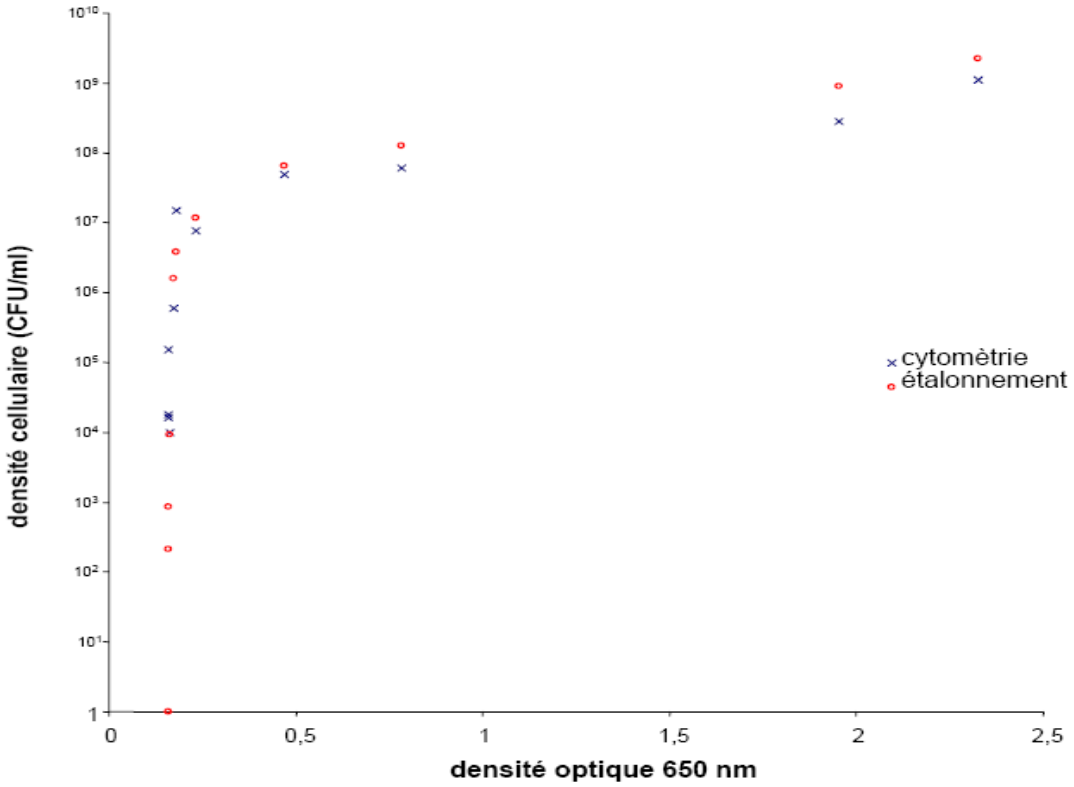


Figure 2 : Relation entre la densité optique à 650 nm et deux mesures directes de densité cellulaires (cytométrie de flux et comptage sur boîte de Petri) pour *Pseudomonas fluorescens SBW25* en milieu KB. Après 24 heurs d'incubation à 28°C et agitation constante (200 r.p.m.) j'ai procédé à des dilutions successives de la culture pure (rapports ½, 1/10, 1/20, 1/100, 1/200, 1/1000, 1/2000, 1/10000, 1/20000) afin d'obtenir un gradient de densité cellulaire. Après la mesure de densité optique, les cultures ont été étalées sur les Boîtes de Petri avec KB.

Une manière très pratique de contourner le problème de la souche et milieu dépendance de la lecture de densité optique consiste à ajouter au milieu de culture un indicateur coloré tel que le tetrazolium violet (Bochner 1989). Lorsque la souche ou le mélange de souches dégrade le milieu de culture (oxydation des sources de carbone) le tetrazolium violet, initialement incolore se réduit en formazan de couleur violet (**Figure 3**). Ce composé est ensuite libéré dans le milieu et sa concentration peut ensuite être mesurée par densité optique à 590 nm, le pic d'absorbance maximale du formazan.

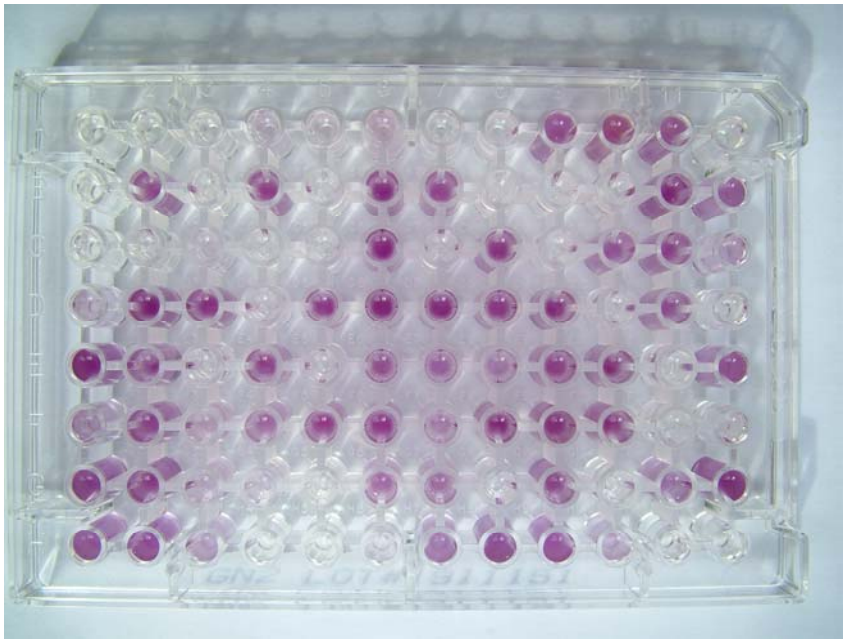


Figure 3 : Photo d'une microplaque Biolog GN2 inoculée avec un génotype de *Pseudomonas fluorescens* SBW25 pris au hasard après 24 heures d'incubation à 28°C. Elle comporte 12 colonnes et 8 lignes pour un total de 96 puits contenant des sources de carbone différentes (Photo : Patrick Venail).

4. Milieu de sélection.

Un des avantages de travailler avec des bactéries telles que *E. coli* et *P. fluorescens* est leur facilité de culture. S'agissant de bactéries aérobies et hétérotrophes, leur croissance au laboratoire requiert la préparation d'un milieu isotonique contenant une source d'azote, de soufre, de phosphore, de magnésium, de potassium, de sodium et de calcium ainsi qu'une source de carbone. Il est également conseillé d'ajouter des éléments trace tels que le fer et le zinc. Un grand nombre de milieux de culture bactérienne sont disponibles (Madigan et al. 2008). Les plus utilisés sont les milieux nutritifs liquides tel que le LB et le KB qui peuvent également être mélangés avec du agar pour créer des milieux gélifiés. On utilise souvent aussi des milieux dits « minimal » car ce sont des milieux très pauvres en nutriments.

Dans les années 80 un système d'identification bactérienne (Biolog) basée sur leurs propriétés métaboliques a été mis au point par la société Biolog.Inc aux Etats-Unis (Bochner 1989). Basé sur le principe d'oxydoréduction de différentes sources de carbone et du tetrazolium violet, ce principe permet d'identifier parmi plusieurs milliers de souches bactériennes, chaque souche disposant d'une empreinte métabolique sur les 95 sources de carbone différentes dont la microplaque dispose (**Figures 3 et 4**). Dans mon travail de recherche c'est ce type de milieu de culture standardisé (Biolog GN2 et Ecoplates) que j'ai privilégié pour cultiver les bactéries.

A1 Water	A2 α-Cyclodextrin	A3 Dextrin	A4 Glycogen	A5 Tween 40	A6 Tween 80	A7 N-Acetyl-D-Galactosamine	A8 N-Acetyl-D-Glucosamine	A9 Adonitol	A10 L-Arabinose	A11 D-Arabitol	A12 D-Cellobiose
B1 I-Erythritol	B2 D-Fructose	B3 L-Fucose	B4 D-Galactose	B5 Gentiobiose	B6 α-D-Glucose	B7 m-Inositol	B8 α-D-Lactose	B9 Lactulose	B10 Maltose	B11 D-Mannitol	B12 D-Mannose
C1 D-Melibiose	C2 β-Methyl-D-Glucoside	C3 D-Psicose	C4 D-Raffinose	C5 L-Rhamnose	C6 D-Sorbitol	C7 Sucrose	C8 D-Trehalose	C9 Turanose	C10 Xylitol	C11 Pyruvic Acid Methyl Ester	C12 Succinic Acid Mono-Methyl-Ester
D1 Acetic Acid	D2 Cis-Aconitic Acid	D3 Citric Acid	D4 Formic Acid	D5 D-Galactonic Acid Lactone	D6 D-Galacturonic Acid	D7 D-Gluconic Acid	D8 D-Glucosaminic Acid	D9 D-Glucuronic Acid	D10 α-Hydroxybutyric Acid	D11 β-Hydroxybutyric Acid	D12 γ-Hydroxybutyric Acid
E1 p-Hydroxy Phenylacetic Acid	E2 Itaconic Acid	E3 α-Keto Butyric Acid	E4 α-Keto Glutaric Acid	E5 α-Keto Valeric Acid	E6 D,L-Lactic Acid	E7 Malonic Acid	E8 Propionic Acid	E9 Quinic Acid	E10 D-Saccharic Acid	E11 Sebacic Acid	E12 Succinic Acid
F1 Bromosuccinic Acid	F2 Succinamic Acid	F3 Glucuronamide	F4 L-Alaninamide	F5 D-Alanine	F6 L-Alanine	F7 L-Alanyl-glycine	F8 L-Asparagine	F9 L-Aspartic Acid	F10 L-Glutamic Acid	F11 Glycyl-L-Aspartic Acid	F12 Glycyl-L-Glutamic Acid
G1 L-Histidine	G2 Hydroxy-L-Proline	G3 L-Leucine	G4 L-Ornithine	G5 L-Phenylalanine	G6 L-Proline	G7 L-Pyroglutamic Acid	G8 D-Serine	G9 L-Serine	G10 L-Threonine	G11 D,L-Carnitine	G12 γ-Amino Butyric Acid
H1 Urocanic Acid	H2 Inosine	H3 Uridine	H4 Thymidine	H5 Phenyethyl-amine	H6 Putrescine	H7 2-Aminoethanol	H8 2,3-Butanediol	H9 Glycerol	H10 D,L-α-Glycerol Phosphate	H11 α-D-Glucose-1-Phosphate	H12 D-Glucose-6-Phosphate

Figure 4 : Distribution des 95 sources de carbone et le blanc (water) de la microplaque Biolog GN2 (Biolog, Inc)

5. Propagation des cultures.

Le maintien de populations bactériennes pendant des centaines, voir des milliers de générations (Lenski et Travisano 1994) dans des conditions contrôlées impose entre autres le renouvellement des ressources et l'élimination des métabolites secondaires issus de la dégradation de ces derniers. Pour cela il existe plusieurs techniques de propagation de cultures. Parmi les plus utilisées sont celles du chemostat et la culture en batch (*batch culture*). Le chemostat est un dispositif « fermé » qui assure l'entrée permanente de nutriments ainsi que la sortie de débris à un flux établi par l'expérimentateur en fonction des besoins des cultures bactériennes (Dykhuizen et Davies 1980). Cette méthode est dite « non invasive » car elle ne nécessite pas la manipulation directe des bactéries ce qui diminue les chances de possibles contaminations. Avec cette technique on garantit également de soumettre les bactéries à un environnement constant et contrôlé. La technique que j'ai utilisée pendant ma thèse est celle dite de culture en batch. Elle impose une plus forte intervention de la part de l'expérimentateur. Les ressources sont renouvelées périodiquement par un transfert d'un échantillon de culture vers un milieu frais. Avant chaque transfert, le milieu de culture doit être homogénéisé afin de garantir que cet échantillon soit représentatif des fréquences des génotypes présents. Il est conseillé de transférer assez de culture pour éviter des goulots d'étranglement qui pourraient aboutir à un phénomène de dérive mais pas trop pour ne pas transférer beaucoup de déchets. Plusieurs variables telles que le volume total de culture, la concentration du milieu, l'espèce bactérienne et les conditions de culture entre autres, doivent être prises en compte pour définir le volume à transférer, souvent on transfère un volume proche du 1% du volume total de culture. Par conséquent, avec cette technique la concentration des ressources est maximale au moment de l'inoculation (transfert) est décroît au fur et à mesure que la densité cellulaire augmente (**Figure 5**).

Le patron de croissance des cultures bactériennes est lui aussi bien connu (**Figure 5**, Dykhuizen 1990). Avant de croître, les bactéries doivent activer leur métabolisme pour l'utilisation des ressources disponibles ce qui se traduit par une période de latence (1). Ensuite la croissance démarre (2, croissance exponentielle), période pendant laquelle la population utilise la majorité de ressources et atteint son taux de croissance ponctuel maximal, qui se trouve au point d'inflexion de la courbe. Après un certain temps, alors que les ressources sont moins disponibles, le taux de croissance et le taux de mortalité sont égaux produisant une période de croissance nulle (3, phase stationnaire). A ce stade la population atteint sa capacité de charge maximale. Enfin, dans certaines cultures lorsque les ressources sont épuisées et les métabolites secondaires trop importants, le taux de mortalité peux être supérieur au taux de croissance et donc la densité cellulaire décroit (4, phase de mortalité).

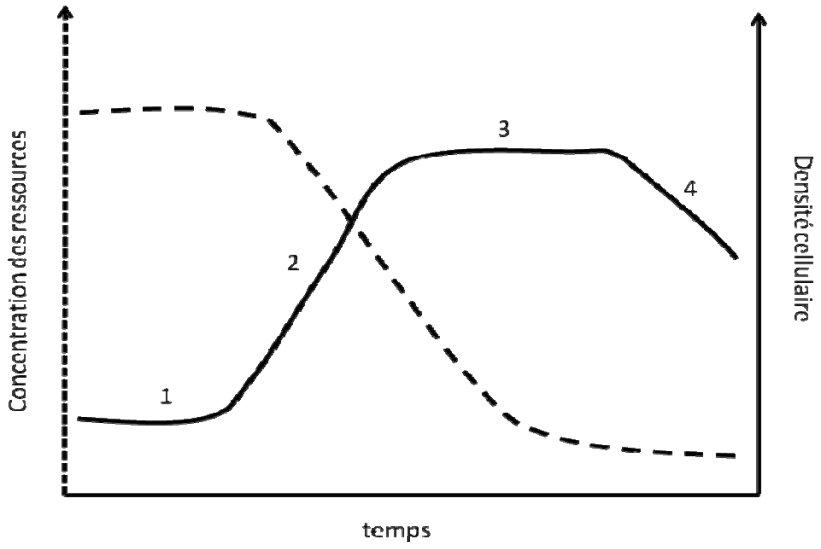


Figure 5 : Schéma de la dynamique d'utilisation des ressources (ligne hachurée) et densité cellulaire (ligne continue) dans un milieu de culture bactérien type (voir texte pour explications, Madigan et al. 2008).

Dans une culture en batch la fréquence des transferts doit être fixée selon les conditions de croissance de la population et du milieu utilisé. Le but

pouvant être de maximiser le numéro de doublements par transfert. Pour cela on établit à partir de la courbe de croissance le moment où la population se trouve en croissance exponentielle (2 dans **Figure 5**). Ainsi, lors de transfert répétés successivement pendant la durée de la période de sélection dans un milieu constant on augmente les possibilités de sélection en favorisant des variants avec une densité plus forte au moment du transfert. Ce processus est connu sous le nom de sélection périodique (Dykhuizen 1990). Un variant avec une plus forte densité cellulaire au moment des transferts va remplacer progressivement un autre variant jusqu'à son exclusion définitive ou jusqu'à ce que la direction de la sélection change ou un autre variant plus performant apparaisse (interférence clonale). La dynamique populationnelle des lignées de sélection bactériennes pendant la période de sélection se caractérise donc par des pics de forte densité suivis d'une forte diminution de la densité lors du transfert (**Figure 6**). Les lignées de sélection peuvent répondre aux pressions de sélection de différentes formes : entre autres par une diminution du temps de latence, par une augmentation du taux de croissance ou par une augmentation dans la capacité de charge.

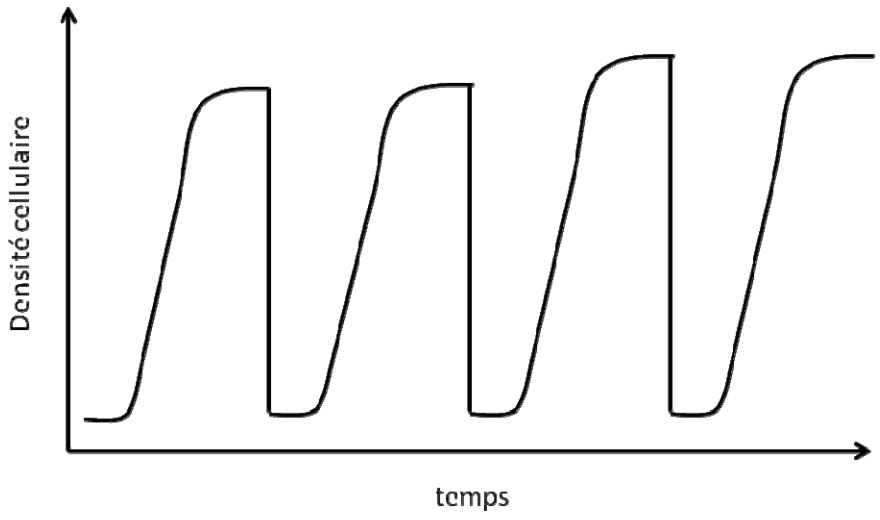


Figure 6 : Dynamique hypothétique de la densité populationnelle dans une expérience d'évolution en culture en batch.

E- Emergence et maintien de la diversité en milieu hétérogène.

« Therefore, progress requires experimental systems that reduce complexity to the point where mechanistic processes can be observed and rigorously tested."

Rainey et al. 2000, Trends in Ecology and Evolution

1. Mutation : source ultime de variabilité.

Les bactéries sont des organismes asexués et haploïdes (elles peuvent dans certaines conditions recombiner et échanger du matériel génétique, Elena et al. 2005). Dans une culture d'un seul clone (isogénique) et fermée à la migration, la seule source de variabilité est la mutation. Les mutations avantageuses, nécessaires pour le processus adaptatif, sont des événements rares (Dykhuizen 1990). Hors, dans des populations avoisinant les 10^{10} individus par millilitre, la probabilité d'apparition d'une mutation avantageuse augmente considérablement. Par exemple lorsque *P. fluorescens* a été soumise à un milieu constant contenant de la serine comme seule source de carbone, la probabilité d'apparition d'une mutation bénéfique a été estimé à 3.8×10^{-8} par division cellulaire (Barrett et al. 2006). En d'autres termes, dans un millilitre d'une population bactérienne avec une densité de 10^8 cellules par ml on s'attend en moyenne à l'apparition de 3.8 mutations bénéfiques par doublement. En plus du taux de mutation, l'avantage sélectif des nouveaux mutants va lui aussi déterminer la vitesse de fixation de nouveaux variants et donc la durée du maintien de cette diversité dite transitoire (Cohan 2005). Plus l'avantage sélectif du mutant augmente, plus vite il sera fixé dans la population. La même étude sur l'adaptation de *P. fluoresnces* à la sérine révéla

une grande variabilité dans la distribution des avantages des nouveaux mutants. Leurs performances étaient entre 20% et 500% supérieures à celles du génotype de départ (Barrett et al. 2006).

2. Sélection en milieu simple et uniforme.

Le processus adaptatif en milieu simple (une seule ressource disponible) et uniforme (constant dans le temps) chez les bactéries se caractérise par une interférence clonale et une apparition et fixation périodique des variants les plus performants. Dans ce type de sélection dite directionnelle, le principe d'exclusion compétitive prédit le maintien du variant plus performant. La possibilité de les congeler et de les conserver sur de longues durées de temps permet d'utiliser les bactéries pour mesurer directement les changements dans la valeur sélective (ou des traits liés à cette dernière) après des centaines ou des milliers de générations. L'expérience de sélection expérimentale la plus longue est celle effectué au laboratoire de Richard Lenski avec la bactérie *E. coli*. Durant déjà plus de 20 ans (45000 générations) ils ont propagé *E. coli* en batch culture et dans un environnement contenant du glucose comme seule source de carbone. Cette étude a permis entre autres de caractériser la dynamique adaptative de la valeur sélective ainsi que de traits phénotypiques tels que le volume cellulaire (Lenski et al. 1991, Lenski et Travisano 1994 ou visitez leur site internet https://myxo.css.msu.edu/ecoli/overview.html). La dynamique adaptative de la valeur sélective se caractérise par une courbe asymptotiquement ascendante révélant des fortes réponses à la sélection en début de période de sélection et des augmentations de moins en moins fortes au fur et à mesure que l'expérience avance (**Figure 7**).

Dans un environnement simple et homogène toute la variabilité observée est donc transitoire et dynamique, résultant d'un remplacement successif de nouveaux variants. Un cas particulier d'émergence et maintien de diversité

stable dans un milieu simple et homogène est celui du *cross-feeding* (Treves et al. 1998, Rozen et Lenski 2000, Doebeli 2002). Dans certains cas, les bactéries capables d'exploiter la seule source de carbone disponible vont produire, comme résultat de la dégradation de cette dernière, une ressource utilisable par un autre variant. L'exemple plus connu de ce type de relation chez les bactéries est celui de la bactérie *E.coli,* laquelle en dégradant le glucose produit du acétate, devenant une nouvelle ressource (Helling et al. 1997, Rozen et Lenski 2000, Spencer et al. 2007). Le *cross-feeding* illustre clairement la nécessité d'une différenciation écologique (différenciation des niches trophiques) afin de permettre une coexistence de formes stables et échapper efficacement au principe d'exclusion compétitive (Gause 1934, Hutchinson 1957, Wright 2002).

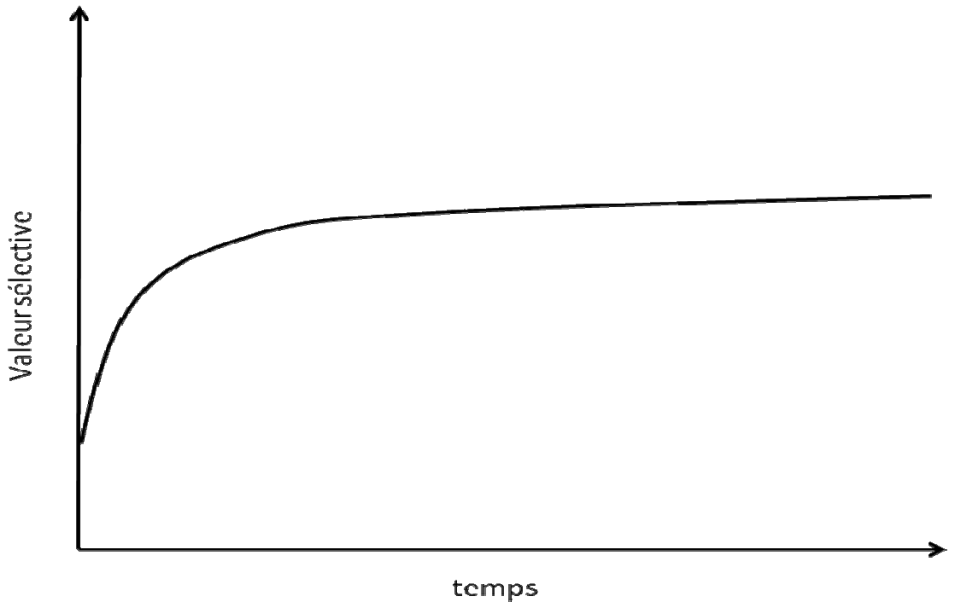


Figure 7 : Représentation simplifiée de la dynamique adaptative d'une population bactérienne dans un milieu homogène (d'après Lenski et Travisano 1994).

3. Sélection en environnement complexe.

Dans un environnement complexe, par exemple en présence de plusieurs ressources, la sélection divergente opère et peut favoriser l'émergence et le maintien d'un polymorphisme stable (et même la spéciation), à condition que des opportunités écologiques (niches vides) soient disponibles (Schluter 2001). Il est formellement établi théoriquement (Amarasekare 2003 pour une revue) et par des expériences avec des microorganismes que des environnements hétérogènes, que ce soit dans l'espace (par exemple Rainey et Travisano 1998) ou dans le temps (par exemple Descamps-Julien et Gonzalez 2005), permettent l'émergence et le maintien d'une plus forte diversité par rapport à des environnements homogènes (Kassen 2002 pour une revue). La théorie de la sélection en milieu hétérogène établit les conditions pour l'émergence et le maintien de la diversité (Levins 1968). Nombreux facteurs tels que la forme du compromis évolutif, le grain environnemental, la dispersion, le type de régulation des populations et la proportion des habitats peuvent influencer la diversité.

a. Compromis évolutifs.

Un compromis évolutif résulte de la relation entre les performances dans deux environnements (Levins 1968, Stearns 1989, Chase et Leibold 2003). Ils traduisent les coûts dans la valeur sélective dans un environnement auxquels un organisme doit se contraindre lorsqu'il augmente sa valeur sélective dans un autre. La **figure 8** présente un cas hypothétique simple avec deux ressources présentes en même proportions. On parle de compromis évolutif faible (courbe verte) ou de compromis évolutif fort (courbe rouge). Dans le premier cas, un généraliste C2 présente une performance moyenne supérieure

à celle des deux spécialistes A et B ; malgré sa plus faible performance par rapport à A dans le milieu 1 et que B dans le milieu 2. Dans ce scénario on attend l'émergence d'un unique généraliste.

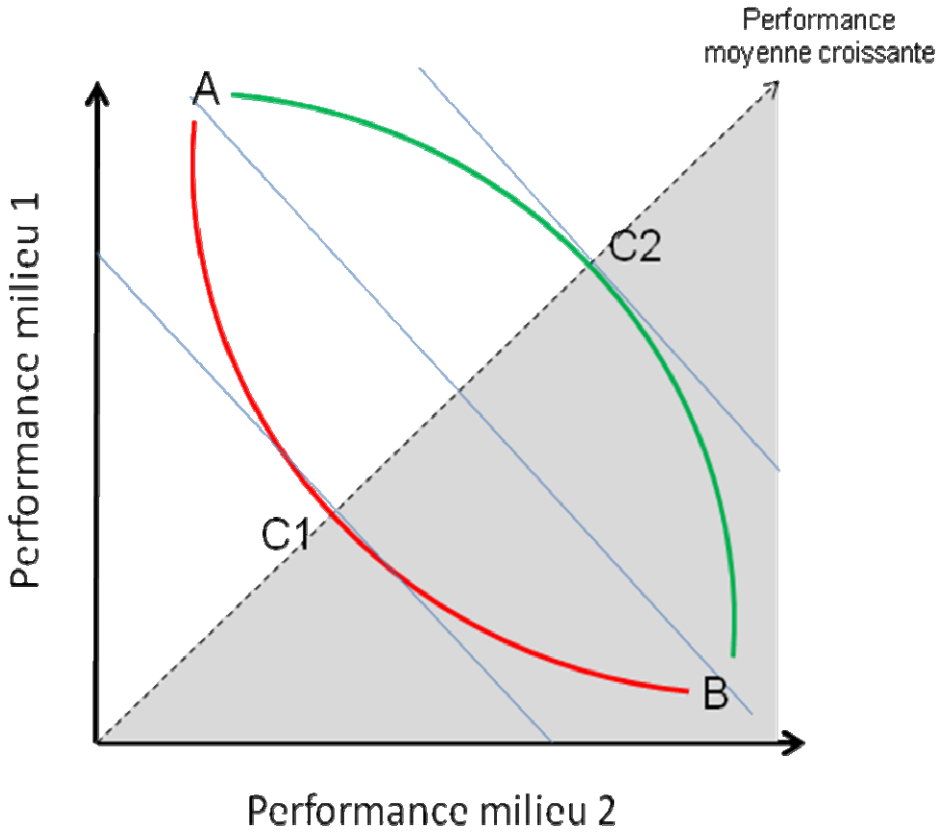


Figure 8 : Représentation graphique du compromis évolutif des performances dans deux milieux différents (voir texte pour explications). La ligne en pointillés représente l'isocline de performances sur laquelle les performances des génotypes sont identiques sur les deux milieux (parfaits généralistes). La région sombre représente les positions des spécialistes du milieu 2 et la région en blanc celle des spécialistes du milieu 1. La ligne rouge représente un compromis évolutif fort. Une augmentation de la valeur sélective du génotype A sur le milieu 2 se traduit par une diminution importante de sa valeur sélective dans le milieu 1. La ligne verte représente un compromis évolutif faible. Une augmentation de la valeur sélective du génotype A sur le milieu 2 se traduit par une faible diminution de sa valeur sélective dans le milieu 1.

Dans le cas d'un compromis évolutif fort, un généraliste C1 présente une performance moyenne inférieure à celle de deux spécialistes A et B en plus d'être moins performant que A dans le milieu 1 et que B dans le milieu 2. Ce coût associé à être généraliste limite son émergence et permettrait la coexistence des deux spécialistes A et B. Un compromis évolutif fort est nécessaire mais pas suffisant pour l'émergence et le maintien de la diversité en milieu hétérogène (Egas et al. 2004, Kneitel et Chase 2004). Un exemple assez commun de compromis évolutif qui sert à expliquer le maintien de la diversité est le « competition-colonization tradeoff » (Levins et Culver 1971, Kneitel et Chase 2004). Ce compromis évolutif prédit que les meilleurs compétiteurs sont des moins bons colonisateurs.

Cadotte et collaborateurs (2006a) ont mis en évidence expérimentalement la présence de ce compromis dans des assemblages artificiels de protozoaires et rotifères. Dans leur expérience, les meilleurs colonisateurs étaient effectivement les moins bons compétiteurs ce qui permettrait d'expliquer leur coexistence.

b. Grain environnemental.

Le grain représente le rang de variabilité environnemental relatif à la durée de vie (variabilité temporelle) et/ou la zone de distribution (variabilité spatiale) d'un organisme donné (**Figure 9**). Dans un environnement spatialement hétérogène, si la distribution de l'organisme lui permet de rencontrer pendant sa durée de vie plusieurs conditions environnementales alors on parle d'un grain spatialement fin (panel haut à gauche). Si par contre la zone de distribution de l'organisme ne présente qu'une unique condition environnementale le grain est dit grossier spatialement (panel haut à droite). Dans un environnement spatialement hétérogène, étudier le grain correspond à étudier l'échelle d'hétérogénéité ; un grain fin correspond à une

hétérogénéité locale alors qu'un grain grossier correspond à une hétérogénéité régionale.

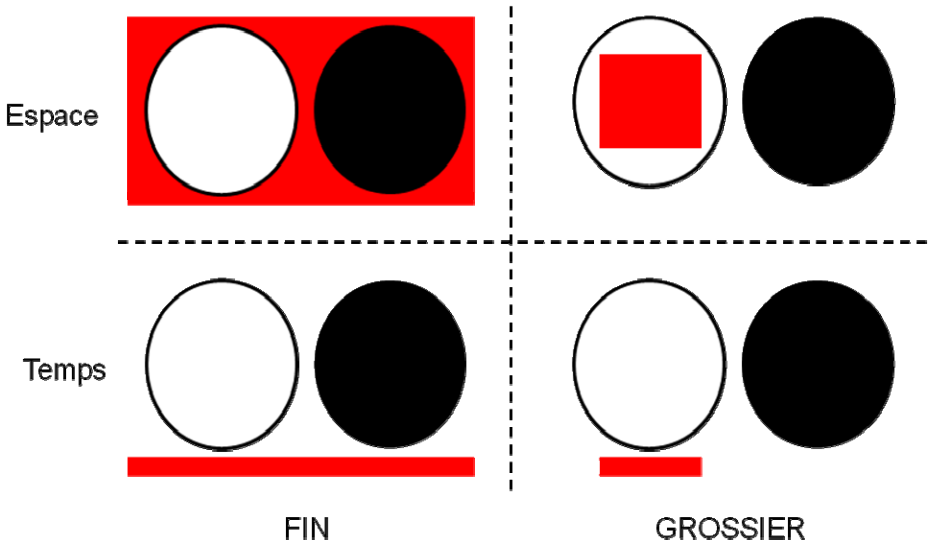


Figure 9 : Représentation graphique du grain environnemental dans un milieu hétérogène dans l'espace (haut) ou hétérogène dans le temps (bas). Les cercles blancs et noirs représentent deux conditions environnementales différentes (ressources). Le rectangle rouge du haut (espace) représente la zone de distribution de l'organisme. Le trait rouge du bas représente la durée de vie de l'organisme (voir plus d'explications dans le texte).

Un grain fin favorise une stratégie généraliste si le compromis évolutif est faible mais si le compromis est fort il favorise deux spécialistes. Un grain grossier par contre favorise la spécialisation (faible diversité locale), indépendamment du compromis évolutif (Levins 1968). Un bon exemple d'un grain fin spatial (hétérogénéité locale) est une culture bactérienne avec deux sources de carbone disponibles. Jasmin et Kassen (2007) ont contrasté le résultat de la sélection après ~600 générations dans un mélange de deux sources de carbone contre des cultures avec une seule source de carbone disponible. Leurs résultats ont montré une grande variabilité, probablement comme conséquence de différents types de compromis évolutifs entre les

performances dans les deux milieux. En effet, plus le contraste environnemental était fort, plus la diversité augmentait.

Dans un environnement temporellement fluctuant (panel bas), si pendant sa durée de vie l'organisme rencontre plus d'une condition environnementale on dit que le grain est fin (panel bas à gauche). Par contre s'il rencontre une même condition environnementale alors le grain est grossier (panel bas à droite). Dans tous les cas on s'attendrait à l'émergence d'un généraliste qui aurait la plus forte valeur sélective moyenne sur les deux environnements (Levins 1968, Kassen 2002). Cependant, plusieurs expériences ont montré qu'un environnement temporellement fluctuant peut maintenir plus de diversité qu'un environnement constant (Kassen 2002). Descamps-Julien et Gonzalez (2005) par exemple ont montré que deux espèces de phytoplancton en compétition pour une seule source étaient capables de coexister dans un environnement avec une température temporellement fluctuante. Très peu d'études empiriques se sont intéressés à l'effet du grain temporel sur la diversité (fin ou grossier), probablement par l'existence d'attendus théoriques très clairs qui suggèrent l'émergence d'une unique stratégie généraliste (voir plus haut). Par exemple, Kassen et Bell (1998) ont observé l'évolution de généralistes dans des populations de *Chlamydomonas reinharti* soumises à un régime de températures fluctuantes dan le temps, indépendamment du grain. Buckling et collaborateurs (2007) ont trouvé le même résultat, mais cette fois avec *Pseudomonas fluorescens* dans un régime de fluctuations dans la qualité du milieu de culture. Ces deux études proposent un manque de coûts (compromis évolutif faible) pour expliquer leurs résultats. La seule expérience mettant en évidence un effet du grain sur la diversité en milieu temporellement fluctuant est celle de Suiter et collaborateurs (2003). Dans leur étude ils ont soumis la bactérie *E. coli* à des fluctuations dans la concentration d'arginine dans le milieu de culture. Les fluctuations de fréquence intermédiaire (4 générations) ont maintenu un polymorphisme génétique plus longtemps que des fluctuations plus (2 générations) ou moins fréquentes (5 et 6 générations).

L'étude expérimentale du rôle du grain dans l'émergence de diversité dans un environnement temporellement fluctuant sera l'objet d'une étude plus approfondie dans cette thèse (voir ***Manuscrit 4***).

c. Dispersion

Comme mentionné précédemment en incluant les processus régionaux dans la compréhension de la structure locale des systèmes biologiques, la métapopulation et la métacommunauté proposent que la dispersion joue un rôle primordial dans l'émergence et le maintien de la diversité en milieu structuré (Leibold et al. 2004). De nombreuses approches théoriques en métacommunautés soulignent l'importance d'une dispersion limitée pour maximiser la richesse spécifique locale (Mouquet et Loreau 2002, 2003). Un faible niveau de dispersion favorise la divergence des communautés (diversité beta maximale) alors qu'un niveau de dispersion trop fort aurait comme effet une homogénéisation du système et une perte de diversité (Mouquet et Loreau 2002).

Dans une méta-analyse des études empiriques sur le rôle de la dispersion sur la diversité des communautés, Cadotte (2006) conclu qu'en général la dispersion augmente la diversité locale. De plus, il trouve que dans les études chez les animaux, la diversité est maximale pour des niveaux de dispersion intermédiaires. A l'échelle régionale, les résultats de sa méta-analyse sont moins concluants car ils révèlent des effets tant nuls comme négatifs de la dispersion sur la diversité.

Les expériences avec des microorganismes permettent de contrôler facilement les facteurs écologiques comme la dispersion pouvant aboutir à des changements à des échelles évolutives (Srivastava et al. 2004, Jessup 2004). Par exemple, Matthiessen et Hillebrand (2006) ont utilisé des

métacommunautés de micro algues pour tester expérimentalement le rôle de la dispersion dans le maintien de la diversité locale. Leur résultat est une diversité locale maximale pour des niveaux de dispersion intermédiaires.

Dans les ***Manuscrits 1*** et ***2*** je développe cette thématique en montrant que des niveaux de dispersion intermédiaires favorisent le processus de diversification aux échelles régionale et locale dans un environnement spatialement hétérogène. Cependant je souligne également la nature dynamique de ce résultat et envisage une diversité régionale maximale à l'équilibre dans des systèmes fermés (pas de dispersion).

d. Autres facteurs.

Un autre facteur couramment évoqué par les modèles de génétique des populations pour permettre un maintien du polymorphisme est la présence d'une sélection de type *soft* (Christiansen 1974, 1975, Karlin et Campbell 1981, Walsh 1984, Ravigné et al. 2004). Ce type de régulation de la densité des différents variants assume que dans un environnement hétérogène, les différentes populations vont contribuer de façon égale au pool régional qui donnera origine à la génération suivante (Levene 1953). Une régulation de la densité des différents variants à l'échelle régionale irait au détriment de la diversité car elle favoriserait le variant adapté au milieu plus productif (sélection type *hard*, Dempster 1955).

Finalement une autre condition *sinequanone* pour le maintien du polymorphisme en milieu hétérogène est une fréquence égale des différents environnements. Si un environnement est plus fréquent, c'est le variant adapté à celui-ci qui finira par dominer dans la métapopulation (Maynard Smith et Hoekstra 1980).

4. *Coexistence*

Beaucoup d'expériences avec des microorganismes ont confirmé le rôle de l'hétérogénéité environnementale comme mécanisme de diversification et de coexistence (Rainey et Travisano 1998, Kassen 2002). Un type environnement complexe très utilisé pour adresser la question de l'émergence et le maintien de la diversité en milieu hétérogène est un mélange de deux ressources. Dans ce cas, les bactéries disposent simultanément et pendant toute la période de sélection des deux ressources différentes (Friesen et al. 2004, Abrams 2006). Ce scénario correspond à ce que je viens de définir comme un environnement avec un grain spatial fin. Dans ce cas de figure, théoriquement deux génotypes (parfois trois génotypes) peuvent coexister stablement à condition que chacun d'entre eux soit avantagé sur une des ressources disponibles. Ceci nécessite, comme nous l'avons vu précédemment, un compromis évolutif fort qui favorise la spécialisation dans les deux environnements. Cette dernière peut aboutir à un type de régulation fréquence-dépendante qui garantit en équilibre stable de la diversité (Lenski 1991). Ces mêmes conditions sont également nécessaires pour l'évolution de la diversité dans un grain spatial grossier comme celui considéré par les modèles en métapopulation ou métacommunauté.

a. *Spécialisation et adaptation locale*.

La spécialisation écologique est donc un mécanisme de coexistence très important dans un environnement hétérogène. Elle peut être étudiée de diverses manières selon le contexte utilisé. Pendant mon travail de thèse j'ai adressée sous forme de revue la question de sa définition et de sa mesure (voir ***Manuscrit 4***). Dans cette section je vais plutôt détailler certains aspects de son évolution.

Nous venons de voir que l'étude de l'émergence et le maintien de la diversité en milieu hétérogène revenait à étudier les conditions de l'évolution de la spécialisation. Théoriquement un spécialiste est celui qui performe mieux dans un environnement que dans un autre. Tout écart par rapport à une performance équivalente dans les différentes conditions environnementales étant signe de spécialisation (variance environnementale, **Figure 8**). Dans ce contexte, l'étude de l'évolution de la spécialisation consiste à étudier les contraintes qui empêchent l'émergence de parfaits généralistes (Kassen 2002). Je ne ferai que mentionner les mécanismes génétiques responsables de la spécialisation écologique (pléiotropie antagoniste ou accumulation de mutations, MacLean et Bell 2002).

Bell (1990) définit trois façons de se spécialiser lorsque deux ressources sont disponibles: par sélection synclinale, par sélection aclinale et par sélection anticlinale (**Figure 10**). Dans le premier cas la réponse à la sélection est positive dans les deux environnements, c'est-à-dire que le variant évolué (B) performent mieux que l'ancêtre (A) dans les deux environnements. Cependant, la réponse est plus importante dans un environnement que dans l'autre. Ce type d'évolution a lieu souvent en présence d'un compromis évolutif faible. Dans le scénario d'évolution aclinale la réponse à la sélection est positive dans un environnement et nulle dans l'autre. Enfin, dans le scénario d'évolution anticlinale la réponse à la sélection est positive dans un environnement et négative dans l'autre. Ce dernier scénario à lieu souvent en cas d'un compromis évolutif fort.

L'évolution de la spécialisation en milieu spatialement hétérogène aboutit souvent à un patron d'adaptation locale. Kawecki et Ebert (2004) ont définit les deux facettes de l'adaptation locale :

1°- « Etre plus performant que les migrants dans son milieu d'origine ». C'est la version Locaux vs. Migrants de l'adaptation locale.

2°- « Etre plus performant dans son milieu d'origine qu'ailleurs ». C'est la version Home vs. Away de l'adaptation locale.

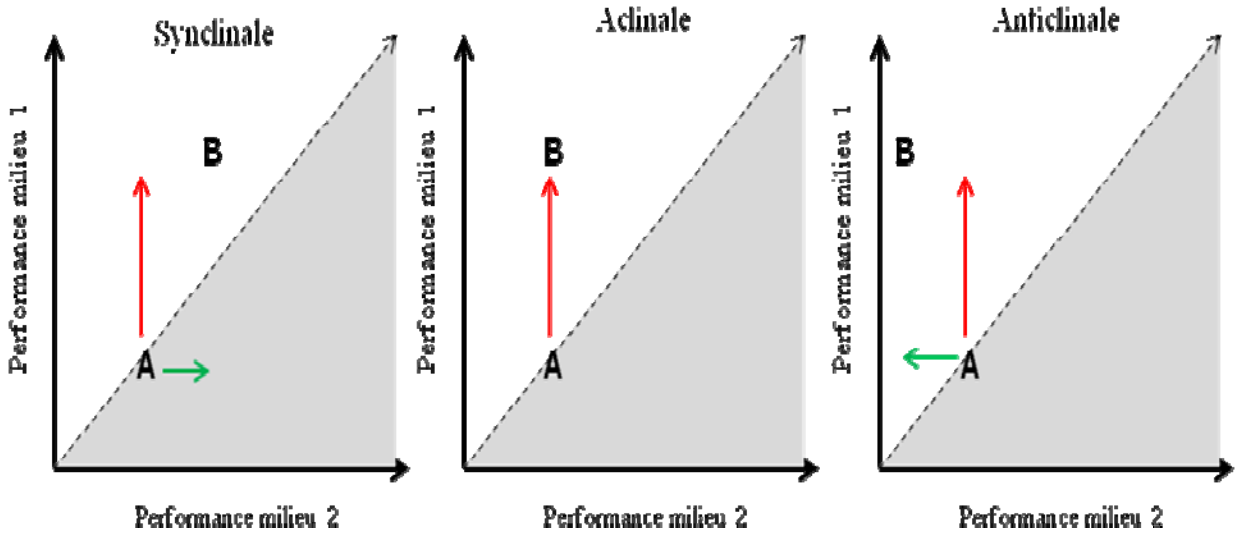


Figure 10 : Trois façons de se spécialiser: par évolution synclinale, aclinale ou anticlinale (modifié d'après Bell et Reboud 1997 ; voir texte pour explications).

Les deux versions sont importantes pour définir le patron d'adaptation, mais seule la version Locaux vs. Migrants de l'adaptation locale nous renseigne vraiment sur le véritable niveau de spécialisation. J'ai traité plus en détail le rôle de la dispersion dans l'évolution de la spécialisation et du patron d'adaptation locale dans un environnement spatialement structuré (voir ***Annexe-Manuscrit en préparation***).

b. Interactions G x E.

Le partitionnement des niches qui définit la spécialisation écologique telle que je l'ai définie permet l'émergence d'interactions du type *génotype x environnement* (*G x E*; Bell 1990). Ce type d'interactions émerge souvent

lorsque la sélection est diversifiante, c'est-à-dire lorsqu'elle agit dans des directions différentes dans les milieux. La présence d'une interaction *G x E* est donc indicateur de la présence de sélection diversifiante et donc garantirait le maintien de la diversité.

c. Fréquence-dépendance négative.

L'ingrédient final pour qu'un polymorphisme ou une coexistence soient stables est la présence de processus de régulation densité ou fréquence dépendants négatifs (Dempster 1955, Ayala 1974, Levin 1988). La sélection fréquence-dépendante négative peut maintenir la diversité d'une manière stable dans un environnement hétérogène (spatial ou temporel). Une sélection fréquence dépendante négative indique que la valeur sélective d'un génotype (espèce) diminue au fur et à mesure que la fréquence de ce dernier augmente (**Figure 11**). On l'appelle également avantage du rare. A base fréquence (proche de zéro), la valeur sélective du génotype rouge est maximale et celle du génotype vert minimale car il domine la population (fréquence 1). Le rouge étant rare présente un avantage sélectif et augmente sa fréquence au détriment du vert jusqu'à ce que la valeur sélective des deux génotypes soit égale. A ce stade le polymorphisme se maintien à une fréquence d'équilibre stable

Ce sont surtout les tests d'invasion qui mettent en évidence une sélection fréquence dépendante négative (Rainey et Travisano 1998). Un génotype rare et généralement marqué est introduit à basse densité dans une population dominée par un autre génotype. Si l'envahisseur persiste dans la population cela indiquerait la présence de sélection fréquence dépendante négative.

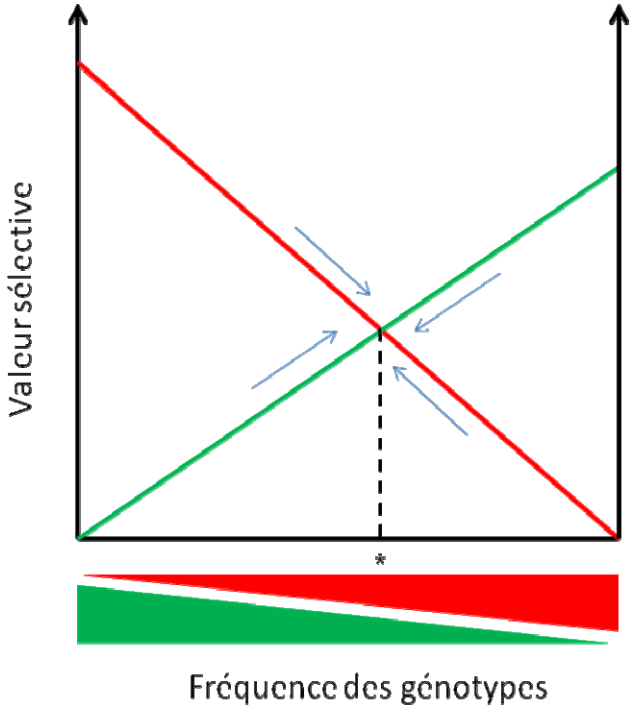


Figure 11 : Schéma de la sélection fréquence dépendante négative (avantage du rare) pour deux génotypes en compétition (voir texte pour explications). Les fréquences des deux génotypes sont inversées.

5. Mesures de diversité.

a. Jardin commun.

Les expériences en jardin commun reçoivent leur nom des pratiques réalisées par les agriculteurs en transplantant réciproquement des plantes vers d'autres conditions environnementales et en mesurant leurs performances. Le but étant d'obtenir des souches plus performantes (productives, résistantes). Dans le cadre de ce travail, j'ai pour la plupart du temps procédé à des tests de performance de génotypes évoluées (et pas au niveau des populations) en respectant le design orthogonal préconisé par le jardin commun : tous les génotypes de toutes les origines étant testés dans tous les environnements.

b. Partition de la variance phénotypique totale.

Nous l'avons vu avant, la technique de partition de la variance sert à mesurer la diversité génétique basée sur des données phénotypiques des individus. Disposant donc d'une matrice orthogonale de performances j'ai procédé à une partition de la variance phénotypique totale inspirée des techniques de partition de la génétique quantitative (Robertson 1959). La variance phénotypique totale se partitionne selon cette technique en trois composants principaux :

$$V_{totale} = V_{génotypique} + V_{environnementale} + \text{interaction}_{GxE} \qquad \text{(Equation 1)}$$

La variance génotypique correspond aux différences entre les performances moyennes des génotypes. Si tous les génotypes sont compétitivement équivalents sur l'ensemble des environnements alors ce composant est nul. La variance environnementale permet de comparer les productivités moyennes des différents environnements et sert à estimer leur similarité. Une forte variance environnementale indique la présence d'environnements très productifs dans lesquels en moyenne les génotypes sont très performants et d'environnements peu productifs dans lesquels les génotypes sont généralement peu performants. L'interaction $G\ x\ E$ regroupe toute la variabilité que les deux autres composants n'expliquent pas.

L'interaction $G\,x\,E$ se calcule selon l'équation générale de Robertson :

$$G\ x\ E = \sum \frac{\left(\sigma_{Ei} - \sigma_{Ej}\right)^2}{2G(G-1)} + \sum \frac{\sigma_{Ei}\ \sigma_{Ej}\left(1 - \rho_{EiEj}\right)}{G(G-1)} \qquad \text{(Equation 2)}$$

Dans cette équation σ_{Ei} et σ_{Ej} sont les écarts types environnementaux des performances du génotype i et j respectivement, G est le nombre total de

génotypes testés et ρ_{EiEj} la corrélation environnementale entre chaque paire de génotypes testés (**Figure 12**). Il s'agit donc d'une moyenne des comparaisons deux à deux de tous les génotypes de la population.

Cette équation devient, lorsque 2 génotypes sont testés sur plus de deux environnements :

$$G \times E = \frac{\left(\sigma_{E1} - \sigma_{E2}\right)^2}{2} + \sigma_{E1}\,\sigma_{E2}\left(1 - \rho_{E1E2}\right)$$

(Équation 3)

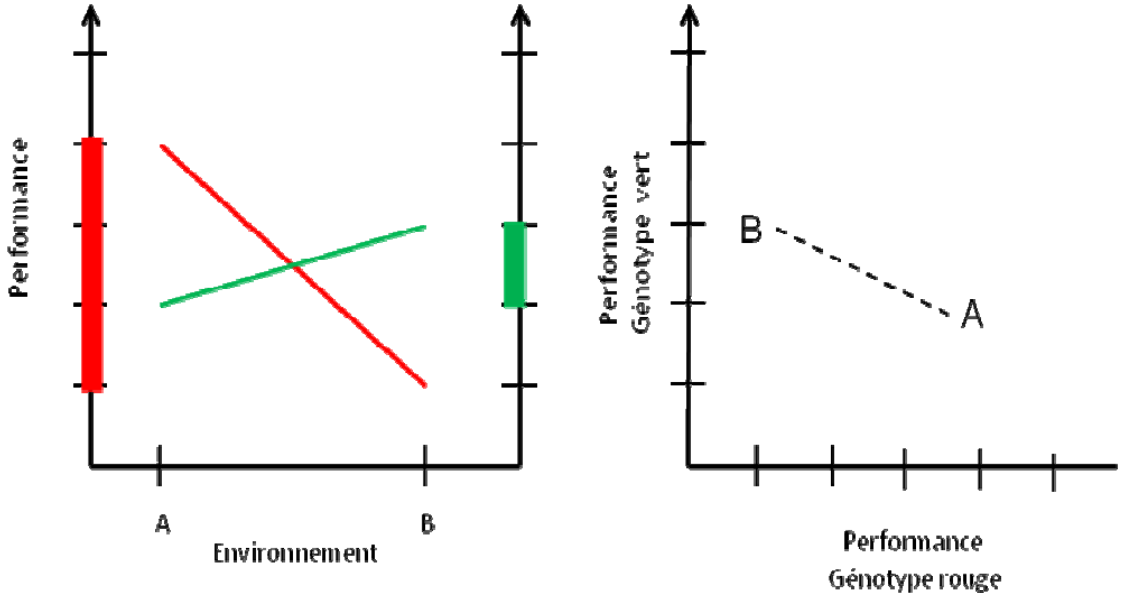


Figure 12 : Représentation graphique avec deux génotypes (rouge et vert) des différents composants utilisés pour calculer l'interaction $G \times E$ lorsque plus de deux génotypes et plus de deux environnements ou lorsque 2 génotypes et plus de deux environnements sont disponibles. Panel de gauche : normes de réaction, panel de droite : corrélation environnementale. Les barres verticales colorées sur les axes des performances du panel de gauche représentent les écarts types environnementaux des deux génotypes (σ_{Ei} et σ_{Ej}). La ligne en pointillés du panel de droite représente la corrélation environnementale de A et B pour ces deux génotypes. Dans ce cas l'indice ρ_{EiEj} est négatif (-1).

Cette équation est uniquement valable lorsque le nombre d'environnements testés est supérieur à 2. La façon de calculer l'interaction $G \times E$ basées sur des résultats d'expériences en jardin commun avec uniquement 2

environnements est différente (Robertson 1959, Barret et al. 2005). L'équation devient :

$$G\ x\ E = \frac{\left(\sigma_{GA} - \sigma_{GB}\right)^2}{2} + \sigma_{GA}\ \sigma_{GB}\left(1 - \rho_{GAGB}\right) \qquad \text{(Equation 4)}$$

Dans ce cas σ_{GA} et σ_{GB} sont les écarts types génotypiques des performances sur chaque environnement A et B et on utilise plutôt une corrélation génotypique et non une corrélation environnementale (Bell 1990, **Figure 13**).

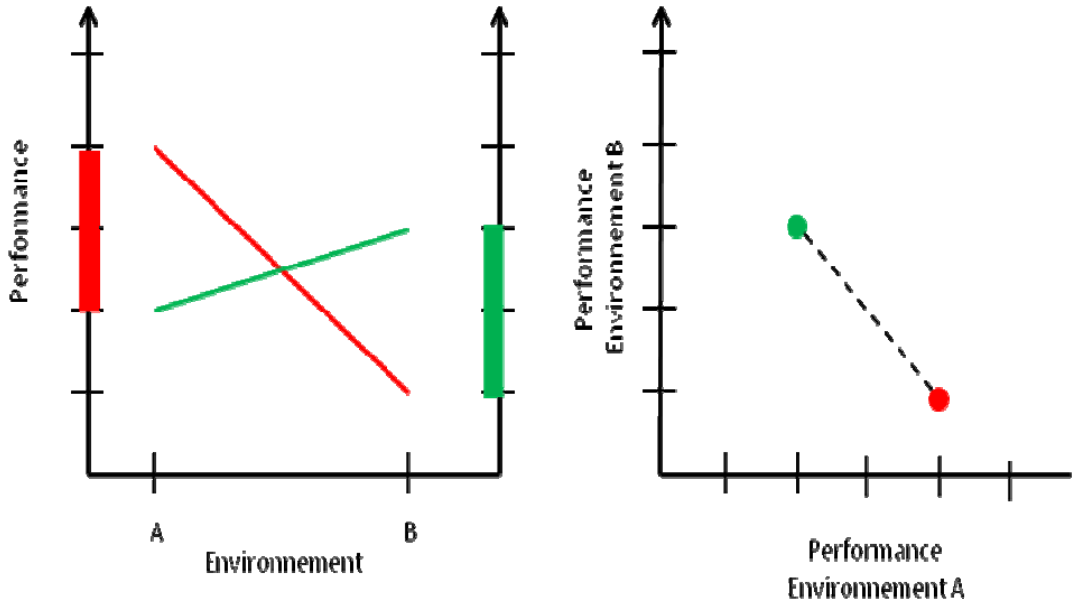


Figure 13 : Représentation graphique avec deux génotypes (rouge et vert) des différents composants utilisés pour calculer l'interaction $G\ x\ E$ lorsque plus de 2 génotypes et uniquement 2 environnements sont disponibles. Panel de gauche : normes de réaction, panel de droite : corrélation génotypique. Les barres verticales colorées sur les axes des performances du panel de gauche représentent les écarts types génotypiques des deux environnements (σ_{Gi} et σ_{Gj}). La ligne en pointillés du panel de droite représente la corrélation génotypique de deux génotypes sur A et B. Dans ce cas l'indice ρ_{GiGj} est négatif (-1).

c. Responsiveness et Inconsistency.

Dans les deux cas que nous venons de voir, que se soit avec une corrélation environnementale (équations 2 et 3) ou avec une corrélation génotypique (équation 4) il est possible de partitionner l'interaction *G x E* en deux composantes appelées *Responsiveness et Inconsistency* (Bell 1990, Barrett et al. 2005). La première renseigne sur des différences dans les écarts types environnementaux (équations 2 et 3) ou sur des écarts types génotypiques (équation 4). L'*inconsistency* renseigne sur l'absence de corrélation entre les génotypes dans les différents environnements (équations 2 et 3) ou de corrélation entre les environnements entre les différents environnements (équation 4).

Biologiquement, dans des équations 2 et 3, une forte valeur de *responsiveness* indique la présence simultanée de génotypes spécialistes (forte σ_E) et généralistes (faible σ_E). Une faible *responsiveness* indique la présence que de spécialistes ou que de généralistes. Dans l'équation 4 elle perd un peu son intérêt car elle indique uniquement si les génotypes ont la même variabilité dans leurs performances dans les deux environnements.

Dans toutes les équations, *l'inconsistency* est un indicateur de diversité écologique car elle mesure le chevauchement des niches des différents génotypes (Bell 1990, Kassen et Bell 2000). Si les génotypes se ressemblent, les corrélations génotypiques et environnementales seront positives et aboutiront à des faibles valeurs *d'inconsistency*. Si les génotypes se sont spécialisés sur des ressources et dons ont des niches non chevauchantes alors *l'inconsistency* sera forte (**Figure 14**). J'utilise *l'inconsistency* comme mesure de diversité fonctionnelle dans la ***Manuscrits 1, 2 et 3*** et également dans le ***Manuscrit 5***.

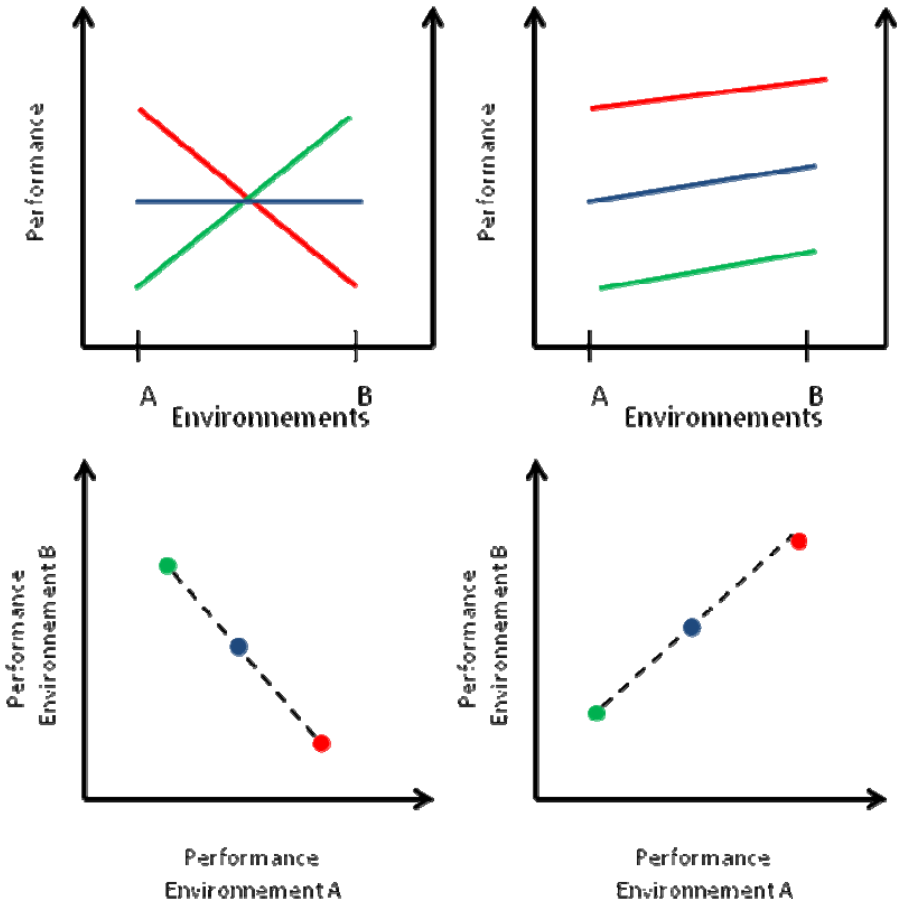


Figure 14 : Représentation graphique d'une forte *inconsistency* qui révèle une forte complémentarité fonctionnelle (partitionnement des niches, panel de gauche) et d'une faible *inconsistency* qui révèle une forte similitude fonctionnelle entre les génotypes (chevauchement des niches, panel de droite). A gauche la corrélation environnementale est négative alors qu'à droite elle est positive.

F. Relation Biodiversité-Fonctionnement.

"From a strictly functional point of view, species matter so far as their individual traits and interactions contribute to maintain the functioning and stability of ecosystems and biogeochemical cycles."

Loreau et al. 2001, Science

1. Un patron général ?

L'étude de la relation entre la diversité contenue dans un système écologique et son fonctionnement a maintenant une longue histoire en écologie (Johnson et al. 1996, Waide et al. 1999). Deux approches différentes se sont développées (Gross et Cardinale 2007). D'une part, beaucoup de ces études ce sont concentrées sur l'effet de certaines propriétés des écosystèmes (surtout la fertilité) sur la diversité (Waide et al. 1999 pour une revue, Kassen et al. 2000, Chase et Leibold 2002, Fukami et Morin 2003, Chalcraft et al. 2004, 2009) alors que d'autres ont plutôt exploré les effets de la biodiversité sur le fonctionnement des écosystèmes (pour des revues Johnson et al. 1996, Schlaepfer et Schmid 1999, Tilman 1999, Chapin et al. 2000, McCann 2000, Schwartz et al. 2000, Cottingham et al. 2001, Hector et al. 2001, Loreau et al. 2001, Naeem 2002, Naeem et Wright 2003, Hooper et al. 2005, Srivastava et Vellend 2005, Balvanera et al. 2006, Cardinale et al. 2006, Duffy et al. 2007, Smith 2007, Stachowicz et al. 2007). Ce sont surtout ces dernières approches qui se sont développés ces 20 dernières années comme résultat du constat du déclin accéléré de la biodiversité (Naeem 2002). La mise en évidence de l'augmentation de certains biens et services écosystémiques comme conséquence d'une plus grande biodiversité est devenue un argument majeur pour sa protection (Schwartz et al. 2000, Srivastava et Vellend 2005) et un axe principal de recherche en biologie de la conservation.

De nombreuses approches théoriques sur l'effet de la biodiversité sur les fonctions et les propriétés écosystémiques sont maintenant disponibles (Tilman et al. 1997, Doak et al. 1998, Yachi et Loreau 1999, Cardinale et al. 2000, 2004, Hughes et Roughgarden 2000, Lehman et Tilman 2000, Ives et Hughes 2002, Mouquet et al. 2002, Gross et Cardinale 2007). La plupart d'entre eux prédisent une relation positive. Cependant, certaines de ces études signalent des cas avec un manque de relation entre diversité et fonctionnement (scénario « idiosyncratique », Lawton 1994) ainsi que des possibles relations négatives (Cardinale et al. 2000, Mouquet et al. 2002). Un exemple de manque d'effet de la diversité sur le fonctionnement est celui de « saturation ». Certaines relations positives entre diversité et fonctionnement sont asymptotiques, révélant une saturation du processus écosystémique qui suggère d'une redondance écologique entre les espèces (Tilman et al. 1997). Au delà d'une certaine diversité, l'ajout de nouvelles espèces ne produit aucun effet sur le fonctionnement d'un écosystème. Plusieurs facteurs tels que l'identité des espèces (Tilman et al. 1997), l'hétérogénéité spatiale, le régime de perturbations (Cardinale et al. 2000), le patron d'interactions interspécifiques (Hughes et Roughgraden 2000) et la présence d'une limitation du recrutement dans un environnement hétérogène (Mouquet et al. 2002) peuvent générer des relations nulles ou négatives. Certaines études signalent également une relation temporellement dynamique, devenant de plus en plus forte pendant le processus l'assemblage des communautés (Cardinale et al. 2004). En conclusion, beaucoup d'études prédisent une relation positive mais soulignent également la difficulté de prédire un seul type de relation.

De nombreuses expériences de terrain sur la relation entre diversité et fonctionnement sont aussi disponibles (par exemple Tilman et Downing 1994, Hooper 1998, Crawley et al. 1999, Hector et al. 1999, 2000, 2002, Tilman et al. 2001, Cardinale et al. 2005, 2007, 2009, Tilman et al. 2006, Gross et al. 2007, Marquard et al. 2009, Romanuk et al. 2009). Ces expériences, qui se basent pour la plupart sur la création d'un gradient « artificiel » de diversité,

produisent des résultats qui sont en général en accord avec les prédictions théoriques. Elles ont confirmé l'importance de la composition (identité des espèces) et non seulement du nombre d'espèces sur le fonctionnement des écosystèmes (Crawley et al. 1999).

Parallèlement, l'étude de la relation entre biodiversité et fonctionnement des écosystèmes a été adressé avec des expériences en laboratoire (par exemple Naeem et al. 1994, Cardinale et al. 2002, Hodgson et al. 2002, Bell et al. 2005, Matthiessen et al. 2007, Weigelt et al. 2008, Weis et al. 2007, 2008, Douglass et al. 2008, Replansky et Bell 2009) avec des résultats très similaires à ceux des expériences « grandeur nature » sur l'importance de l'identité des espèces avec une forte tendance vers des claires relations positives entre diversité et fonctionnement (Schwartz et al. 2000, Jiang et al. 2009). Ces études se basent également en une manipulation directe de la diversité, par assemblage de communautés de diversité croissante (Bell et al. 2005) ou par suppression progressive d'espèces (Naeem et al. 1994).

Ces études ont souvent été critiquées par le non respect des règles « naturelles » d'assemblage des communautés ce qui aboutit à des assemblages « immatures », limitant une possible extrapolation vers des systèmes naturels (Cadotte et al. 2005, Jiang et al. 2009) ou par le fait de considérer des échelles spatiales trop petites (Cardinale et al. 2004, Srivastava et Vellend 2005). Cependant, des arguments en faveur de ce type d'expériences dites « simplifiées » sont aussi disponibles (Cadotte et al. 2005). Par leur simplicité, elles permettraient de mieux comprendre l'essence des systèmes naturels. Expérimentalement, la possibilité de manipuler facilement et dans un petit espace des gradients considérables de diversité de jusqu'à plusieurs dizaines d'espèces ainsi que la possibilité de suivre l'émergence des propriétés des systèmes biologiques (voir *Manuscrit 1*) représentent deux atouts majeurs de l'utilisation des microorganismes comme modèle pour étudier les conséquences de la biodiversité sur le fonctionnement des écosystèmes.

Dans mon travail de thèse j'ai disposé d'une manière simple et originale de mesurer la productivité (la densité optique). La densité optique est une bonne approximation de la biomasse cellulaire et donc de la productivité d'une population bactérienne car elle reflète une quantité de ressources transformées en une durée de temps limitée.

Récemment, l'histoire évolutive des communautés a commencé à être prise en compte pour étudier la relation entre diversité et fonctionnement des écosystèmes (Cadotte et al. 2008). La diversité phylogénétique d'une communauté semble mieux expliquer la productivité d'un écosystème que d'autres mesures de diversité comme le nombre d'espèces et la diversité fonctionnelle (voir aussi Cadotte et al. 2009). Dans mon travail de thèse j'ai mis en évidence l'émergence par évolution d'une relation positive entre diversité et productivité à l'échelle régionale (voir *Manuscrits 1 et 2*).

Déchiffrer l'importance de la biodiversité nécessite non seulement une meilleure compréhension de ces conséquences sur les écosystèmes mais aussi celle des mécanismes qui déterminent son émergence et son maintien (Tilman 2000, Mouquet et al. 2002, Cardinale et al. 2004, Van Ruijven et Berendse 2005). Différents patrons de la relation biodiversité - fonctionnement sont obtenus selon le mécanisme de coexistence considéré ce qui suggère un manque de relation générale entre diversité et fonctionnement (Mouquet et al. 2002). Par exemple, basées sur la variabilité des mécanismes responsables de la coexistence, certaines études ont souligné l'importance de l'échelle spatiale considérée sur le type de relation reliant la biodiversité au fonctionnement des écosystèmes (Bond et Chase 2002, Mouquet et Loreau 2003, Cardinale et al. 2004, Harrison et al. 2006, Gonzalez et al. 2009 in Naeem et al. 2009). L'influence des mécanismes locaux et régionaux de coexistence peut altérer les relations entre les espèces et générer différents types de relations entre diversité et fonction selon l'échelle spatiale considérée (Bond et Chase 2002). Dans le *manuscrit 2* j'aborde plus en détail cette problématique.

En conclusion, des approches théoriques et expérimentales montrent des tendances en faveur d'un effet positif de la diversité (qu'elle soit mesurée en

nombre d'espèces, en nombre de fonctions, en nombre de groupes fonctionnels ou en diversité phylogénétique) et les propriétés écosystémiques tels que la biomasse, la production primaire, la productivité, la stabilité, le flux de matière, la résistance aux invasions et à la sécheresse. Cependant, certaines incertitudes obligent encore à réaliser des recherches complémentaires avant d'utiliser cet effet positif comme argument pour sa protection (Schwartz et al. 2000, Srivastava et Vellend 2005). Par exemple, plus d'études expérimentales pour confirmer certains attendus théoriques sont nécessaires (Hooper et al. 2005),

2. *Mécanismes*.

La plupart des mécanismes utilisés pour expliquer la relation entre diversité et fonctionnement supposent la différenciation écologique des espèces (Hillebrand et Matthiessen 2009). Principalement, deux mécanismes peuvent être évoqués pour comprendre l'amélioration du fonctionnement des écosystèmes avec une augmentation de la biodiversité : la complémentarité et l'effet « sampling » (Loreau et al. 2001, Hector et al. 2002). L'hypothèse de complémentarité suppose qu'un système plus divers fonctionne mieux (i.e. plus productif) comme conséquence d'une meilleure utilisation des différentes conditions environnementales (i.e. ressources) car chaque condition sera dominée par l'espèce la mieux adaptée. On peut s'attendre à ce que les performances des assemblages de plusieurs espèces soient supérieures à celle des espèces individuellement (phénomène connu comme *overyielding ;* Loreau et Hector 2001). Ce principe est utilisé pour mettre en évidence des phénomènes de *complémentarité* tels que la facilitation (**Figure 15**). Dans ce cas, aucune espèce ne produit individuellement ce que produit l'assemblage le plus productif (panel gauche). Le *sampling* suppose que plus on augmente la diversité, plus il est probable que la communauté contienne une espèce très

productive individuellement. Dans ce cas, on s'attend à ce qu'une espèce soit aussi performante que le plus performant des assemblages (panel droit).

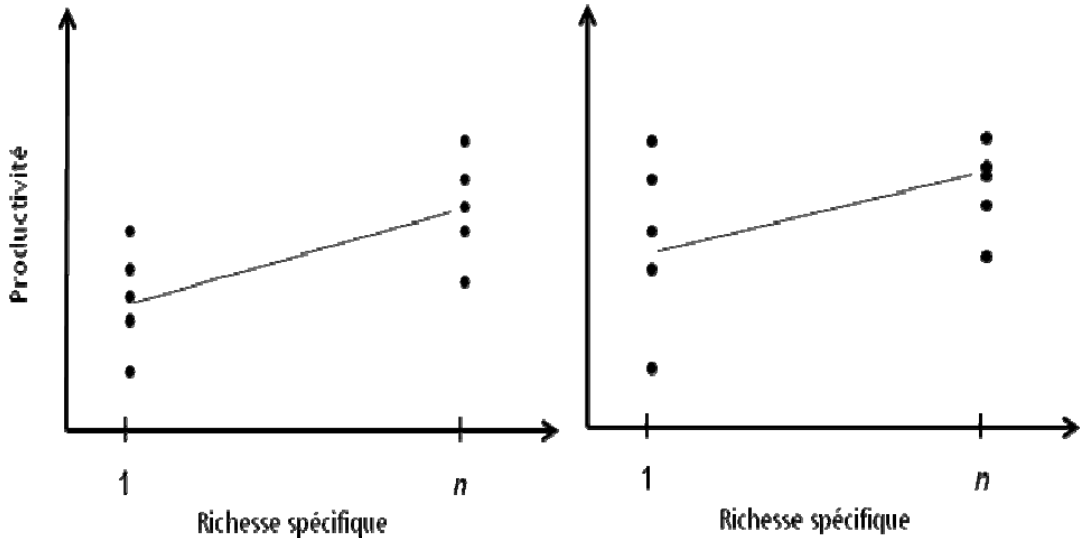


Figure 15 : Schéma simplifié du protocole expérimental utilisé pour différencier entre la *complémentarité* et le *sampling* comme mécanismes responsables d'une relation positive entre biodiversité (i.e. richesse spécifique) et le fonctionnement d'un écosystème (i. e. productivité, voir texte pour explications, modifié d'après Loreau et Hector 2001).

Cardinale et collaborateurs (2000) proposent une approche théorique pour montrer que le mécanisme responsable d'une relation positive entre diversité et fonctionnement dépend des conditions environnementales. L'hypothèse de la complémentarité ne pouvant être évoquée que dans le cadre d'un environnement hétérogène. Dans un environnement homogène c'est une augmentation des chances d'inclure une espèce productive qui explique une plus grande productivité des assemblages plus divers (effet *sampling*). Dans un environnement hétérogène c'est souvent une plus forte complémentarité dans l'exploitation des ressources qui est évoquée.

Dans le ***manuscrit 2*** j'explore comment la forme de la relation entre diversité et productivité change en fonction de l'échelle spatiale considérée. Dans un environnement homogène (une communauté avec une seule ressource) la relation est nulle alors que dans un environnement spatialement complexe

(métacommunauté avec 95 ressources) la relation entre diversité et productivité est positive. Ceci résulte de la possibilité donnée par un environnement hétérogène pour l'expression d'une forte complémentarité écologique.

II-ARTICLES

Manuscrit 1

Diversity and productivity peak at intermediate dispersal rate in evolving metacommunities

P. A. Venail, R. C. MacLean, T. Bouvier, M. A. Brockhurst, M. E. Hochberg & N. Mouquet,

Nature 2008, Vol. 452:210-214

Résumé en Français

Un effet positif de la diversité biologique sur le fonctionnement des écosystèmes a été démontré pour un grand nombre de systèmes biologiques et plusieurs mécanismes ont été proposés pour l'expliquer. Cependant, la plupart de ces études sont purement écologiques et ne considèrent pas l'influence des processus évolutifs sur la forme de cette relation. Egalement, les approches expérimentales de la relation entre diversité et fonctionnement se sont pour la plupart basées sur la construction d'assemblages artificiels de diversité croissante ou sur le retrait d'espèces.

Cet article présente les résultats d'une expérience en évolution expérimentale de ~500 générations avec la bactérie *Pseudomonas fluorescens* SBW25 dans un environnement spatialement structuré composé de 95 sources de carbone différentes. En partant d'un clone unique, ce travail explore l'effet du niveau de dispersion sur l'émergence et le maintien de la diversité sous l'effet de la sélection divergente. La nature asexuée des bactéries nous permet considérer ce système comme une véritable métacommunauté.

Le résultat est une diversité régionale maximale pour des niveaux de dispersion intermédiaires, associée à une plus forte productivité des métacommunautés. La dispersion ce comporte comme une source alternative de variabilité en plus de la mutation qui favorise le processus adaptatif et par conséquent la divergence entre les communautés. Beaucoup de dispersion par contre homogénéise la métacommunauté et limite sa diversification. Le gradient de diversité obtenu, crée par l'assemblage des

communautés soumises à différents niveaux de dispersion, présente une relation positive avec la productivité des métacommunautés. Un niveau de dispersion intermédiaire a favorisé la différenciation des niches, permettant une meilleure complémentarité entre les espèces dans l'exploitation des ressources.

Ce résultat illustre l'importance de considérer à la fois des processus évolutifs (adaptation des espèces à leur environnement) et des processus écologiques (dispersion) dans l'étude des conséquences de la diversité sur le fonctionnement des écosystèmes.

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Diversity and productivity peak at intermediate dispersal rate in evolving metacommunities

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Abstract

Positive relationships between species diversity and productivity have been reported for a number of ecosystems[1,2]. Theoretical and experimental studies have attempted to determine the mechanisms that generate this pattern over short time scales[1,2], but little attention has been given to the problem of understanding how diversity and productivity are linked over evolutionary time scales. Here, we investigate the role of dispersal in determining both diversity and productivity over evolutionary time scales using experimental metacommunities of the bacterium *Pseudomonas fluorescens* assembled by divergent natural selection. We show that both regional diversity and productivity peak at an intermediate dispersal rate. Moreover, we demonstrate that these two patterns are linked: selection at intermediate rates of dispersal leads to high niche differentiation between genotypes, allowing greater coverage of the heterogeneous environment and a higher regional productivity. We argue that processes that operate over both ecological and evolutionary time scales must be jointly considered when attempting to understand the emergence of ecosystem-level properties such as diversity-function relationships.

Current understanding of the relationship between diversity and productivity is based on the ecological effects of "complementarity" and "sampling"[1]. The former suggests that species richness enhances productivity because of niche differentiation (e.g., complementarity) or positive interactions (e.g., facilitation) between species and therefore more of the available resources are exploited. In "sampling", more diverse communities are, by chance, more likely to contain species with a higher average productivity than communities with low diversity. Disentangling these two effects has been a major challenge in biodiversity research because both lead to a positive relationship between diversity and productivity, but differ considerably in their implications for understanding the consequences of diversity loss[1]. It is less clear, however, how the ecological mechanisms driving species coexistence (e.g., environmental heterogeneity, dispersal) are responsible for the shape of this relationship[3,4] and the underlying evolutionary mechanisms remain largely unexplored.

Population genetic[5] and ecological models[6] consider environmental heterogeneity and intermediate dispersal as central both to the emergence and the maintenance of genetic polymorphism within metapopulations and/or species diversity within metacommunities[7-10]. Given that diversity and productivity are likely to be correlated[1], dispersal rate should also play a key role in determining community structure and function (e.g., productivity) over both ecological and evolutionary time scales. Some experimental studies support the idea that dispersal in heterogeneous environments is important in the emergence and maintenance of diversity[11,12], but the simultaneous consideration of ecological and evolutionary mechanisms controlling diversity has yet to be considered. Here we report the results of a long-term experiment in heterogeneous metacommunities where the level of dispersal between patches was manipulated. This experimental design enabled us to investigate the effect of dispersal on both biodiversity and productivity.

Bacteria offer the unique opportunity to obtain rapid ecological and evolutionary responses using experimental designs that fulfill the assumptions of theoretical models[13]. We allowed a single clone of *Pseudomonas fluorescens SBW25* to evolve for ~500 generations in a highly spatially heterogeneous environment (i.e., Biolog® GN2 microplates containing 95 unique sources of carbon) under four levels of dispersal. All the genetic and metabolic variability that appeared during the experiment evolved *de novo* by mutation so that each emerging genotype with its own functional properties can

be considered equivalent to an ecological "species" and the contents of a Biolog microplate therefore represent a metapopulation of genotypes or a metacommunity of species[14,15]. Performance on each carbon source was estimated every 24h (+/- 20 min) by measuring optical density with a spectrophotometer. The sum of absorbances over a Biolog microplate therefore reflects the resource use capacity of each metacommunity, considered as a proxy for its productivity (for further details see Methods)

Metacommunity productivity significantly increased during the selection period in all treatments (Fig. 1a) and this increase was greatest at an intermediate dispersal rate, as determined by quadratic regression and multiple means comparison tests (Fig. 1b). Frequency distributions of productivity on individual substrates help to link this result to the pattern of resource utilization. Distributions were initially bimodal (Fig. 1c to f), revealing high inequality in resource exploitation by the ancestral genotype and a source-sink like structure of the metacommunity. The distribution of productivity on individual substrates remained bimodal in the non-dispersal treatment (Fig. 1c), revealing that selection could not generate local adaptation on "poor" substrates in the absence of dispersal. The increase in productivity in metacommunities with dispersal (Fig. 1b) was primarily driven by an increase in productivity on substrates that were initially poorly exploited, as revealed by the switch to unimodality in the distribution of productivity on individual substrates (Fig. 1d, e and f).

To investigate the mechanisms underlying the hump-shaped dispersal-productivity relationship (Fig. 1b), we estimated the functional composition of evolved metacommunities at the end of the selection period by assaying the productivity of 16 randomly chosen genotypes from each metacommunity on each substrate. One potential explanation for this hump shaped pattern is that highly productive genotypes are selected at intermediate dispersal rates. To test this hypothesis, we estimated the genotypic productivity for each clone as the sum of productivity scores from each individual substrate. Across all dispersal treatments, average genotypic productivity was lower than that of the ancestral clone (Fig. 2) and there were no significant differences in mean genotypic performances between dispersal treatments or between replicates within treatments.

Alternatively, it is possible that productivity peaks at intermediate dispersal rate because of increased diversity at the scale of the metacommunity. To test this

hypothesis, we partitioned the total within-metacommunity variance of individual performances into effects of genotype (G), environment (E), and genotype-by-environment interaction (GxE).

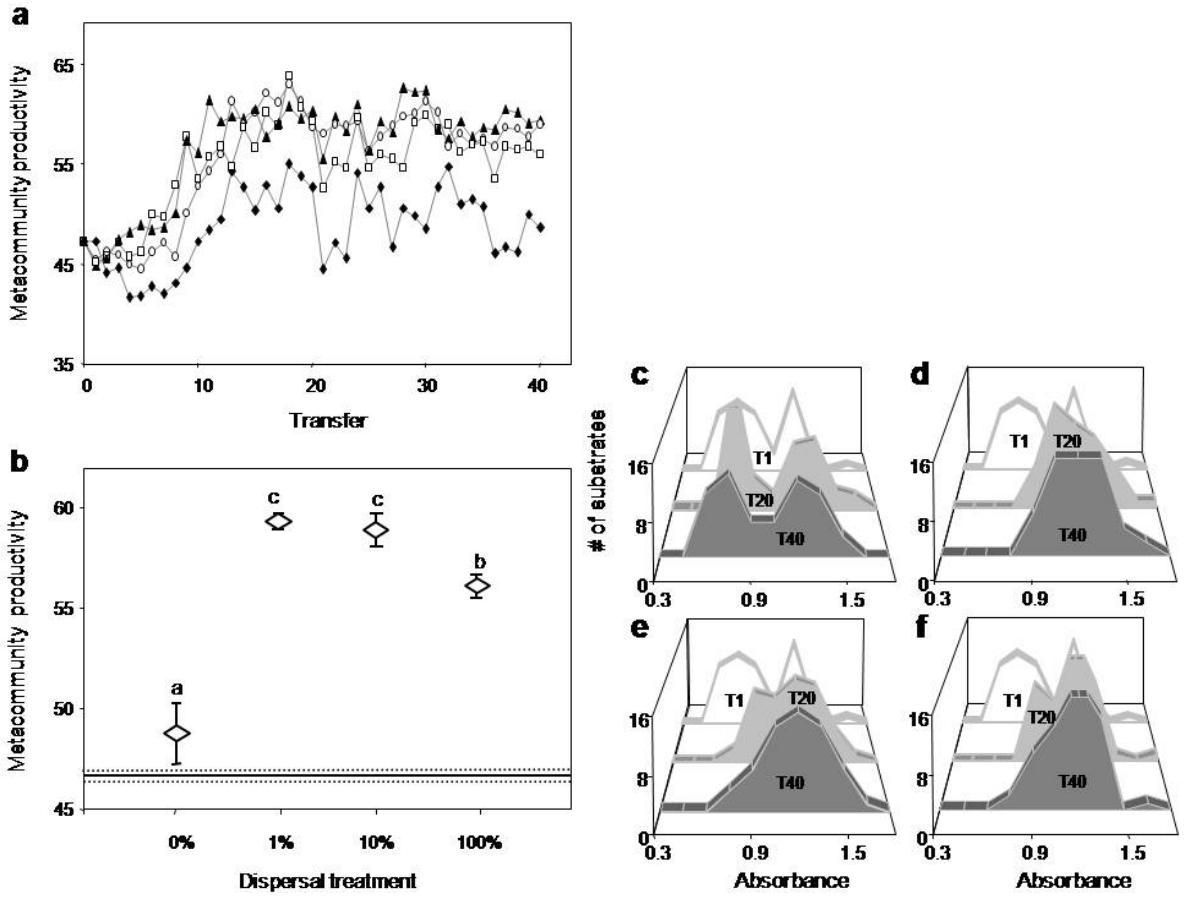


Figure 1: Metacommunity productivity as a function of dispersal rate: a) Plotted points show the temporal variation in mean productivity of metacommunities (n = 3) with 0% dispersal (♦), 1% dispersal (▲), 10% dispersal (○), and 100% dispersal (□). In all cases a plateau in productivity was observed in the second half of the selection period suggesting that maximal metacommunity productivity was attained. **b)** Mean metacommunity productivity (+/- s.e.m; n = 3) as a function of dispersal rate at the end of the selection period. Productivity peaks at intermediate dispersal rates as judged by quadratic regression (i.e., transfer 40; Quadratic test: $F_{2,9}$ = 47.8, P < 0.0001, quadratic parameter t = -7.98, P < 0.0001) and multiple means comparison (Tukey's test, q = 3.2; α = 0.05). The solid line represents the mean productivity of the replicate measures of the ancestral clone (+/- s.e.m; dotted lines, n = 12). This pattern is consistent over the plateau period (i.e., summed metacommunity productivity values over the 20 last transfers; Quadratic test: $F_{2,9}$ = 34.337, P < 0.0001, quadratic parameter t = -6.52, P = 0.0001; Tukey's test, q = 3.202; α = 0.05) **c** to **f)** Frequency distribution of mean productivity on individual substrates of the three replicates of each dispersal treatment at the beginning (T1), middle (T20) and at the end of the selection period (T40) for **c**) no dispersal, **d**) 1% dispersal, **e**) 10% dispersal and **f**) 100% dispersal treatments.

The E component measures variation in growth between substrates (i.e., environmental heterogeneity) while the G component measures variation in mean individual productivity among genotypes[16,17]. The GxE component reflects differences in the response of different genotypes to different environmental conditions; this is a measure of the amount of niche variation within metacommunities. GxE variance can be further decomposed into inconsistency (I), which measures niche differentiation (i.e., complementarity) or functional diversity within the metacommunity[17] and responsiveness (R), which measures diversity of niche breadth within the metacommunity (see Box 1).

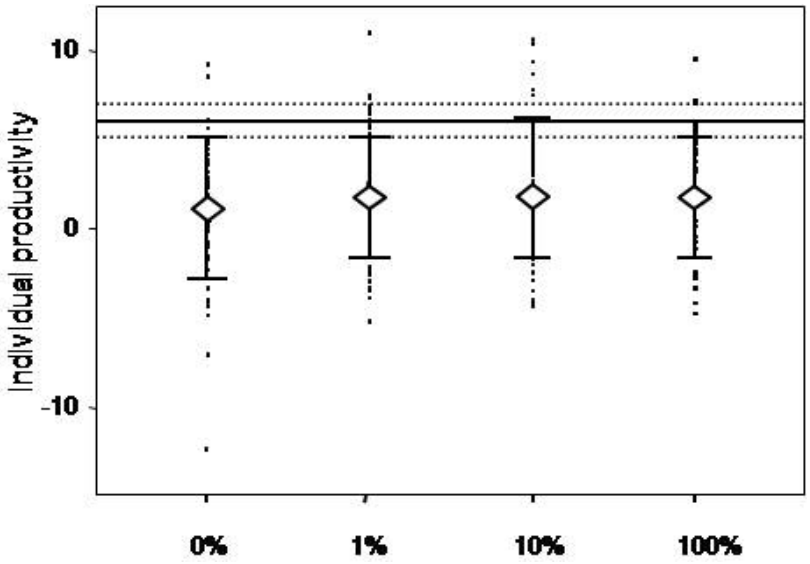


Figure 2: Individual genotypic productivity for each dispersal level: plotted points represent the productivity of individual genotypes at the end of the selection period for each dispersal treatment (n = 48); diamonds are the mean genotypic productivity for each dispersal treatment (+/- s.e.m; n = 3) and the solid line represents the mean productivity of replicate measures of the ancestral clone (+/- s.e.m; n = 12). We observed no differences in mean individual genotypic performances between dispersal treatments or between replicates within treatments (Nested ANOVA: $F_{3,180} = 0.46$, $P = 0.46$ and $F_{8,180} = 1.75$, $P = 0.09$, n = 192).

The absolute quantities of G, E, and GxE do not differ between dispersal treatments (Fig. 3a). However, the proportion of total variance attributable to inconsistency varies among dispersal treatments, with maximal functional diversity (i.e., inconsistency) at an intermediate dispersal rate (Fig. 3b). The proportion of total variance attributable to responsiveness declines with increasing dispersal (see Supplementary Figure) but only

represents 2 to 3 % of total phenotypic variance. Given that both functional diversity and productivity peak at an intermediate dispersal rate, this analysis supports the idea that differences between metacommunities in productivity stem from differences in diversity. Further support for this comes from the observation that the rank-order diversity and productivity of individual metacommunities are reasonably well correlated (Fig. 4; n = 12, $P = 0.04$, $r^2 = 0.34$).

In the absence of dispersal, the community present on each substrate behaves as a closed system, where *in situ* mutation is the only source of variation. Under such conditions, adaptation to poorly exploited habitats is obstructed by low effective population size, which reduces the rate of supply of beneficial mutations and increases the effect of genetic drift. Evidence for this constraint can be seen by the observation that adaptation to 'poor' substrates was impossible without dispersal (Fig. 1c). At the metacommunity scale, this results in low productivity, because only a minority of substrates make substantial contributions to total production, and low diversity, because the metacommunity is dominated by genotypes that are specialized on a narrow range of productive resources. With dispersal, beneficial mutations generated *ex situ* are constantly introduced by migration, allowing adaptation to 'poor' quality habitats[18] (see Supplementary Discussion).We argue that the consequences of adaptation to poor substrates, in terms of both diversity and productivity, depend on the strength of selection for specialists with high productivity on a narrow range of substrates, and generalists with intermediate productivity on a wide range of substrates.

At low and intermediate dispersal rates, selection favors resource specialization[15]. At the scale of the metacommunity this results in high diversity because of substrate specialists, and high productivity since each substrate is exploited efficiently. Consistent with this argument, functional diversity (i.e., inconsistency) is high in the 10% migration treatment (Fig. 3b) and average niche breadth is low, reflecting resource specialization (Fig. 3c). At high dispersal rates, selection favors generalists across multiple substrates instead of resource specialists[15], resulting in intermediate diversity and productivity at the scale of the metacommunity. In support of this argument, inconsistency is low in the 100% dispersal treatment (Fig. 3b), reflecting lower functional diversity, and average niche breadth is high (Fig. 3c), demonstrating the presence of generalists.

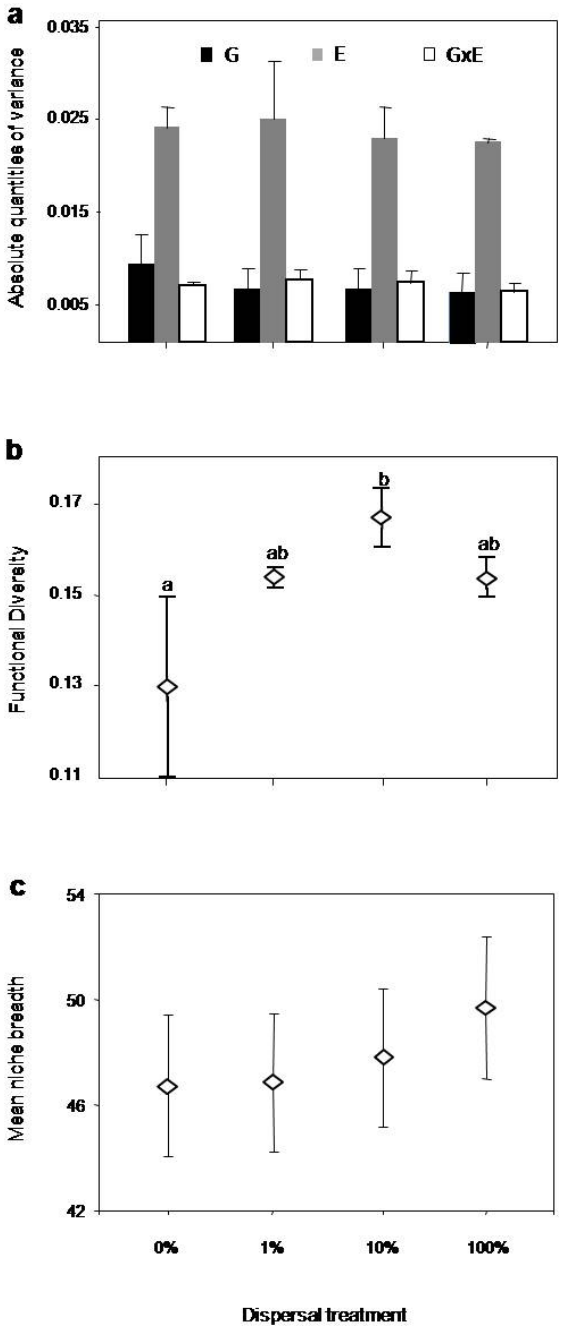


Figure 3: Phenotypic variance partitioning and niche breadth for each dispersal treatment: a) Bars show the mean quantity of phenotypic variance (+/- s.e.m; n = 3) attributable to effects of genotype (G), environment, (E) and genotype by environment interaction (GxE). The absolute quantities of G, E, and GxE do not differ between dispersal treatments (one-way ANOVA, n = 12, $P > 0.05$) **b)** Plotted points show functional diversity measured as a percentage of total phenotypic variance attributable to inconsistency. Functional diversity peaks an intermediate dispersal level as judged by quadratic regression (Quadratic test: $F_{2,9} = 9.84$, $P = 0.05$, n = 12; quadratic parameter $t = -3.16$, $P = 0.012$) and multiple means comparison (Tukey's test, $q = 3.2$; $\alpha = 0.05$). **c)** Diamonds show the average genotypic niche breadth for each dispersal treatment (+/- s.e.m; n = 48), calculated as the number of substrates giving positive growth scores for each genotype. Average niche breadth increases exponentially with dispersal rate ($F_{1,3} = 420$, $P = 0.0024$, $r^2 = 0.99$) and there are no differences in mean niche breadth among replicate lines nested within treatments ($F_{3,8} = 1.43$, $P = 0.19$).

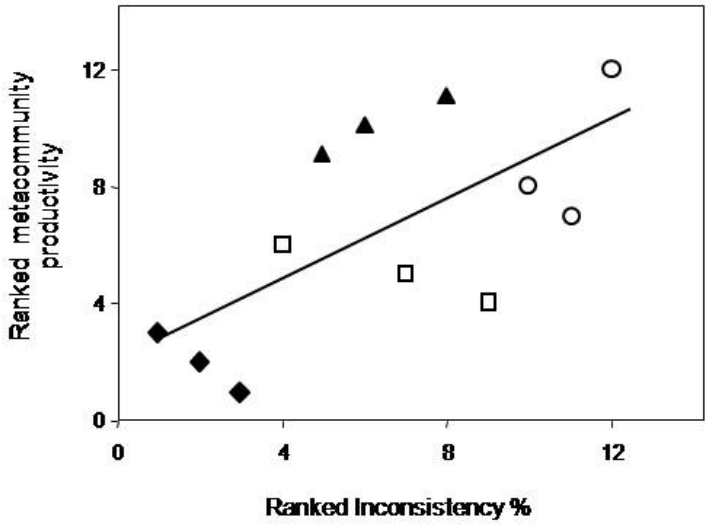


Figure 4: Metacommunity diversity and productivity: plotted points show the rank-order productivity and functional diversity of metacommunities with 0% dispersal (♦), 1% dispersal (▲), 10% dispersal (○), and 100% dispersal (□). Diversity and productivity are positively correlated (Spearman's test: $F_{1,10} = 5.26$, $P = 0.04$, $r^2 = 0.34$, n = 12).

In summary, we have shown that both functional diversity and productivity peak at an intermediate dispersal rate in bacterial communities assembled by natural selection resulting in a positive correlation between diversity and productivity (Fig. 4). The productivity of individual genotypes did not differ between dispersal treatments (Fig. 2), implying that a "sampling" effect cannot account for this pattern. Instead, our results indicate that communities with higher functional diversity evolve at an intermediate dispersal rate (Fig. 3b), demonstrating that "niche differentiation" (i.e., complementarity) contributes to the observed differences in productivity between dispersal treatments (Fig.4).

It is important to point out, however, that the correlation between diversity and productivity is not perfect: we estimate that 34% of the variation in productivity among communities can be accounted for by variation in functional diversity. An intriguing possibility is that facilitative interactions between genotypes[19,20] play an important role in determining productivity. Distinguishing the effects of niche differentiation (i.e., functional diversity) and facilitation is difficult in practice, and they are usually referred to collectively as complementarity[21]. Disentangling these two effects may be an interesting prospect for future research. It is important to note, however, that

facilitation via biochemical mechanisms such as cross-feeding[22] requires niche differentiation into different metabolic phenotypes, suggesting that the potential for facilitation was also greatest at an intermediate dispersal rate.

Most of our understanding of biodiversity and ecosystem function comes from models of niche differentiation driven by exploitative competition[23,24] and experiments on ecological time scales (reviewed in refs. 1 and 2). This work differs from previous studies in two important respects. Firstly, previous empirical studies have manipulated either diversity or productivity as the independent variable and then measured the response of the dependent variable (again, either diversity or productivity)[1,2]. Here, we have manipulated an independent variable (dispersal) that is predicted to play an important role in the emergence and maintenance of diversity, and independently measured the response of both diversity and productivity. Secondly, previous experimental studies of the relationship between diversity and productivity have focused on communities that are artificially assembled by choosing species from pre-existing pools (see, for example, the BIODEPTH experiment[25]), whereas we have studied the properties of communities that are naturally assembled by *in situ* evolutionary diversification. Although it is not entirely clear if the results of microbial experiments can be extrapolated to understand patterns in communities of multicellular eukaryotes[13], the controlled nature of microbial experiment has allowed us to gain insights into some of the most complex interactions in natural systems: how ecological and evolutionary mechanisms combine to determine community structure and function[26].

Methods Summary

We selected populations derived from a single clone of *Pseudomonas fluorescens* SBW25 on Biolog GN2 microplates under 4 different dispersal treatments (3 replicate lines per treatment). Every 24 hours (+/-20 minutes) we measured the absorbance at 590 nm on each line on every substrate, which provides an accurate measure of both cell density and productivity on each substrate. Following the assay migration was allowed to occur between substrates in each line such that 0%, 1%, 10%, or 100% of the population on each substrate was derived from a pool of immigrants derived from

all substrates (further details in Methods). Finally, we transferred 1uL from each substrate to a fresh Biolog plate. Microplates were incubated at 28C in a humidified incubator.

We measured the growth of 16 randomly sampled genotypes from each selection line at transfer 40 across all 95 Biolog substrates in 2 large assays (8 clones/line/assay). As a control, we also assayed 6 colonies of the ancestral clone in each assay. The ancestral clone gave equivalent growth scores in both assays across all substrates.

Methods

Ancestral Strain

To initiate our experiment we used an isolate of a *Pseudomonas fluorescens* SBW25 isogenic strain that had previously evolved for *c.* 900 generations in a complex environment of 8 carbon sources[16].

Selection Experiment

Biologs contain 95 different carbon sources from 11 different chemical families: polymers, carbohydrates, esters, carboxylic acids, amides, amino acids, aromatic compounds, amines, alcohols, phosphorylated compounds and bromhydric acid.

A single colony of our ancestral strain was used to found three replicates for each selection line (i.e., dispersal level). Each colony was initially grown for 24h in 6 ml of M9KB media (NH_4Cl 0,1 g/L; Na_2HPO_4 0,6 g/L; KH_2PO_4 0,3 g/L; NaCl 0,05 g/L; Glycerol 1 g/L, Proteose peptone # 2 2g/L) under constant orbital shaking (200 r.p.m.). One ml of this culture was centrifuged (1min at 13000 rpm) and washed by eliminating the M9KB and adding 1ml of M9 minimal salts (NH4Cl 1g/l; Na2HPO4 6g/l; KH2PO4 3g/l; NaCl 0,5g/l). 125 µl of this solution was diluted into 25ml of M9 minimal salts and starved for two hours at 28°C. 140 µl of this "starved cells" solution was then used to inoculate each well of the Biolog. The Biologs were incubated in the dark at 28°C during 24 hours in humid chambers. Daily transfers to new Biologs maintained maximal growth rate and allowed control of the amount of dispersal between wells. A transfer consisted of

inoculating each well of a new recipient Biolog with 1µl of the content of an old donor Biolog. Before each transfer, new Biologs were prepared by adding 140 µl of M9 minimal salts to each well. In the 0% dispersal treatment, transfers from old wells to new wells were directly carried out with a 96 pin replicator (Boekel 96 pin/well model #140500) that transfers 1µl of culture and 'prints' the populations grown on each selection Biolog to the new selection Biolog. We did not wash the cultures before each transfer since we estimated that after 24 hours the carbon remaining in the used Biolog was rather small and/or the volume of substrate transferred was also very small (1µl) to have any effect. For the 1% treatment we serially filled each well of a sterile 96 well microplate (Falcon 96 well microplate) with 99 µl of each old well plus 1 µl of immigrants obtained by mixing the content of each old well of the same Biolog. We then used the pin replicator to inoculate the new Biolog with the content of this intermediate microplate. For the 10% dispersal treatment we followed the same procedure, but with 90µl plus 10µl immigration. For the 100% dispersal treatments, we sampled all 140 µl from each old well to prepare a mixture. We then refilled the old wells with 140µl of that mixture and used the pin replicator to inoculate the new Biologs. All treatments thus result in the same quantity of culture (1µl) transferred every 24h into new Biologs.

We measured light absorbance at 590 nm (absorbance peak of tetrazolium) every 24 hours (+/- 20 minutes) prior to each transfer, using a FLUOstar Optima spectrophotometer (BMG®). Usually absorbances measured in Biolog microplates are corrected by a blank well (with no carbon source but very few nutrients and tetrazolium). However, in our experiment the correction against the control well was not possible, because the genetic composition of the control wells varied during the selection period. For each metacommunity, the sum of absorbances measures the total catabolic performances over the substrates of the Biolog. Because this corresponds to the quantity of carbon metabolised during 24h it can be used as a proxy of metacommunity productivity. The experiment ran for 40 transfers (*c.* 500 bacterial generations). At the 40th transfer, the content of all wells of each Biolog were mixed and frozen at -80 °C in 50% glycerol (w/v).

Assays

The assays measured individual genotypic performances on each carbon source at the end of the experiment. Prior to the assays, frozen mixtures from the end of the selection period were reconditioned in 6 ml of M9KB media for 24 h at 28°C under constant orbital shaking (200 r.p.m.). Sixteen genotypes (after serial dilution and plating) were randomly chosen from each replicate of each dispersal treatment (192 genotypes in total). We individually amplified these genotypes in M9KB media for 24 h at 28°C under constant orbital shaking (200 r.p.m.). For each genotype, 1 ml of culture was sampled, centrifuged and cells were washed by eliminating the M9KB and adding 1ml of M9 minimal salts. Then, 125 µl of washed culture was diluted in 25 ml of M9 minimal salts and starved for two hours (at 28°C) before their inoculation into the 96 wells of the Biologs (140µl). We scored absorbance at 590 nm for each genotype in each carbon source (16 genotypes × 4 dispersal treatments × 3 replicates × 95 carbon sources) after 24h of incubation in the dark at 28°C in humid chambers. We also reconditioned and assayed (same protocol) 12 genotypes of the ancestral clone used to inoculate the Biologs at the beginning of the selection period. We corrected absorbances by subtracting control well scores, because the genotypic composition of each well was the same.

Statistical analysis

On the first day of the selection period, we calculated the mean coefficient of variation in absorbances for each carbon source. Forty-one substrates were excluded because repeated measurements on the ancestor were statistically unreliable (coefficient of variation >1; see ref. 27), leaving 54 informative substrates. Analysis with all 95 substrates showed no qualitative differences to that used in this study with the 54 informative substrates (data not shown). For each dispersal treatment, we analyzed the total phenotypic variance in absorbances within metacommunities by partitioning environmental, genotypic and genotype-by-environment interaction components[28]. The interaction component ($G\times E$) can be further decomposed into *responsiveness* and *inconsistency*[16,28] as described in Box 1. We calculated the proportions of each component of total phenotypic variance to compare dispersal treatments (One-way

ANOVA). To compare relative proportions of *inconsistency,* we used an angular transformation of the data. To link metacommunity productivity at the end of the selection period vs. functional diversity we performed a Spearman rank correlation test (because the data were not normally distributed) and present the ranked data in Fig. 4. Data were analyzed with JMP 5.0 software (SAS Institute, Cary, NC).

BOX 1

Responsiveness and Inconsistency as relative measures of the diversity of resource exploitation strategies and niche differentiation (functional diversity)

In our 'common garden' assays, the performances of different genotypes over different environments (V_P) can be decomposed into genotypic (V_G), environmental (V_E) and genotype-by-environment interaction (V_{GE}) components as follows:

$$V_P = V_G + V_E + V_{GE} \quad (1)$$

The genotypic variance is the variance of average performances of a given genotype across all environments, and the environmental variance is the variance of environmental deviations (deviation of each phenotypic value from the mean performance of the genotype across all environments). The covariance term arises if genotypic values and environmental deviations are correlated and the interaction component arises as genotypes react differently to different environments[28]. Furthermore, the interaction component ($G\times E$) can be partitioned into *responsiveness* and *inconsistency*[16,28]: $V_{GE} = R + I$. The responsiveness component (R) indicates differences in environmental variances and thus measures diversity of resource exploitation strategies (specialists and generalists):

$$R = \sum \frac{\left(\sigma_{Ei} - \sigma_{Ej}\right)^2}{2G(G-1)} \quad (2)$$

The inconsistency (I) component indicates non-correlations between genotypes over environments:

$$I = \sum \frac{\sigma_{Ei}\ \sigma_{Ej}\ (1 - \rho_{EiEj})}{G(G-1)} \qquad (3)$$

where G is the number of genotypes tested, σ_{Ei} and σ_{Ej} the standard deviations of environmental responses for each genotype, and ρ_{EiEj} the environmental correlation among each pair of genotypes tested. The lack of correlation implies that each genotype is fittest in a different environment or, more generally, that reaction norms are negatively correlated over the different environments. The *inconsistency* is a measure of niche differentiation or functional diversity[17] since it reflects specialization on different resources. Measures of functional diversity based on trait diversity are probably more informative than simple measures of diversity *per se*[29].

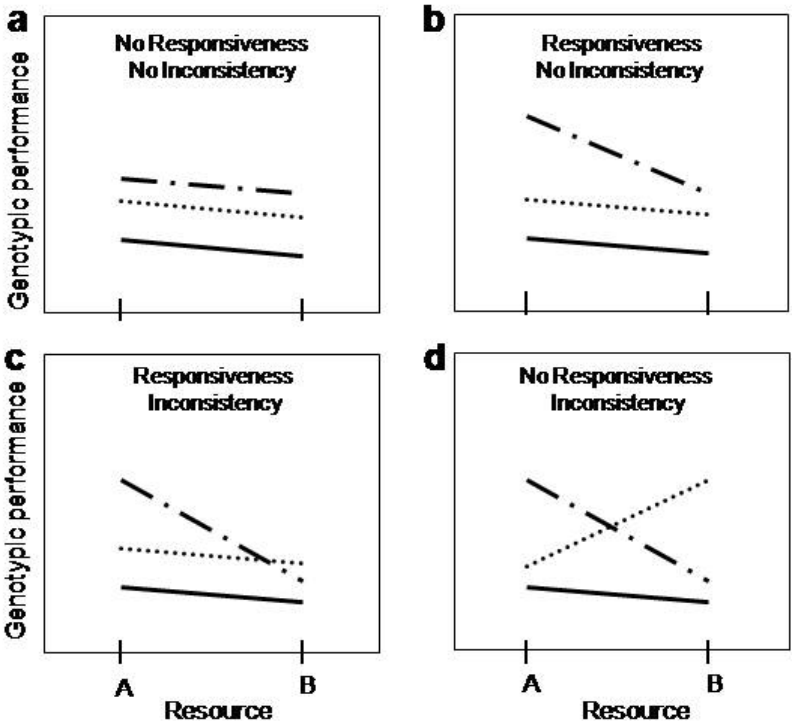
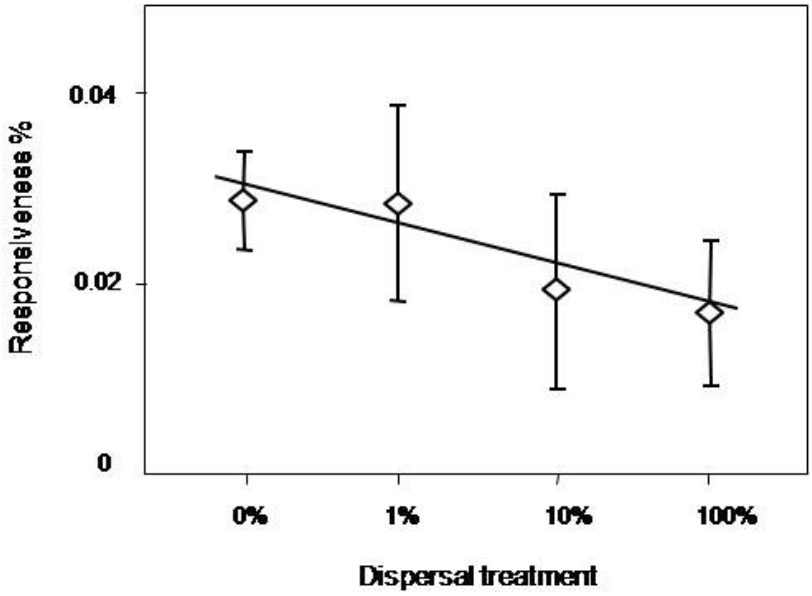


Figure Box 1: Each dashed line represents the reaction norm over resources A and B of two genotypes that have evolved in A and B respectively from the same isogenic population (solid line). In **a**) the evolved genotypes have parallel reaction norms and there is no *GxE* interaction. The environmental variances of two genotypes are equal (no *responsiveness*) and reaction norms are parallel (no *inconsistency*). In **b**) genotypes do not respond equally to different environments, they have different environmental variances (*responsiveness*) but their responses are highly correlated (no *inconsistency*). In both cases regional functional diversity is low because genotypes are regionally redundant in their resource utilization. In **c**) the reaction norms intersect

(*inconsistency*) and environmental variances differ (*responsiveness*). In this case, *responsiveness* and *inconsistency* account for the interaction component of total phenotypic variance. Finally in **d**) the ranking of genotypes changes over resources (*inconsistency*), but in this case they have the same environmental variation (no *responsiveness*). In **c**) and **d**) regional functional diversity is high because genotypes exploit different resources.

We present in the figure various possible outcomes of selection in the simplest metacommunity, constituted of two wells (with different resources) and no dispersal. Depending on the reaction norms obtained after evolution, one can differentiate between situations with no responsiveness and/or no inconsistency, leading to contrasted ecological interpretations.



Supplementary figure: Responsiveness proportion of total variance: Plotted points show the mean responsiveness (+/- s.e.m; n=3) expressed as a percentage of total phenotypic variance for each dispersal treatment (linear test: $F_{1,10} = 4.75$, $P = 0.054$, $r^2 = 0.32$).

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Manuscrit 2

Dispersal scales up the biodiversity-productivity relationship in an experimental source-sink metacommunity

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Résumé en Français

La théorie des métacommunautés prédit une variation dans le patron de la relation entre diversité et fonctionnement selon l'échelle spatiale considérée comme conséquence des variations dans les mécanismes de coexistence. Pourtant, l'étude de la relation entre diversité biologique et le fonctionnement des écosystèmes (relation BDEF) s'est souvent effectuée sans prendre en compte les mécanismes qui expliquent la diversité. A l'échelle locale, la dispersion peut maintenir une forte diversité via l'immigration (*mass-effect*). Cette diversité, maintenue par la dispersion ne favorise pas les processus écosystémiques et on s'attend à une relation BDEF nulle ou négative. A l'échelle régionale, la coexistence qui résulte d'une partition des niches (*species sorting*) entraîne une complémentarité qui entraîne une relation BDEF positive. La dispersion peut favoriser la diversification en apportant la variabilité nécessaire pour l'adaptation aux différentes conditions environnementales et favoriser l'émergence d'une relation positive entre diversité et fonctionnement.

Cet article étudie expérimentalement l'influence de la dispersion sur la diversité locale ainsi que l'importance de l'échelle spatiale sur la relation entre diversité et productivité avec la bactérie *Pseudomonas fluorescens*. Après ~500 générations d'évolution en milieu spatialement hétérogène la diversité locale est maximale pour des niveaux de dispersion intermédiaires et la fréquence de génotypes mal adaptés

augmente linéairement avec la dispersion, ce qui indique la présence d'un « *mass-effect* ». A l'échelle locale, l'augmentation de la diversité ne produit aucun effet sur la productivité. Par contre à l'échelle régionale l'augmentation de la diversité génère une plus forte productivité qui s'explique par une différenciation écologique des génotypes.

Malgré sa simplicité, ce travail illustre comment l'étude de la relation entre diversité et fonctionnement ne peut s'effectuer sans prendre en considération l'échelle spatiale à laquelle s'expriment les différents mécanismes de coexistence. Ces derniers vont déterminer le type d'effet que la diversité peut avoir sur le fonctionnement des écosystèmes. Ces résultats peuvent servir à expliquer les fortes variations observées dans les études sur le patron de la relation entre diversité et fonctionnement.

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Dispersal scales up the biodiversity-productivity relationship in an experimental source-sink metacommunity

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Abstract

The influence of biodiversity on ecosystem functioning is a major concern of ecological research. However, the biodiversity-ecosystem functioning relationship has very often been studied independently from the mechanisms allowing coexistence. By considering the effects of dispersal and niche partitioning on diversity, the metacommunity perspective predicts a spatial scale-dependence of the shape of the relationship. Here we present experimental evidence of such scale-dependent patterns. After ~500 generations of diversification in a spatially heterogeneous environment we measured functional diversity and productivity at both local and regional scales in experimental source-sink metacommunities of the bacterium *Pseudomonas fluorescens* SBW25. At the regional scale, environmental heterogeneity yielded high levels of functional diversity and we observed a positive correlation between diversity and productivity. At the local scale, dispersal increased local functional diversity through a mass-effect but we failed to observe any correlation between diversity and productivity. These experimental results underline the importance of considering the mechanisms maintaining biodiversity and the appropriate spatial scales in understanding its relationship with ecosystem functioning.

Keywords: biodiversity-ecosystem functioning relationship, spatial scale-dependence, source-sink metacommunity, mass-effect, dispersal, productivity.

1. INTRODUCTION

Under the current decline of biodiversity, understanding its influence on ecosystem properties such as productivity and stability has become a central issue in ecology (Loreau *et al.* 2001; Hooper *et al.* 2005). To this aim, ecologists have developed theoretical approaches and performed massive field and controlled laboratory experiments (Hooper *et al.* 2005; Balvanera *et al.* 2006). Such studies have revealed high variability in the shape of the biodiversity-ecosystem functioning (BEF) relationship. Some support a positive effect of biodiversity (i.e. positive BEF relationship) while others present evidence of negative and null patterns (Schwartz *et al.* 2000; Hooper *et al.* 2005; Srivastava & Vellend 2005; Jiang *et al.* 2008). Consequently, and despite the large body of work, controversy remains on the influence of biodiversity in ecosystem function (Srivastava & Vellend 2005; Smith 2007).

Previous studies have revealed how numerous factors such as the ecosystem type, the ecosystem function or type of diversity measured and the history of community assembly may influence the shape of the BEF relationship, making difficult to obtain a general pattern (Cardinale *et al.* 2000; Fukami & Morin 2003; Petchey *et al.* 2004; Van Ruijven & Berendse 2005; Balvanera *et al.* 2006; Smith 2007; Replansky & Bell 2009). Some studies have also pointed out a spatial scale-dependence in the shape of the BEF relationship (Bond & Chase 2002; Chase & Leibold 2002; Mouquet & Loreau 2003; Cardinale *et al.* 2004; Harrison *et al.* 2006), based on the multi-factorial nature of the mechanisms responsible for species coexistence (Mouquet *et al.* 2002; Cardinale *et al.* 2004). More specifically, the combined influence of local and regional mechanisms maintaining species diversity may alter the characteristics of communities and generate different *BEF* patterns at different spatial scales (Bond & Chase 2002).

Metacommunity ecology provides a general framework linking local and regional determinants of diversity maintenance and helps understanding some aspects of the scale-dependence of the *BEF* relationship (Mouquet & Loreau 2003; Leibold *et al.* 2004). It is now well established that diversity results from the interaction between local and regional processes (Ricklefs 1987; Hubbell 2001; Leibold *et al.* 2004) and communities are represented as embedded within complex interactive networks connected by

dispersal (Leibold *et al.* 2004). In a spatially heterogeneous environment, the source-sink perspective predicts that dispersal may increase local diversity through mass-effects (Shmida & Wilson 1985; Loreau & Mouquet 1999). This is because sustained immigration from areas of high success (i.e. sources) may prevent the exclusion or extinction from unfavorable areas (i.e. sinks), allowing the establishment of species at sites where they cannot be self-maintaining without dispersal (Amarasekare & Nisbet 2001; Mouquet & Loreau 2002). This results in an increase in local diversity relative to what would be expected in a closed system. Too much dispersal, however, may be detrimental to diversity as the metacommunity becomes increasingly homogenized, reducing both local and regional diversity.

In a source-sink metacommunity, different mechanisms of diversity maintenance are expressed, so it is likely that the shape of the BEF relationship will depend on the spatial scale considered (i.e. local or regional). Regional coexistence is based on niche differentiation while local coexistence results from constant immigration (Mouquet & Loreau 2002). Since niche complementarity leads to a positive BEF relationship (Tilman et al. 1997; Loreau 1998), it is likely that a positive effect of biodiversity on ecosystem function will be found at the regional scale (Venail et al. 2008). At the local scale, diversity will strongly depend on (i) immigration rate and (ii) the diversity of immigrants, which reflects diversity at the regional scale. There is no *a priori* reason for thinking that immigrants will increase functional complementarity at the local scale, and a null or even a negative BEF relationship may arise when the proportion of the most productive species declines with increasing immigration (Loreau & Mouquet 1999; Mouquet & Loreau 2003). The scarce experiments conducted in source-sink systems have revealed a positive effect of diversity on productivity at both local (Matthiessen & Hillebrand 2006) and regional scales (Venail *et al.* 2008). We are unaware of any experimental study simultaneously addressing the BEF relationship at both local and regional scales.

To explicitly test the hypothesis that the shape of the BEF relationship varies at different spatial scales, we experimentally measured functional diversity and productivity at both local and regional scales in a bacterial source-sink metacommunity. Bacteria are haploid and asexual organisms so all the measured genetic diversity

evolved during the experiment and each emerging genotype can be functionally considered as an ecological "species". In a previous experiment (Venail *et al.* 2008) we allowed a single clone of the bacterium *Pseudomonas fluorescens* SBW25 to naturally diversify in a spatially heterogeneous environment under four different levels of dispersal. After ~500 generations of adaptive radiation we observed a positive relationship between regional functional diversity and productivity, with maximal values at intermediate levels of dispersal. In the present study, we measured the realized functional diversity and productivity at the local (i.e. community) and regional (i.e. metacommunity) scales. At a local scale, our results support the action of a mass-effect, resulting in a gradient of local diversity but with no consequences in terms of local productivity. On the contrary, the BEF relationship was positive at the regional scale as the result of regional niche differentiation. These results represent the first empirical evidence of the predicted scale-dependence in the diversity-productivity relationship by explicitly including dispersal and spatial heterogeneity as the main mechanisms of coexistence.

2. MATERIAL AND METHODS

(a) Selection experiment

A single clone of the bacterium *P. fluorescens* SBW25 was allowed to diversify for ~500 generations (40 days) over a highly heterogeneous environment (Biolog GN2® microplates) by controlling the amount of dispersal among patches (Venail *et al.* 2008). The Biolog system represents a highly structured environment composed of 95 wells, each containing a different carbon substrate (i.e. patch) belonging to 11 different chemical families plus one blank control well. Each well also contains a dye (tetrazolium violet) which turns violet as the extant carbon substrate is metabolized. The strain we employed had previously evolved for ~900 generations in a complex environment of 8 carbon sources (Barrett *et al.* 2005). Each of 3 replicates of this unique 'ancestral' strain was used to inoculate four independent Biolog microplates. And submitted to 4 different dispersal levels among the patches: 0%, 1%, 10% and 100% dispersal (i.e. 3 replicates x 4 dispersal treatments = 12 Biolog microplates). We transferred the bacteria to new Biolog GN2 microplates every 24 hours to renew the available nutrients in each patch during a selection period of 40 days. This transfer technique (batch

culture) enhances bacterial growth rates and allowed the control of dispersal between patches. After 24h of incubation in the dark at 28°C, and prior to each transfer, we scored light absorbance at 590 nm for each patch using a FLUOstar Optima spectrophotometer (BMG®). Absorbance is related to the metabolic capacities of bacteria and was used as a proxy of productivity. We allowed dispersal of bacteria between patches in each microplate such that 0%, 1%, 10%, or 100% of the culture used to inoculate each patch of a new microplate came from a pool of immigrants derived from a mixture of all the 95 patches. At the end, a new recipient Biolog was inoculated with 1µl of the content of a donor Biolog by using a pin replicator (Boekel 96 pin/well model #140500). After the forty days of selection, we transferred 40 µl of the content of each well of the Biolog microplates to new empty microplates and added 60 µl of 50:50 glycerol/M9 salts solution before storing them at -80°C.

(b) Phenotypic assays

A phenotypic assay consisted of measuring the performances of individual genotypes over the 95 carbon substrates of the Biolog system. We randomly sampled five genotypes from 10 evolved communities (i.e. wells containing a single carbon source) of the 95 available in the Biolog system after streaking out the content of the well in KB agar Petri dishes (120 communities in total: 10 wells x 4 dispersal levels x 3 replicates). For logistic reasons we were not able to include more communities in our analysis. We assayed a total of 600 evolved genotypes in 8 blocks (i.e. days; 75 genotypes per day) using a randomized block design. Each genotype was first amplified in 1 ml of M9KB media for 24h at 28°C under constant orbital shaking (200 r.p.m) and then stored at -80°C in 30% glycerol solution. For each genotype, one ml of culture was unfrozen, centrifuged (3 min at 8000 r.p.m) and then washed by eliminating the liquid media and adding 1ml of M9 minimal salts. 125 µl of this solution was diluted into 25 ml of M9 minimal salts and starved for two hours at 28°C before inoculation into the Biolog microplates (140µl). After 24h of incubation in the dark at 28°C in humid chambers we scored absorbance at 590 nm for each genotype in each carbon source. As a control for any 'block-effect' we also assayed 5 replicates of the ancestral clone each day (same protocol). Measured absorbances were corrected by the blank well (with no

carbon source, but with tetrazolium violet). Corrected absorbance on each well measures genotypic catabolic performance.

(c) Statistical analysis

Before any analysis on the effect of dispersal on diversity, we tested for an eventual 'block effect' in absorbance measurements made on different days by comparing the eight sessions of the five replicated assays of the ancestral strain.

In order to quantify the functional diversity within each evolved population, we partitioned the total phenotypic variance into genotype, environment and genotype-by-environment interaction components (Bell 1990). This partitioning technique requires a complete genotype by environment matrix (i.e. common garden experiment). The interaction component (*GxE*) indicates the occurrence of genotypes adapted to different substrates (i.e. niche diversification). The interaction was further decomposed into *inconsistency* and *responsiveness* (Bell 1990; Barrett *et al.* 2005; Venail *et al.* 2008). *Inconsistency* (*I*) indicates the lack of correlation among genotypes over environments:

$$I = \sum \frac{\sigma_{Ei}\ \sigma_{Ej}\left(1 - \rho_{EiEj}\right)}{G(G-1)}$$

with G being the number of genotypes tested, σ_{Ei} and σ_{Ej} the standard deviations of environmental responses for each genotype, and ρ_{EiEj} the environmental correlation among each pair of genotypes tested. The lack of correlation implies reaction norms are negatively correlated, revealing niche complementarity among genotypes. We used the *inconsistency* proportion of total phenotypic variance in performances over the 95 carbon substrates as a measure of functional diversity (*FD*). We estimated local *FD* by calculating *inconsistency* using the corrected absorbance of the five randomly selected genotypes from each community. We measured regional *FD* by including in the calculation of *inconsistency* the fifty genotypes belonging to the same metacommunity (10 communities x 5 genotypes).

We discriminated among 'source' and 'sink' genotypes by using their corrected absorbance in the carbon substrate from which they were sampled. Positive corrected absorbances represent genotypes able to grow in the environment from which they

were isolated and were considered as 'sources'. On the contrary, a negative corrected absorbance means the genotype cannot exploit the substrate from which they were sampled. We calculated the proportion of source and sink genotypes for each community.

As a measure of local productivity we employed the mean absorbance over the three last transfers from our previous data set (Venail et al. 2008). When calculating regional productivity we averaged local productivity over the 54 "informative" carbon substrates after 41 were excluded because repeated measurements of the ancestor were statistically unreliable (coefficient of variation >1).

We used a two factor mixed effects ANOVA to test for dispersal (fixed) and community (random) effects on local *FD*. We used a one-way ANOVA to test for an effect of dispersal on regional *FD* and also to compare the proportion of sink genotypes (after angular transformation) among dispersal treatments. We fitted quadratic models to the dispersal-diversity relationships, a linear model for the effect of dispersal on the proportion of sink genotypes, and linear and log-linear models to the *FD*-productivity relationship. We used R and JMP5 software for statistical analyses.

3. RESULTS

(a) *No block effect on assays*

We did not observe any "block-effect" on the replicated absorbance measurements of the ancestral strain ($F_{7,39}$ = 0.7, P = 0.67), allowing all the assayed genotypes (n = 600) to be pooled for a global analysis of the effect of dispersal on diversity.

(b) *Regional diversity peaks at intermediate dispersal rates*

Dispersal significantly affected the amount of regional functional diversity (*FD*) after ~500 generations of diversification in our source-sink metacommunities. (r^2 = 0.65, $F_{3,8}$ = 4.987, P = 0.0308). Regional *FD* was maximal for intermediate levels of dispersal (Fig. 1) as revealed by quadratic regression ($F_{2,9}$ = 8.15, P = 0.0096, quadratic

parameter t = -3.96, P = 0.0033). These results confirm a previous study (Venail *et al.* 2008) where we employed a different sampling technique of genotypes for functional diversity calculation.

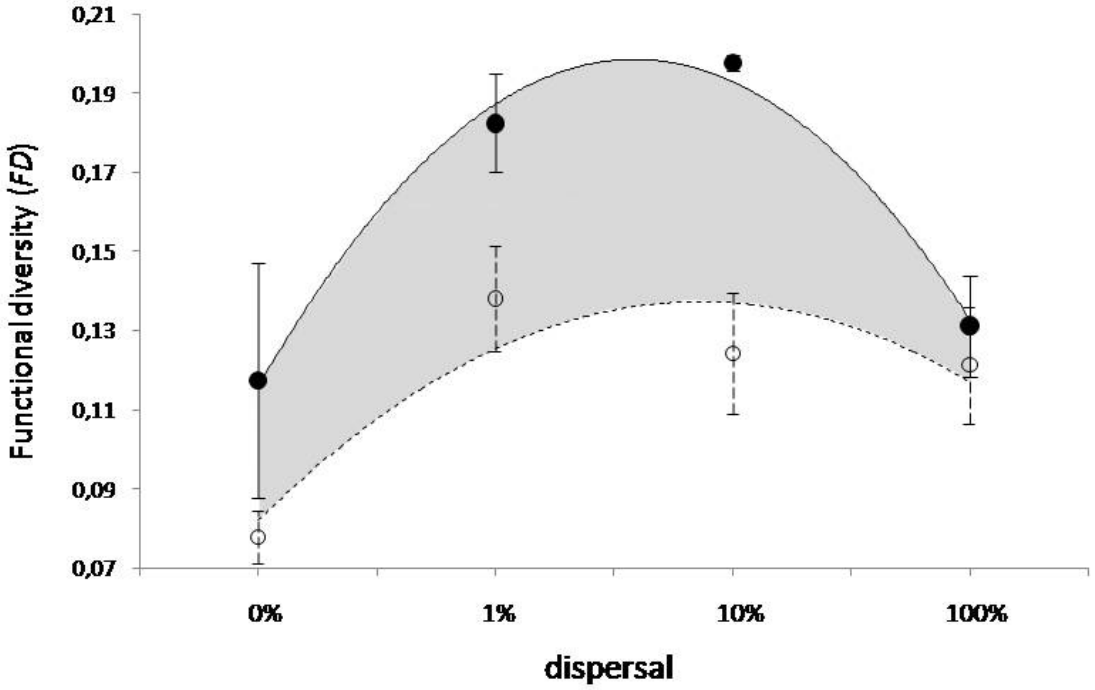


Figure 1: Effect of dispersal rate on regional and local functional diversity (*FD*) after ~500 generations of diversification. Both local diversity (empty circles) and regional diversity (filled circles) peaked for intermediate dispersal levels (local quadratic regressions: $F_{2,37}$ = 4.8479, P = 0.0135, quadratic parameter t = -2.40, P = 0.0213 and $F_{2,9}$ = 8.15, P = 0.0096, regional quadratic parameter t = -3.96, P = 0.0033 respectively). For regional *FD*, each point represents the averaged diversity and standard error bars over three independent replicates, each containing fifty genotypes. For local *FD*, each point represents the averaged diversity and standard error bars over the ten assayed communities, each containing five genotypes. Lines represent quadratic model fits.

(c) *Local diversity peaks at intermediate dispersal rates*

Both dispersal and community had an effect on local *FD* (Table 1). Local *FD* peaked at intermediate dispersal rates (Fig. 1) as revealed by quadratic regression ($F_{2,37}$ = 4.8479, P = 0.0135, quadratic parameter t = -2.40, P = 0.0213). At 100% dispersal, local and regional *FD* converged (Fig. 1; t-test: t = -0.590, P = 0.5876).

(d) Dispersal limits local adaptation

The proportion of 'sink' genotypes linearly increased with dispersal (Fig. 2, r^2 = 0.76, $F_{1,10}$ = 32.47, P = 0.0002) starting at 0.127 (+/- 0.0067) for 0% dispersal and increasing up to 0.48 (+/- 0.023) for 100% dispersal; thus, dispersal prevented local adaptation in our experimental system.

(*e) The BEF relationship is scale-dependent*

The observed gradient of diversity created by dispersal had different effects on productivity depending on the spatial scale considered (Fig. 3). At the local scale, *FD* had no effect on productivity (Fig. 3a, r^2 = 0.0078, $F_{1,38}$ = 0.2983, P = 0.5881), whereas at the regional scale *FD* positively affected productivity (Fig. 3b, linear fit : r^2 = 0.3369, $F_{1,10}$ = 5.08, P = 0.0479 ; log-linear fit : r^2 = 0.3628, $F_{1,10}$ = 5.6947, P = 0.0381).

Table 1: Results of a two factor mixed effects ANOVA with dispersal and community as fixed and random effects respectively.

Effect	D.F.	M.S.	F	p-value
Dispersal	3	0.02047	5,6376	**< 0.001**
Community	9	0.00931	4,2456	**0.002**
Dispersal x community	27	0.003631	1,6563	**0.0438**
Residual	80	0.002193		

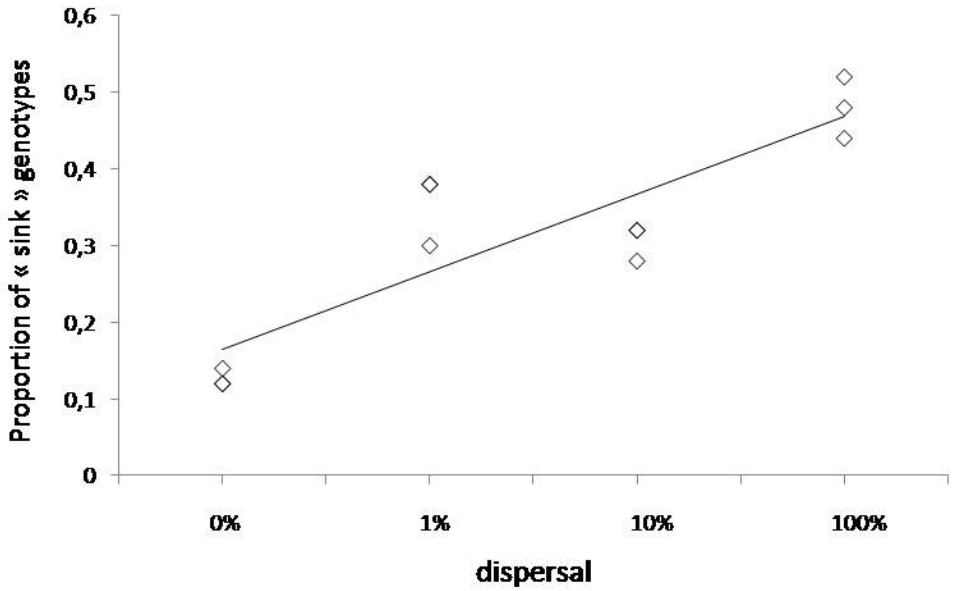


Figure 2: Effect of dispersal rate on mean proportion of sink genotypes (as defined in the Methods) after ~500 generations of evolution in highly heterogeneous environments. The mean proportion of sink genotypes linearly increased with dispersal (r^2 = 0.76, $F_{1,10}$ = 32.47, P = 0.0002). Mean proportions are: 0.127 (+/- 0.0067), 0.353 (+/- 0.0267); 0.307(+/- 0.013) and 0.48 (+/- 0.023) for 0%, 1%, 10% and 100% dispersal, respectively. Each dot represents an independent replicate for the same level of dispersal and is a mean value over ten independent populations.

4. DISCUSSION

We initiated our experiment with a single clone of *P. fluorescens* SBW25, allowing emergent communities to naturally assemble by only controlling the amount of dispersal between them, so all the observed diversity exclusively emerged by mutation during the experiment. Migration has contrasting effects on local specialization and diversity. On the one hand, the increase in mutation supply rate provided by immigration increases the rate of adaptation to poor environments, resulting in an increase in productivity in patches that support poor growth of the ancestral clone. At higher dispersal, however, the massive arrival of maladapted mutants was detrimental for local adaptation (i.e. migration load), and the composition of the entire metacommunity becomes homogenized, resulting in a decrease of diversity and productivity (Venail *et al.* 2008).

(a) *Evidence for source-sink dynamics*

The consequences of dispersal on species coexistence have previously been tested (Cadotte 2006b). However, the predicted positive effect of dispersal on local diversity has only been well documented under homogeneous environmental conditions (Kneitel & Miller 2003; Cadotte 2006b; Matthiessen & Hillebrand 2006). Empirical evidence of true source-sink dynamics (i.e. a combination of mass-effects and regional species sorting) is still scarce (Forbes & Chase 2002; Cottenie *et al.* 2003). Here we present clear evidence of source-sink dynamics in experimental metacommunities based on combined actions of regional environmental heterogeneity, species sorting and dispersal as mechanisms for species coexistence.

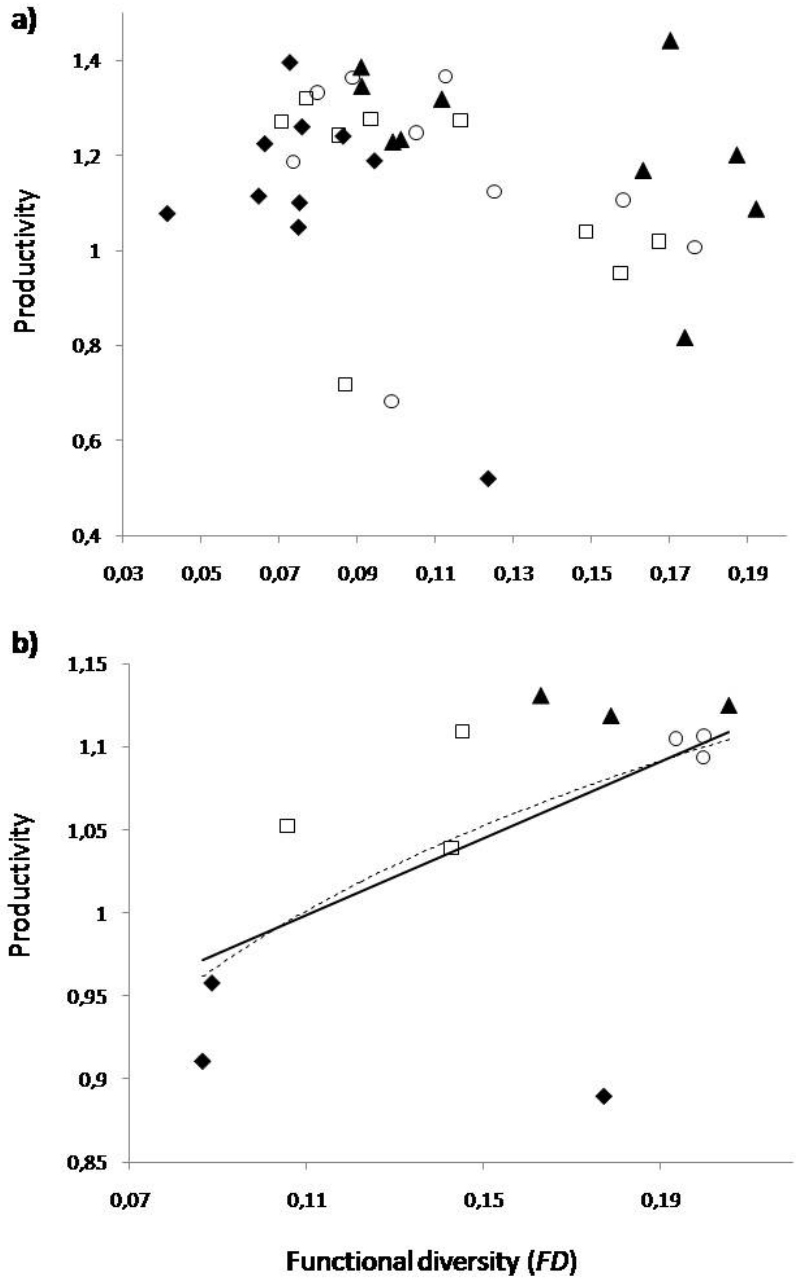


Figure 3: Functional diversity and productivity relationship at local (**a**) and regional (**b**) scales. Local functional diversity (*FD*) and local productivity are not related (r^2 = 0.0078, $F_{1,38}$ = 0.2983, P = 0.5881). Regional functional diversity (*FD*) and productivity are positively related (linear fit: r^2 = 0.3369, $F_{1,10}$ = 5.08, P = 0.0479 ; log-linear fit : r^2 = 0.3628, $F_{1,10}$ = 5.6947, P = 0.0381). At the local scale each point represents the mean community values over three replicates. At the regional scale points represent average metacommunity values over three replicates. Filled diamonds, filled triangles, empty circles and empty squares represent 0%, 1%, 10% and 100% dispersal, respectively. The continuous line represents a linear fit and the dashed line represents log-linear fit.

Local diversity was shown to be highly correlated with regional diversity, exhibiting the same hump-shaped pattern as a function of dispersal. Given that local communities experienced single homogeneous environments (i.e. only one carbon source per well), no local niche partitioning was expected. It is therefore likely that the hump-shaped pattern observed at this scale was due to immigration from the regional pool. This is confirmed by the increase in the proportion of 'sink' genotypes with dispersal, showing that continuous dispersal from 'sources' maintained genotypes not adapted to local conditions. At higher dispersal, local and regional diversity converged, confirming the homogenization pattern predicted from source-sink theory (Mouquet & Loreau 2003).

Our results differ from purely ecological models of source-sink metacommunities (Mouquet & Loreau 2002, 2003), which predict maximal regional diversity in closed communities at equilibrium. In these models, higher regional diversity is based on higher differentiation among communities (i.e. beta diversity). Our experiment addressed the emergence of diversity by starting with a single clone and by measuring diversity after ~500 generations of evolution. It is probable that our communities were not at evolutionary equilibrium (i.e. complete regional niche differentiation). Differentiation among closed communities is slower compared to open ones, and thus we suggest that the hump-shaped dispersal-regional diversity pattern observed may be evolutionarily transient. Despite this transient pattern, we found evidence for 'ecological' source-sink dynamics. This illustrates that source-sink dynamics can also result in more complex patterns for the relationship between local (i.e. alpha) and regional (i.e. gamma) diversity than expected from equilibrium ecological models.

Predictions of the relationship between alpha, beta and gamma diversity exist for alternative metacommunity models such as the neutral model (Economo & Keitt 2008). The mechanisms generating the patterns are, however, fundamentally different. In neutral metacommunities, the differentiation between communities observed without dispersal is a consequence of the action of both local and regional stochastic effects (i.e. drift). As dispersal increases, the communities converge because gamma diversity decreases and alpha diversity increases, due to mass-effect (Economo & Keitt 2008). Our experimental results, while sharing some of the tendencies found for neutral metacommunities, differ from this perspective because we found clear evidence of local

specialization and niche differentiation at the regional scale (Venail *et al.* 2008), as well as the presence of non-adapted genotypes increased with dispersal. In the future, it would be interesting to compare our results with experiments performed with purely neutral metacommunities based on the same experimental design but with a homogeneous environment at the regional scale (e.g. a single carbon source in every well).

(b) *Scaling up the BEF relationship*

We addressed the BEF relationship at both regional and local scales. If the mechanisms determining species assemblages vary with spatial scale, it is likely that different BEF relationships will be obtained for each scale considered (Bond & Chase 2002; Mouquet & Loreau 2003; Cardinale *et al.* 2004; Gross & Cardinale 2007). Our results strongly support this prediction. In our experimental metacommunities, local coexistence is based on mass-effects while regional coexistence is based on niche differentiation (i.e. specialization on different resources). As expected, we found a positive BEF relationship only at the regional scale where niche differentiation occurs. Under the complementarity hypothesis (Tilman et al. 1997; Loreau 1998), multiple resources are required for a positive biodiversity-productivity relationship, and the more potential niches available the more the positive effects of diversity on productivity should be expressed (Cardinale *et al.* 2000; Weis *et al.* 2008; Wacker *et al.* 2009; Replansky & Bell 2009). In our experiment, it is at the regional scale that most diversity has evolved and that niches have differentiated. Thus, even if by increasing diversity dispersal shifted the effects of regional habitat heterogeneity to the local scale, this did not result in a positive relationship between diversity and productivity.

The source sink metacommunity model developed by Mouquet and Loreau (2003) predicts a positive BEF relationship at the regional scale, while a more complex pattern may emerge at the local scale with both positive and negative relationships. In their model, maximal local productivity is expected at equilibrium for closed communities when species optimally fit environmental conditions. The arrival of poor competitors through the mass-effect hinders local productivity resulting in a negative BEF relationship. Our results confirms a previous experiment (Mouquet *et al.* 2004)

showing that even if the mass-effect can be strong enough to increase local species diversity, it will not necessarily result in the expected decrease of local productivity.

Other scale dependent relationships have been proposed between species richness and productivity (Loreau *et al.* 2001; Schmid 2002; Chase & Leibold 2002). For instance, it has been hypothesized that by considering feedbacks between productivity (e.g. fertility) and biodiversity, some counter-intuitive scaling relationships might emerge (Waide *et al.* 1999; Chase & Leibold 2002; Gross & Cardinale 2007). At the local scale, diversity ought to be maximal for intermediate productivity, while diversity and productivity are positively linked at larger scales (Dodson *et al.* 2000; Chase & Leibold 2002; Chase & Ryberg 2004). Even if these empirical results consider another definition of productivity (i.e. closer to fertility), they also suggests that the BEF relationship cannot be understood without considering the appropriate scale for the mechanism controlling biodiversity and productivity. In the future, it would be interesting to investigate how all these different scale dependent relationships interact to shape the realized BEF relationship observed in the field.

The experiment presented in this paper is obviously an oversimplification of real environments. Controlled experimentation was central however in disentangling the combined actions of regional species sorting and mass-effect in shaping the BEF relationship. Moreover, we have considered only one aspect of the BEF relationship (i.e. productivity-diversity), while many other ecosystem functions could be associated with species diversity (Hooper *et al.* 2005). For example, the 'insurance hypothesis' proposes that species which are functionally redundant for an ecosystem process might show some kind of complementarity in temporally fluctuating environments, increasing long-term community productivity and stability (Yachi & Loreau 1999). It is thus likely that the local diversity maintained in our experiment through the mass-effect could buffer local communities from environmental fluctuations. This could lead to a positive relationship between local diversity and mean temporal productivity, such as suggested in the spatial and temporal insurance hypothesis (Yachi & Loreau 1999).

Two decades of intense work have revealed that there is no single pattern linking biodiversity to ecosystem functioning. Our study supports the spatial scale-dependence

of the BEF relationship in source-sink metacommunities by manipulating the mechanisms of species coexistence and allowing communities to assemble through natural ecological and evolutionary processes. Spatial heterogeneity generated regional diversification while dispersal produced a hump-shaped pattern of functional diversity at both local and regional scales. We found that the relationship between biodiversity and productivity was null at the local scale and positive at the regional scale, where complementarity among species occurred. More complex experiments combining other factors controlling species diversity over short and large scales are now needed. For instance, understanding diversity organization also requires encompassing different temporal scales (Johnson & Stinchcombe 2007; Fussman *et al.* 2007; Urban *et al.* 2008). Our experiment suggests a transient evolutionary state for the relationship between regional and local species richness. We expect interesting results to emerge from experiments combining longer ecological and evolutionary time scales.

Acknowledgements

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Manuscrit 3

Diversification in temporally fluctuating environments: effect of the environmental grain in experimental bacterial populations.

P. A. Venail, O. Kaltz, I. Olivieri, T. Pommier & N. Mouquet.

En préparation

Résumé en Français

L'hétérogénéité environnementale a souvent été évoquée comme un facteur déterminant dans la diversification au niveau génétique et au niveau spécifique. Les approches théoriques ont bien défini les conditions pour un tel scénario et soulignent la nécessité d'un compromis évolutif fort. De même, ils suggèrent que l'hétérogénéité spatiale entrainerait plus facilement la diversification qu'une hétérogénéité temporelle car cette dernière agirait plutôt en faveur de l'émergence de stratégies généralistes. Dans un environnement temporellement fluctuant, on peut s'attendre à ce que seuls des grains intermédiaires et en présence d'un compromis évolutif fort soient en mesure de favoriser la diversité. Le grain représente le rang de variabilité environnemental relatif à la durée de vie (variabilité temporelle) et/ou la zone de distribution (variabilité spatiale) d'un organisme donné.

Dans cette étude nous avons testé expérimentalement le rôle du grain environnemental dans l'émergence et le maintien de la diversité avec la bactérie *Pseudomonas fluorescens* SBW25. Un clone unique de départ à été exposé pendant ~300 générations à un environnement temporellement fluctuant. N'ayant pas d'à priori sur la forme du compromis évolutif entre les performances sur les différents milieux, nous avons utilisé 20 paires de sources de carbone différentes comme milieux de

sélection. Nous espérions ainsi inclure une gamme assez large de formes de compromis évolutifs pour générer un résultat général sur le rôle du grain.

Nous avons trouvé une diversité génétique plus importante dans un environnement spatialement hétérogène que dans un environnement temporellement fluctuant. De plus, dans les environnements temporellement fluctuants, la plus forte diversité correspondait au traitement de grain intermédiaire. L'étude approfondie de l'interaction génotype x environnement et des corrélations génotypiques nous ont montrées que la spécialisation écologique réciproque dans un environnement temporellement fluctuant est rare. Seul 20% des corrélations génotypiques étaient négatives pour un grain intermédiaire. Même dans un environnement spatialement hétérogène, les cas de spécialisation n' 'étaient pas nombreux (30%). Ceci suggère une basse fréquence de compromis évolutifs forts, lesquels sont nécessaires pour la spécialisation réciproque. Il est aussi probable que la durée de la période de sélection ne fut suffisante pour permettre la spécialisation. La diversité observée résulterait d'un polymorphisme transitoire résultant d'un équilibre entre mutation et sélection.

Ces résultats confirment le rôle de l'hétérogénéité temporelle comme scénario pour la diversification et soulignent l'importance du grain pour sa mise en place. Cet article ouvre des pistes de recherche pour d'autres études sur la diversification en milieu temporellement fluctuant. Par exemple sur l'importance du patron de fluctuations : aléatoire vs. périodique dans le processus de diversification.

In preparation

Diversification in temporally fluctuating environments: effect of the environmental grain in experimental bacterial populations.

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Abstract

Environmental fluctuations have been proposed as an explanation for both genetic and species diversity. Theory of selection clearly established the necessary conditions for the evolution and maintenance of diversity in temporally heterogeneous environments but the empirical evidence remains scarce and controversial. One possible explanation for this is the difficulty of designing experiments including the relevant range of temporal grains and employing environments with the appropriate trade-off functions. Here we experimentally explore, after ~300 generations of evolution, the impact of the environmental grain on the diversification of the bacterium *Pseudomonas fluorescens* in a temporally fluctuating environment by including 20 different pairs of resources. In conformity with theory, our results present clear evidence of higher levels of genotypic diversity at an intermediate environmental grain in temporally fluctuating environments. This general pattern resulted in part from the enhanced capacity of disruptive selection to generate more negative genotypic correlations for fitness. However, the evolution of reciprocal specialization was an uncommon outcome. Alternatively, some of this polymorphism may be maintained by the incapacity of selection to eliminate non-adapted genotypes at intermediate fluctuation frequencies. Our findings suggest that provided the proper conditions, temporal heterogeneity can play a major role in the evolution and maintenance of diversity.

Key-words: *Temporal heterogeneity, experimental evolution, Pseudomonas fluorescens, diversity, specialization, genotype by environment interaction.*

1. INTRODUCTION

Environmental heterogeneity in space or in time can be a major determinant of species diversity within communities (Hutchinson 1961; MacArthur & Levins 1964; Levins 1968; Chesson 2000a) and of genetic variation within species (Levene 1953; Dempster 1955; Hedrick 1986). It is generally thought that diversity can be generated and maintained by divergent selection in contrasting environments, leading to ecological specialization and concomitant emergence of genotype-by-environment fitness interactions (Felsenstein 1976, Bell 1990).

Levins (1968) showed that stable diversification in heterogeneous environments requires strong evolutionary constraints (trade-offs) on the traits involved in adaptation, such that specialist types perform well in their own, but poorly in foreign environments and that intermediate types (generalist) have a lower overall mean fitness than the specialists (concave fitness sets, *sensu* Levins 1968). Diversification further depends on the environmental grain, that is, on the temporal and spatial scales of environmental heterogeneity relative to the 'home range' of an individual (Levins 1968). A fine grain means that the individual encounters more than one environmental condition during its lifetime. A coarse grain means that individuals spend their entire life-time, or even multiple generations, in the same environment. Thus, specialists may evolve under a relatively coarse environmental grain; otherwise, a generalist strategy may be favored. Strong trade-offs and coarse grain environments are both necessary conditions for specialization (Levins 1968).

Under such conditions, spatial heterogeneity may be more efficient in maintaining genetic diversity than temporal heterogeneity. Classical population genetics models show that conditions for protected polymorphism in temporally fluctuating environments are more restricted than in spatially heterogeneous environments (Dempster 1955; Haldane 1963; Gillespie 1972; Hedrick 1974, 1986; Felsenstein 1976; Dean 2005). This is because spatial heterogeneity provides refuges for different

specialized types allowing their coexistence, whereas temporal variability regularly exposes all types to all environments, thus favoring the fixation of the single generalist genotype (or species) with the highest geometric mean fitness across all environmental conditions (Levins 1968, Wilson & Yoshimura 1994). As mentioned above, the scale of the temporal grain may be particularly important. One may expect that very slow and coarse-grained fluctuations lead to the sequential evolution and fixation of specialists, adapted to the prevailing environment, whereas very fast and fine-grained fluctuations may select for generalists, maximizing fitness through phenotypic plasticity rather than specialization. This suggests that intermediate temporal grains allow a maximum of genetic diversity.

Still relatively few experiments have tested the effect of temporal environmental variation on the evolution and maintenance of genetic/species diversity (reviewed in Kassen 2002). Consistent with general predictions, temporal fluctuations in nutrient or resource supply facilitate competitive coexistence in bacterial or phytoplankton model systems, as compared to single constant environments (Kassen 2002; Flöder et al 2002; Suiter et al 2003, Descamps-Julien & Gonzalez 2005). However, experimental manipulation of the temporal grain of fluctuations has produced equivocal results. For example, in an environment cycling between high and low nutrient supplies, intermediate cycle length facilitated coexistence of a wild-type strain and a metabolic mutant strain of the bacterium *Escherichia coli*, whereas finer or coarser grains lead to the predominance of one or the other strain (Suiter et al. 2003). Similarly, Flöder and colleagues (2002) showed that phytoplankton diversity peaked at intermediate rates of fluctuation between high and low light intensities. Descamps-Julien and Gonzalez (2005) observed the stable coexistence of two diatoms species under fluctuating but not constant temperature. In other studies, however, the temporal grain of fluctuations had no obvious impact on the amount of genetic diversity (Riddle et al. 1986, Grover 1988, Scheiner & Yampolsky 1998; Litchman 1998) or on the evolution of specialist vs. generalist strategies (Kassen & Bell 1998; Buckling et al. 2007).

One explanation for these variable outcomes is the difficulty of designing experiments that cover the relevant range of temporal grains and employing environments with appropriate trade-off functions. In fact, information on the trade-off shape may only rarely be available *a priori*. Alternatively, rather than focusing in detail on a single set of fluctuating environments, one may therefore use a 'shotgun' approach,

replicating a large number of sets of environments over several temporal grains. This approach relies on the idea that different trade-offs evolve in different environments and therefore general properties of the evolution of diversity can be inferred as an average phenomenon over many replicated experiments.

Here, we used such a "shotgun" approach to study the impact of temporal environmental fluctuations on the evolution of genotypic diversity in experimental populations of *Pseudomonas fluorescens*, adapting to pairwise combinations of alternating carbon substrates. Populations were propagated either continuously on each single substrate or were alternated between the two substrates. Substrates were alternated at five different temporal grains, from 1-day (finest grain) to 8-day intervals (coarsest grain); thus representing fluctuation frequencies ranging from one every ~9 generations to one every ~70 generations. The experiment was replicated over 20 independent pairs of substrates from different chemical families. After ~300 generations, we assayed within-population genotypic diversity in fitness (i.e., performance) on the two substrates and estimated the strength of genotype-by-environment interactions (*inconsistency*, Bell 1990). We found that, on average, higher genotypic diversity evolved at an intermediate temporal grain. This differentiation likely resulted from specialization of genotypes to the two substrates.

2. MATERIAL AND METHODS

Study organism

Pseudomonas fluorescens SBW25 has become a bacterial model system for the study of adaptation and diversification in heterogeneous environments (Buckling et al. 2009). In particular, *P. fluorescens* rapidly specializes when propagated on different carbon sources (MacLean et al. 2004; Barrett et al. 2005; Venail et al. 2008). The present experiment was initiated from a single clone of *P. fluorescens* SBW25, previously selected for ~900 generations in a complex environment containing eight carbon sources (Barrett et al. 2005).

Selection environments

In a serial transfer experiment (batch culture), we allowed replicate bacterial populations to diversify for 32 days (~300 generations) in 20 independent pairs of environments. These pairs of environments consisted of 2 arbitrarily chosen carbon substrates on Biolog GN2® 96-well microplates. The list of pairs of substrates and their arrangement on the Biolog plate are provided in the Supplementary Information.

Temporal grain

The experiment was designed such that all replicate populations spent a total of 16 days (~150 generations) on a given substrate. For each pair of environments, we used four frequencies of cyclical temporal fluctuations, with the two carbon substrates alternating in 1-day, 2-day, 4-day and 8-day intervals, over the total of 32 days. Thus, the 1-day interval represented the finest temporal grain (daily change of substrate ≈ every 9 generations) and the 8-day interval the coarsest temporal grain (change of substrate every 8 days ≈ every 70 generations). We further included a *random* fluctuation treatment, where the temporal sequence of the two substrates was randomized instead of cyclically alternating. This *random* treatment included 1- to 3-day intervals, with a mean alternation of substrates every 1.7 days. In addition to these *temporal* treatments, we established a control treatment for each pair of environments, where replicate populations were constantly cultured on each single substrate for 16 days. This is equivalent to a spatially heterogeneous environment (referred to as *spatial* treatment, hereafter), with two spatially separated subpopulations evolving independently in the two environments. Because the environmental grain is maximal, we expected a maximum of (allopatric) diversification between subpopulations in this *spatial* treatment. We established three replicate populations for each pair of environments and treatment (20 pairs x 6 treatments x 3 replicate populations = 360 populations in total), spread out over 13 Biolog plates.

Selection protocol

Crystals from a frozen stock (-80°C) of the initial clone were inoculated into 13 sterile glass vials with 6 ml of M9KB solution (NH_4Cl 0.1 g/L; Na_2HPO_4 0.6 g/L; KH_2PO_4 0.3 g/L; NaCl 0,05 g/L; Glycerol 1 g/L, Proteose peptone # 2 2 g/L). After 24h of growth under constant orbital shaking (200 r.p.m.) at 28°C, 1 ml of culture from each vial was centrifuged (3 min at 6900*g*), the supernatant removed and replaced with 1 ml of M9 minimal salts medium (NH4Cl 1 g/l; Na2HPO4 6 g/l; KH2PO4 3g/l; NaCl 0.5 g/l). Then, 125 µl of this washed solution were diluted into 25 ml of M9 medium and starved for two hours at 28°C. For the first inoculation of the 13 Biolog plates, we added 140 µl of starved cells to each well. For this and all subsequent transfers, plates were incubated for 24 hours in the dark at 28°C without shaking. To renew the available nutrients and to alternate between environments, small samples of the bacterial populations were transferred to new Biolog plates in daily intervals. At each transfer, we homogenized the content in each well by gently taking up and releasing 100 µl of volume with a pipette (20 times); then a pin replicator (Boekel 96 pin/well model #140500) was used to transfer ≈ 2µl of culture to the respective substrate on a new Biolog plate (each well on the new plate was previously filled with 140 µl of M9 medium). This batch culture technique ensured nearly constant bacterial growth. After 32 transfers (16 in the *spatial* treatment), 80-µl samples from each replicate population were mixed with 80 µl of a 50:50 glycerol/M9 medium and stored at -80°C.

Fitness assay

To obtain individual genotypes for the fitness assays, we streaked out samples from each evolved bacterial population on KB-agar Petri dishes. Eight randomly picked colonies (= genotypes) per population were individually amplified on KB and 400 µl of overnight cultures were frozen in 50% Glycerol at -80°C. Prior to the assay, genotypes were amplified for 24h on 96-well plates in 140 µl of M9KB medium at 28°C, under constant orbital shaking (200 r.p.m). Then, the plates were centrifuged (5 min at 3050*g*) and wells washed by removing the supernatant and adding 140 µl of M9 medium. After two hours of starvation, we used the pin replicator to inoculate the Biolog plates for assays. Genotypes from fluctuating treatments were tested on the two

carbon substrates (say, substrates A and B) on which they had evolved; the genotypes from the corresponding *spatial* treatment (separate evolution on either A or B) were also tested on these two substrates. Each genotype was measured once on each substrate (20 pairs of environments x 6 temporal treatments x 3 replicate populations x 8 genotypes = 2880 tests). The assays were carried out on four days, with two of the eight genotypes of a given replicate population tested on each day. After 24 hours in the dark at 28°C, we scored the optical density (absorbance) at 590 nm, using a FLUOstar Optima spectrophotometer (BMG®). Light absorbance measures the capacity of the genotype to exploit the carbon source and thus is used as a proxy of bacterial performance (MacLean & Bell 2003). Some genotypes were able to grow in the blank well, so we decided not to correct the data by subtraction of the blank. To assess potentially confounding effects due to uncontrolled variation among the four assay dates, we included five replicates of the ancestral clone on each day.

Estimates of genetic diversity

Our estimates of within-population genetic diversity were based on the performance of the 8 genotypes of a given replicate population on two carbon substrates (e.g., substrates A and B from each carbon substrate pair, Fig. 1). Our main estimator of this genotypic diversity is, *inconsistency,* which is one component of the G x E interaction for fitness (Bell 1990, Barrett et al. 2005, Venail et al. 2008):

$$inconsistency = \sigma_{G1}\sigma_{G2}(1-\rho_{G1G2}) \quad \text{(Eq. 1)}$$

where σ_{G1} and σ_{G2} are the genotypic standard deviations in performance (i.e., absorbance) in the two environments, respectively, and ρ_{G1G2} is the genotypic correlation between the two environments. Genotypes are ranked in terms of performance on each substrate. *Inconsistency* indicates variation among genotypes of the order of substrate rankings, suggesting their specialization to different environments (Hall & Colegrave 2006). It is null if the rank order is perfectly positively correlated between environments (i.e., parallel reaction norms, ρ_{G1G2} = +1). Highly positive correlations indicate the potential for selection of generalist genotypes with high fitness in both environments. *Inconsistency* increases as the genotypic correlation coefficient becomes < +1, that is, when there is an increasing number of rank order

inversions between the two environments. Genotypic correlations become negative with substantial rank order inversions (crossing reaction norms) and as the best available genotypes are specialists.

To further describe the genotypic within-population variation, we calculated the mean *genotypic variance* (*GV*) in performance as:

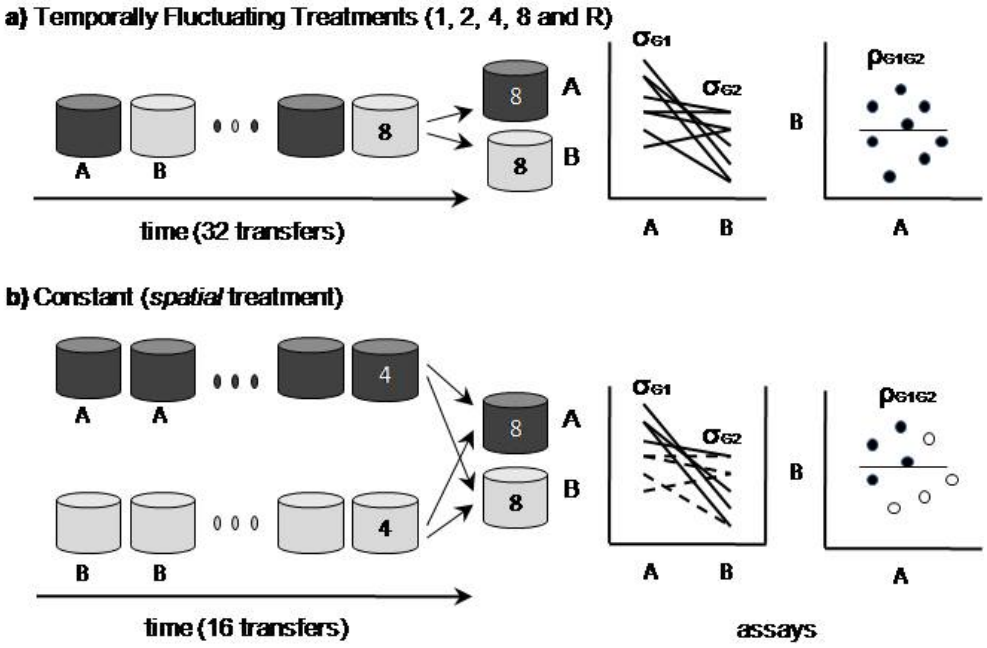


Figure 1: Flow chart of the experimental design used for calculations of *inconsistency* and *genotypic variance* (*GV)*. After 32 transfers, for all the fluctuating treatments **a)** (1, 2, 4, 8 and R) we randomly sampled eight genotypes from each selection line and assayed them on the two sources of the environmental pair (A and B). We calculated *inconsistency* as the product of genotypic standard deviations in the two environments by one minus the genotypic correlation (Eq. 1) and mean *GV* as the sum of genotypic variances (Eq. 2). A negative genotypic correlation implies that reaction norms are negatively correlated over the different environments and represents rank order shifts in the performance of the genotypes over the two environments. We also measured *inconsistency* and *GV* by randomly picking 4 genotypes from each evolved line and pooling the eight genotypes. **b)** This treatment represented a spatially divided population and was used as a theoretical upper boundary for diversification (*spatial* treatment).

$$GV = \frac{\left(\sigma^2_{G1} + \sigma^2_{G2}\right)}{2} \qquad \text{(Eq. 2)}$$

where σ^2_{G1} and σ^2_{G2} are the genotypic variances in the two environments. Note that *inconsistency* and *GV* are expected to be correlated, as the former increases with the product of the genotypic standard deviations (σ) and the latter with the sum of the variances (σ^2).

Inconsistency and *GV* were calculated for the replicate populations from all *temporally* variable environments. To estimate diversity for *spatially* variable environments, we used genotypes from populations constantly propagated on a single substrate. Thus, for a given pair of constant environments (e.g., substrates A and B, Fig. 1), we randomly chose four of the 8 genotypes from substrate A and four from substrate B to re-assemble a virtual group of 8 genotypes, for which we calculated *inconsistency* and *GV*. This mimicked sampling from two subpopulations in a spatially heterogeneous environment (i.e., substrates A and B separated in space).

Response to selection

For each environmental grain and environment, we quantified the response to selection as the difference in performance between evolved genotypes and the ancestral clone. Thus, we calculated the response to selection in the '*contemporary*' environment, from which genotypes were isolated after the last growth cycle; it was also calculated for the '*previous*' environment populations had encountered one cycle earlier (for constant treatments the environment that was never encountered). Response to selection in the *contemporary* environment approximates the direct response to selection and thus the efficacy of selection in this environment, at least for the more coarse-grained treatments. Response to selection in the *previous* environment approximates the correlated (indirect) response to selection and can thus hint at potential costs of adaptation. The difference in the responses to selection among the two environments is an indicator of the degree of specific adaptation.

Statistical analysis

We used factorial analyses of variance (ANOVA) to test effects of the environmental grain (all *temporal* treatments and the *spatial* treatment) and the

environmental pair on *inconsistency and genotypic variance (GV)*. To test for non-linear relationships among the temporally alternating treatments, we also carried out analyses of covariance (ANCOVA), with temporal grain as a covariate (first- and second-order polynomial). *Inconsistency* was log-transformed and *GV* square-root-transformed for analysis. Finally, we fitted linear models to test for a relationship between the mean response to selection (direct and correlated) and the environmental grain. We used R (R development core team, 2009) and JMP5 (SAS 2003) software for statistical analysis.

3. RESULTS

Reference measurements of absorbance of the ancestral strain did not significantly differ among the four experimental blocks ($F_{3,95} = 0.67$, $P = 0.58$), indicating the absence of a "day effect" on bacterial performance. Therefore, results among evolved lines were analyzed without correction for block (day) effects.

Inconsistency

After ~300 generations of selection, we found a significant effect of the environmental conditions on *inconsistency*. Overall, *inconsistency* in the *spatial* treatment was substantially higher than in any of the *temporal* treatments (contrast *spatial* vs. highest *temporal* (4-day interval) treatment: $F_{1, 95} = 10.17$, $p = 0.0019$; Fig. 2a). Thus, rank order changes of genotypes between environments were more pronounced in the *spatial* treatment than in the *temporal* treatments. Further analysis, restricted to the *temporal* treatments (Table 1), revealed significant effects of environmental pair and of temporal grain.

Table 1: Results of the two full factorial mixed ANOVA tests on the effect of temporal grain (fixed factor) and the environmental pair (random factor) on Log *Inconsistency* and on the square root of *Genotypic Variance*, *GV* (the random treatment was not included in the analysis).

Log *Inconsistency*

Effect	M.S.	D.F.	F	p-value
Grain	2.019	4	2.58	**0.0439**
Pair	7.621	19	8.66	**< 0.0001**
Grain x Pair	0.783	76	0.89	0.719
Residual	0.88	200		

Square root *GV*

Effect	M.S.	D.F.	F	p-value
Grain	0.0185	4	5.97	**0.0003**
Pair	0.0557	19	16.38	**< 0.0001**
Grain x Pair	0.0031	76	0.90	0.6887
Residual	0.0034	200		

Generally, environments fluctuating in a 1-day manner produced the lowest levels of *inconsistency*; more coarse grains produced higher rank inversions, with a peak for 4-day intervals. *Inconsistency* in randomly fluctuating environments (mean interval length = 1.7 days) was similar to that in environments strictly alternating in 2-day intervals (Fig. 2a).

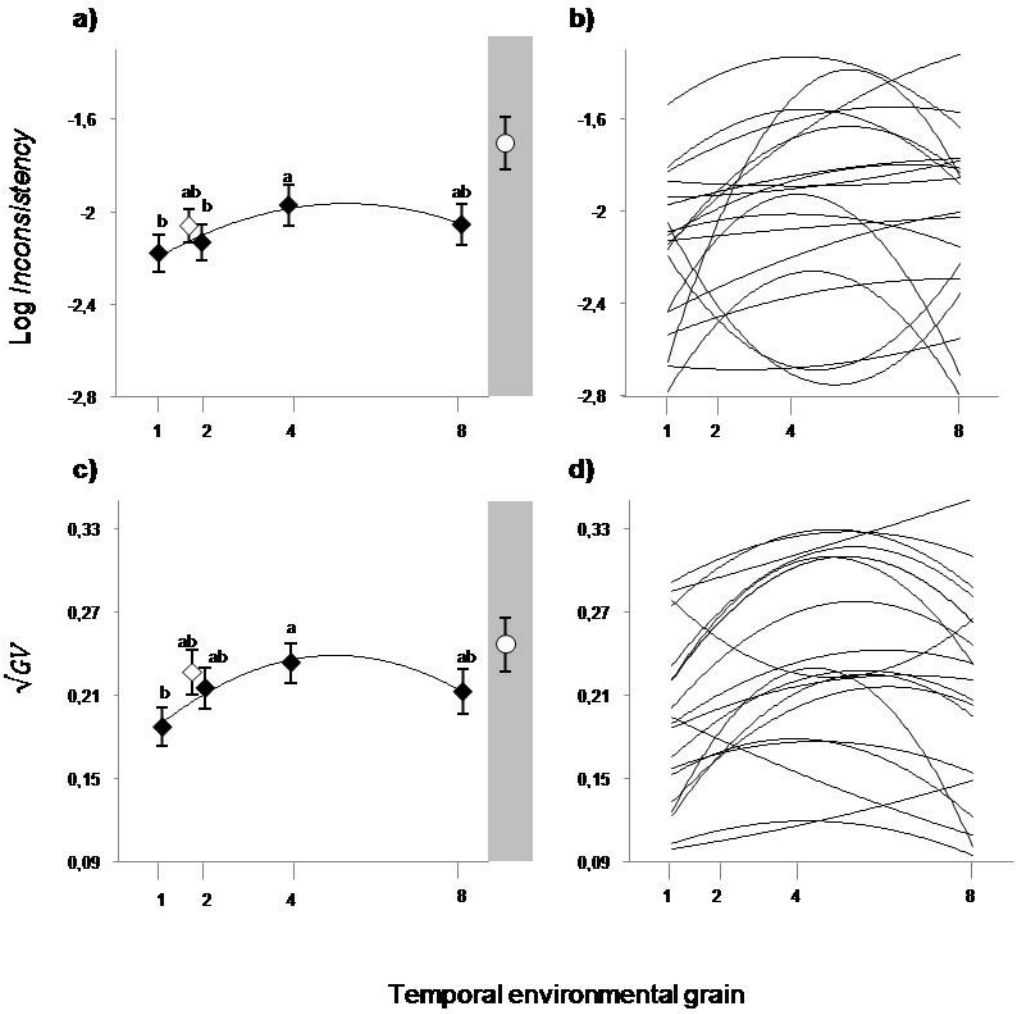


Figure 2: Effect of temporal grain on Log *Inconsistency* and $\sqrt{GV}$. **a)** Log *Inconsistency* peaked at intermediate temporal grain and was significantly higher when mixing genotypes from two selection lines (*spatial* treatment, open circle in shaded region) than in any other treatment. Each dot represents the mean Log *Inconsistency* (+/- S.E.) over the 20 environmental pairs replicated each three times. The line represents a quadratic model fit. Empty diamond represents the *random* treatment. **b)** Polynomial fits between *Inco* and temporal grain for each environmental pair (*n = 20*), quadratic fits revealed 15 relationships presented a unimodal pattern. **c)** $\sqrt{GV}$ peaked at intermediate temporal grain and was significantly higher when mixing genotypes from two selection lines (*spatial* treatment, open circle in shaded region) than in any other treatment. Each dot represents the mean $\sqrt{GV}$ (+/- S.E.) over the 20 environmental pairs replicated each three times. The line represents a quadratic model fit. Empty diamond represents the *random* treatment. **d)** Polynomial fits between $\sqrt{GV}$ and temporal grain for each environmental pair (*n = 20*), quadratic fits revealed 16 relationships presented a unimodal pattern.

Using temporal grain as a continuous covariate in an ANCOVA, we found significant linear (positive) and second-order (negative) effects of temporal grain on *inconsistency* (Table 2). Thus, there was a general hump-shaped relationship between temporal grain and *inconsistency*, with a maximum for an intermediate grain (Figure 2a). This relationship was largely consistent across environmental pairs (non-significant

grain x pair interactions, Table 1, 2): convex relationships between temporal grain and *inconsistency* were found for 15 of the 20 environmental pairs, and another two pairs showed at least a positive linear relationship (Fig. 2b).

Table 2: Results of the two full factorial mixed ANCOVA tests on the effect of temporal grain (as a covariate) and the environmental pair (random factor) on Log *Inconsistency* and on the square root of *Genotypic Variance*, *GV* (the random treatment was not included in the analysis).

Log *Inconsistency*

Effect	M.S.	D.F.	F	p-value
Grain	1.3727	1	8.9486	**0.0032**
Pair	0.5772	19	3.7629	**<0.0001**
Grain x Pair	0.1975	19	1.2880	0.1958
Grain^2	0.8915	1	5.8121	**0.0169**
Grain^2 x Pair	0.2249	19	1.466	0.1024
Residual	0.1534	180		

Square root *GV*

Effect	M.S.	D.F.	F	p-value
Grain	0.0533	1	16.257	**<0.0001**
Pair	0.0163	19	4.9733	**<0.0001**
Grain x Pair	0.0028	19	0.8683	0.6225
Grain^2	0.0522	1	15.9194	**<0.0001**
Grain^2 x Pair	0.0026	19	0.8013	0.7038
Residual	0.5905	180		

Genotypic Variance (GV)

As for *inconsistency*, the *spatial* treatment produced higher levels of *genotypic variance* (*GV*) than did *temporal* treatments (contrast *spatial* vs. all *temporal* treatments: $F_{1, 95} = 11.51$, $p = 0.0010$). Both the temporal grain and the environmental pair had a significant impact on *GV,* and there was a hump-shaped relationship between temporal grain and *GV*, with a maximum of genetic variation in bacterial performance at

4-day fluctuation intervals (Tables 1, 2; Fig 2c). Again, the pattern was relatively consistent across environmental pairs, with concave curves in 16 of the 20 cases (Fig 2d).

Taken together, our two measures of genetic within-population diversity peaked at intermediate temporal grains. Alternating carbon substrates in 4-day intervals produced the strongest variation in the relative fitness (*inconsistency*) and in the mean fitness (*GV*) of genotypes on these substrates.

Genotypic correlation coefficients

We further inspected in more detail one component of *inconsistency*, the genotypic correlations. Overall, negative genotypic correlations were relatively rare (Fig. 3). On average, 30% (6/20) of the pairs in the *spatial* treatment showed negative genotypic correlations, indicating substantial specialization of genotypes on these substrates. Because specialization was more pronounced in the spatially variable environments, genotypic correlations were less positive in the *spatial* treatment (0.28 ± 0.10) than in the temporally variable environments (0.47 ± 0.04). The strength of genotypic correlations did not significantly differ among temporal grains ($F_{4, 76} = 0.85$, $p > 0.4$), with only 5 to 20% of the pairs in the temporal treatments producing negative correlations (Fig. 3). Thus, although analysis of *inconsistency* revealed a clear signal of the temporal grain, patterns for the genotypic correlations indicate that highly differentiated specialists were rare in the *temporal* treatments.

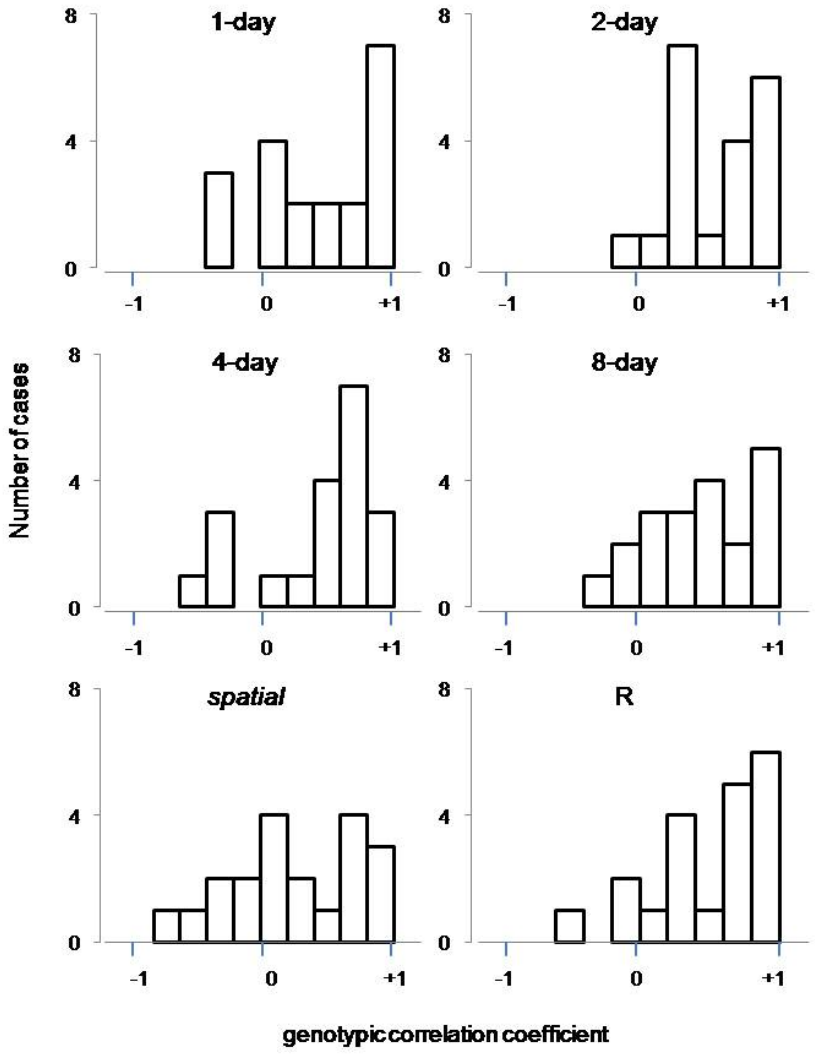


Figure 3: Frequency distributions of genotypic correlation coefficients for each environmental treatment. Each distribution represents 20 pairwise comparisons of environmental conditions over 8 genotypes.

Response to selection

On average, responses to selection were positive on all substrates (0.284 ± 0.006, n = 5760), indicating considerable bacterial adaptation to the growth conditions. Patterns of responses to selection differed between *contemporary* and *previous* environments. First, genotypes showed stronger adaptation to the substrates they had last encountered in the long-term experiment (*contemporary* environment: 0.448 ± 0.008, n = 2880) than to the environments they had encountered one cycle back (*previous* environment: 0.120 ± 0.008, n = 2880; $t_{1,\ 5758}$ = -30.404, p < 0.0001). Second, responses to selection linearly increased with environmental grain on *contemporary*

environments (main effect of grain in ANCOVA: F = 9.609; p = 0.024), but decreased when measured on *previous* environments (F = 8.7124; p = 0.038; Fig. 4). Thus, the longer the time continuously spent on the last substrate they encountered, the stronger was the degree of adaptation to that *contemporary* environment. Conversely, the more time had elapsed since genotypes had encountered the other environment for the last time, the less they were adapted to the *previous* environments, resulting in an overall negative correlation between the two types of responses to selection (Fig. 4).

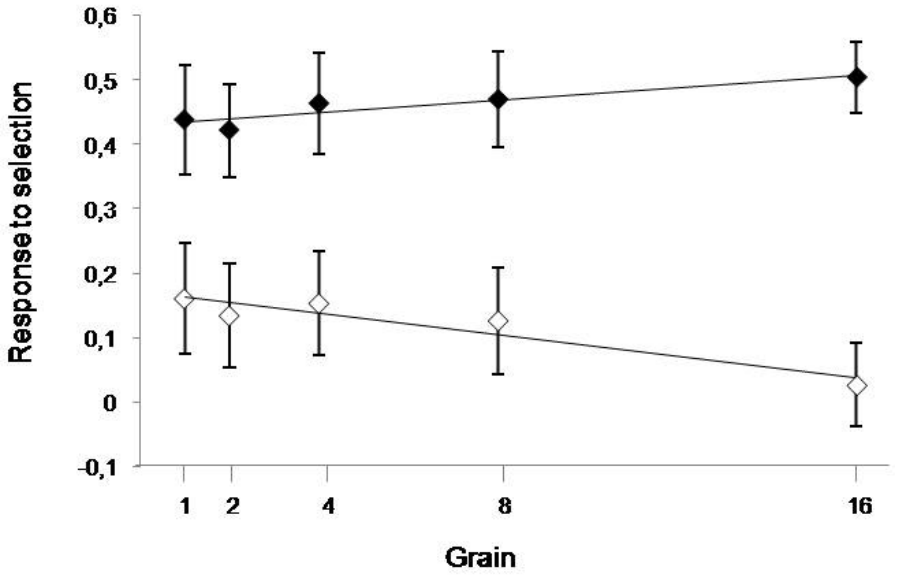


Figure 4: Effect of temporal grain on the mean response to selection calculated as the difference in performance between the evolved genotypes and the ancestral clone. Filled diamonds represent the response to selection in the last environment (*contemporaneous* +/- S.E.). Empty diamonds represent the response to selection to the other environment (*previous* +/- S.E.). Lines represent linear fits. In the case of the *contemporary* environment, the grain represents the number of continuous days the genotypes have been exposed to that environment. In the case of the *previous* environment, the grain represents the number of days the genotypes have not been exposed to that environment.

4. DISCUSSION

In this experiment, we explored the evolution of genotypic diversity and specialization in the bacterium *Pseudomonas fluorescens* SBW25 when evolving in temporally fluctuating environments. After ~300 generations, the highest degree of genotypic diversity was observed at an intermediate environmental grain (i.e., 4-day

alternation of two carbon substrates), a pattern that held for most of the pairs of substrates tested (15 out of 20).

Population genetic theory predicts that protected polymorphism will emerge in heterogeneous environments as a result of specialization, given the appropriate shape of the trade-off functions (strong trade-off) and of environmental grain (coarse). We tested for signatures of specialization, by estimating the strength of genotype x environment interactions (*inconsistency*) in our evolved populations. *Inconsistency* describes the extent of rank order changes in performance of genotypes between environments, indicative of differential adaptation of genotypes to the selection environments. We found that the magnitude of such rank order changes varied with our experimental treatments.

First, consistent with theory (Dempster 1955; Haldane 1963; Gillespie 1972; Hedrick 1974, 1986; Felsenstein 1976; Dean 2005), the *spatial* treatment produced substantially higher levels of diversity (for both *inconsistency* and *GV*), than did *temporal* treatments. The *spatial* treatment mimicked sampling from two subpopulations in a spatially heterogeneous environment; unlike in the *temporal* treatments, each subpopulation was continuously selected in a single environment, and therefore this treatment was expected to set the upper boundary for the evolution of genetic diversity in our experiment. Substrate specialization as a driver of this differentiation is further suggested by the observed patterns of responses to selection. Among all treatments, the *spatial* treatment showed the strongest mean direct response to selection on the local (or: *contemporary* for the temporal treatments) substrate and the weakest correlated response to selection on the foreign (or: *previous*) substrate. The negative relationships between *contemporary* and *previous* responses to selection indicate costs of adaptation and concomitant specialization (MacLean & Bell 2002, 2003). These patterns corroborate results from previous studies with *Pseudomonas fluorescens* SBW25, demonstrating the importance of spatial heterogeneity in shaping the emergence and maintenance of diversity (Rainey & Travisano 1998; Maclean & Bell 2003; Barrett et al. 2005; Venail et al. 2008).

Second, we found a hump-shaped relationship between the temporal grain of environmental fluctuation and within-population genotypic diversity, with average peak levels of *inconsistency* and *genotypic variance* for intermediate fluctuations (4-day

interval). Indeed, low diversity at very fine temporal grains is expected if frequent switching between environments disfavors differentiation into specialists. Instead, a fine grain selects for generalists capable of withstanding frequent transitions and with the highest mean fitness across both environments. When fluctuations become more coarse-grained, periods of selection in a given environment may be long enough to select for genotypes adapted to that environment, but still short enough to allow the persistence of genotypes adapted to the other environment. Thus grains allowing the coexistence of differentially adapted genotypes will have higher values of *inconsistency*. Further increasing temporal grains provide long periods of constant environmental conditions, allowing efficient selection for genotypes adapted to the contemporary environment and against genotypes adapted to the *previous* environment, resulting in lower levels of *inconsistency*. This idea that more coarse-grained fluctuations allow more efficient sorting of adapted (or maladapted) genotypes is consistent with our observation of linear relationships between environmental grain and response to selection on *contemporary* or *previous* environments. Namely, the more time a population spent on a given (*contemporary*) substrate, the stronger was the increase in fitness relative to the ancestor on this substrate.

While a substantial fraction (30%) of the environmental pairs showed negative genotypic correlations for fitness (i.e., strong crossing reaction norms), most of them produced weak to moderate positive genotypic correlations. This suggests that many carbon sources did not represent highly contrasting selection environments, required for the evolution of strong reciprocal specialization (Levins 1968, Jasmin & Kassen 2007). This explanation is consistent with the generally positive responses to selection in the *previous* environment, indicating fitness increases even in environments populations were not selected on. Similar patterns of synclinal selection were obtained elsewhere with *P. fluorescens* (MacLean & Bell 2002, Barrett et al. 2005, Jasmin & Kassen 2007). Our results are also comparable to observations in a study on genotype x environment interactions in *Chlamydomonas reinhardtii,* where *inconsistency* was largely due to incomplete genetic correlations (i.e., < +1), but did not necessarily entail negative genetic correlations (Bell 1990). Hence, our finding of higher levels of *inconsistency* at intermediate temporal grains, but still with positive genotypic correlations, suggests that the ranking of genotypes varied to some degree between

environments, but that the evolution of complete rank reversals was by far not the rule. In other words, these populations seem to harbor a mix of moderately specialized genotypes and generalists. Unless stronger trade-offs and negative correlations arise after longer time spans of selection on these substrates (Buckling et al. 2007), this would imply that some of the observed diversity is only transient and that generalists could become fixed in the long run.

The idea of transient coexistence has a long tradition in ecological models of species coexistence. Asexual haploids, such as *Pseudomonas*, are conceptually very close to species, as defined in ecological models, and thus parallels can be found with the ecological literature concerned with fluctuating environments. Traditional hypotheses hold that long-term coexistence is possible as long as the time to exclusion and the period of fluctuation are comparable (Hutchinson 1961). Under these circumstances, time under constant environmental conditions is not sufficient to allow exclusion. This theory, called the “Intermediate Disturbance Hypothesis” (IDH), predicts that maximal diversity is expected at intermediate rate and/or intensities of perturbations. While being not stable in the long-term (Chesson & Huntly 1997), exclusion is likely to occur very slowly and thus transient diversity is expected almost indefinitely, given the appropriate environmental fluctuations (Huston 1979). An equilibrium alternative to the IDH is the "storage effect" hypothesis (Chesson 1983; Chesson 2000b). It states that coexistence is possible as long as species have complementary non-linear growth curves along a resource gradient. As the resources fluctuate, each species recover from unfavorable periods during more favorable ones. If the periods of recovery counteract the harsh periods, coexistence is possible. Recently it has been shown that the storage effect could be well suited to explain de maintenance of genetic polymorphism in haploids (Dean 2005).

These ecological theories are concerned with the maintenance of already evolved diversity, while our experiment explored the conditions for its emergence. Nonetheless, the conditions for the emergence and maintenance of diversity are very similar and strong parallels can be found between model of genetic diversity in population and ecological diversity in communities (Vellend & Geber 2005). Evolutionary and ecological processes may be combined to explain the dynamics of diversity in our experiment, where polymorphism is results of a balance between mutation and selection. We have

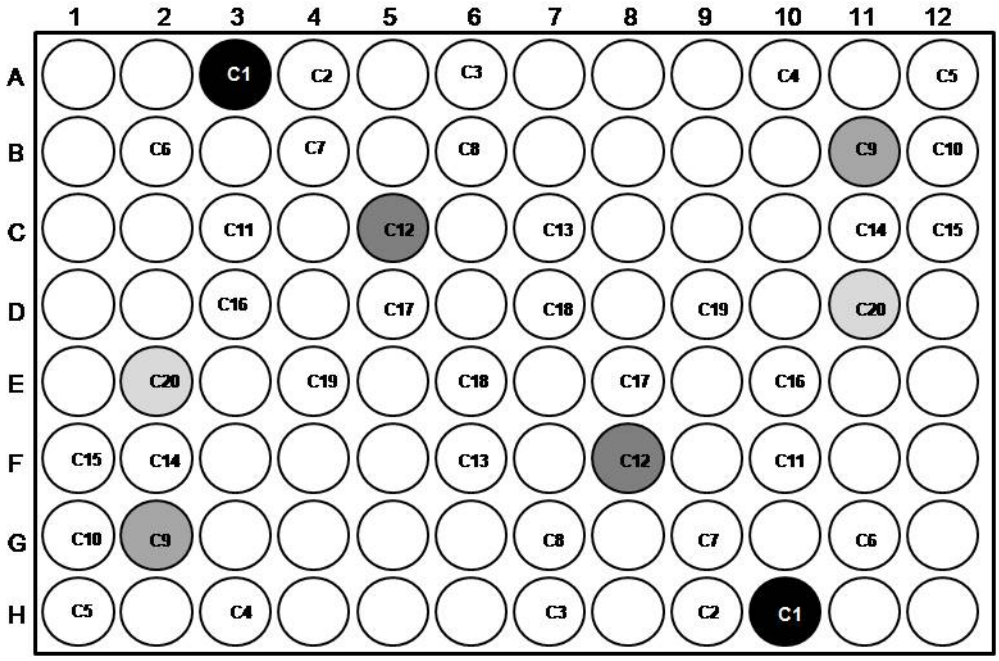
found that some level of specialization has evolved, and thus it is likely that some of the observed diversity was stably maintained because genotypes had complementary growth curves (as predicted by the storage effect). Also, some of this polymorphism was probably transient as selection is less efficient at intermediate temporal grain at eliminating non-adapted genotypes. The present study represents a single snapshot in time; whether polymorphism can be stably maintained or not under the different temporal grains would require repeated assays over longer periods of time.

Both theoretical and empirical work indicates that the autocorrelation structure of fluctuating environmental conditions can strongly affect population growth and persistence as well as community dynamics (Caswell & Cohen 1995, Gonzalez & Holt 2002; Massin & Gonzalez 2006). For example, cyclic fluctuations are expected to maintain higher genotypic diversity than random fluctuations (Hedrick 1974; Scheiner 1993). Here, we were mainly interested in alternating environmental conditions (i.e., negative autocorrelation, or "blue noise", Caswell & Cohen 1995), but had added a random fluctuation treatment (no autocorrelation, or "white noise"). This *random* treatment, where substrates alternated in an average interval of 1.7 days, produced similar levels of genetic diversity than the truly alternating 1-day and 2-day treatments, suggesting that the degree of autocorrelation had no strong impact on the evolutionary processes in our experiment. However, this result must be interpreted with caution because our experiment was not explicitly designed to test random vs. cyclical treatments. As natural populations may be exposed to variable spectra of fluctuations (from “white” to “blue” to “red noise”), this represents an interesting aspect for future research.

Several experiments both in evolution and ecology have found that intermediate fluctuations of the environment could lead to maximum genotypic or specific diversities (Mackay 1980; Gottschall et al. 1981; Litchman 1998; Suiter et al. 2003; Hiltunen et al. 2008). In these experiments, different factors such as the species identity (Gottschall et al. 1981), the average light (Litchman 1998), the phenotypic trait considered (Mackay 1980) and the grain (Suiter et al. 2003) have been shown to shape the effect of temporal variations. In accordance to some of these results, we found that temporal fluctuation had a positive impact on the emergence and maintenance of diversity and we have evidence that diversity was maximal for an intermediate environmental grain. This

confirms results found by Suiter and colleagues (2003) who observed that maximal diversity emerged in populations of the bacterium *Escherichia coli* at intermediate environmental grains compared to more fine (2 generations) or coarse (5 and 6 generations) grains. Some other experiments however did not found any effect of temporal fluctuation on species diversity (Scheiner & Yamplosky 1998; Buckling et al. 2007; Hughes et al. 2007) suggesting some complexity in understanding the effect of temporal heterogeneity (Kassen 2002). Our experiment is the first to combine a high number of environmental scenarios (20 pairs) along a gradient of fluctuating environmental grains. The contrasted patterns of our results clearly illustrate the complexity of the possible outcomes of diversity in temporally fluctuating environments. Depending on the carbon sources and environmental grain, we have obtained different outcomes with cases of stable or transient coexistence of specialists and generalist, cases with exclusion by generalists and/or specialist as well as intermediate situations. However, despite this heterogeneity of outcomes our "shotgun" design has allowed us to highlight some kind of general pattern as we found a hump shape relationship between temporal grain and the amount of genotypic diversity that emerged. Our result combined with previous ecological and evolutionary experiment suggests that the emergence and maintenance of diversity in a temporally heterogeneous is multifactorial and may not be driven by a simple equilibrium theory based on reciprocal specialization as suggested in theoretical literature (Levins 1968). Despite the complexity in the outcome of diversity in temporally fluctuating environments, our experimental approach has lead to a single general pattern!

Supplementary Information:



Supplementary Figure: Arrangement of the 20 pairs of environments on the 96-well Biolog plate. Each well contains a different carbon substrate. For each environment on the upper half of the plate, the corresponding environments were determined by inverting the order of from 20 to 1 on the lower half of the plate. Four examples of pairs of environments are shown in different tones. For example, for the pair c1, selection lines were grown on the carbon substrates with coordinates A-3 (Dextrin) and H-10 (D, L-alpha-Glycerol Phosphate), in alternating sequences of different temporal grains. The constant treatment, without alternation, consisted of 16 days of exclusive culture on A-3 or on H-10.

List of environmental pairs:

C1: Dextrin (*I*) / D, L-alpha-Glycerol Phosphate (*XI*); **C2**: Glycogen (*I*) / Glycerol (*X*): **C3**: Tween 80 (*I*) / 2-Aminoethanol (*IX*); **C4**: L-Arabinose (*II*) / Uridine (*VIII*); **C5**: D-Cellobiose (*II*) / Urocanic Acid (*VIII*); **C6**: D-Fructose (*II*) / D, L-Carnitine (*VII*); **C7**: D-Galactose (*II*) / L-serine (*VII*); **C8**: Alpha-D-Glucose (*II*) / L-Pyroglutamis Acid (*VII*); **C9**: Hydroxy-L-Proline (*II*) / D-Mannitol (*VII*); **C10**: D-Mannose (*II*) / L-Histidine (*VII*), **C11**: D-Psicose (*II*) / L-Glutamic Acid (*VII*); **C12**: L-Rhamnose (*II*) / L-Asparagine (*VII*); **C13**: Sucrose (*II*) / L-Alanine (*VII*); **C14**: Pyruvic Acid Methyl Ester (*III*) / Succinamic Acid (*VI*); **C15**: Succinic Acid Mono-Methyl-Ester (*III*) / Bromosuccinic Acid (*V*); **C16**: Citric Acid (*IV*) / D-Saccharic Acid (*IV*); **C17**: D-Galactonic Acid Lactone (*IV*) / Propionic Acid (*IV*); **C18**: D-Gluconic Acid (*IV*) / D,L-Lactic Acid (*IV*); **C19**: D-Glucuronic Acid (*IV*) / Alpha-Keto Glutaric Acid (*IV*); **C20**: Beta-Hydroxybutyric Acid (*IV*) / Itaconic Acid (*IV*).

(roman numerals represent chemical families: I-Polymers, II-Carbohydrates, III-Esters, IV-Carboxylic Acids, V-Bromidic Acid, VI-Amides, VII-Amino Acids, VIII-Aromatics, IX-Amines, X-Alcohols, XI- Phosphorylated Chains).

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Manuscrit 4

Defining and measuring ecological specialization

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Résumé en Français

La spécialisation écologique est un concept avec une longue histoire en écologie et biologie de la conservation. Par conséquent, plusieurs définitions sont disponibles dans la littérature ainsi qu'un grand nombre de manières de la quantifier. Le but de cette revue est d'éclaircir le concept de spécialisation écologique en explorant les avantages et les inconvénients des différentes approches proposées dans le passé.

Cette revue présente la spécialisation écologique comme un concept lié à celui de la niche écologique. Un spécialiste a souvent été définit comme un organisme avec une niche restreinte ou étroite. Par conséquent, cette synthèse montre que la niche peut être définie selon les besoins d'une espèce (niche Grinnellienne) ou selon son impact sur l'environnement (niche Eltonienne). Le premier objectif de cet article est d'expliquer cette dichotomie. Cette revue de la littérature sur la spécialisation nous a également permis d'identifier la spécialisation comme un concept échelle-dépendant : il dépend de l'échelle spatiale ainsi que de l'échelle temporelle des observations. Le concept de spécialiste est donc très large : applicable tant aux individus comme aux communautés, en passant par les espèces.

Cet article propose aussi une synthèse des différentes méthodes de quantification de la spécialisation écologique selon les besoins de chaque étude et montre que selon le type de niche considérée (Grinnelienne ou Eltonienne) différentes façons de la calculer sont disponibles.

En proposant une synthèse des différentes approches disponibles sur le concept de spécialisation écologique, cette étude cette doit servir pour générer des meilleures prédictions écologiques applicables dans le domaine de la conservation.

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Defining and measuring ecological specialization

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Summary

1. Ecological specialization is one of the main concepts in ecology and is increasingly used in conservation to investigate whether specialist and generalist species are equally at risk when facing global changes. However, this concept has become highly context-dependent and is now obscured by the great variability of existing definitions and methods used to characterize ecological specialization.

2. Here, we clarify this concept by reviewing the strengths and limitations of different approaches commonly used to define and measure ecological specialization. We show that ecological specialization can either be considered as reflecting species' requirements or species' impacts and as a species-specific characteristic or as the result of local and contingent environmental constraints. We also show why and how ecological specialization should be scaled across spatial and temporal scales, and from individuals to communities.

3. We then illustrate how this review can be used as a practical toolbox to classify widely used metrics of ecological specialization in applied ecology, depending on the question being addressed, the method used, and the data available.

4. *Synthesis and applications*. Clarifying ecological specialization is useful to make explicit connections between several fields of ecology using the niche concept. Defining this concept and its practical metrics is also a crucial step to better formulate predictions of scientific interest in ecology and conservation. Finally, understanding the different facets of ecological specialization should help to investigate the causes and consequences of biotic homogenization and to derive relevant indicators of biodiversity responses to land-use changes.

Keywords: Biotic homogenization, Ecological niche, Elton, Grinnell, Hutchinson, Indicator, Niche breadth, Niche metrics, Specialist-generalist, Species distribution models

Introduction

Specialist species are increasingly shown to be declining and experiencing higher extinction risk relative to generalist species (e.g., marsupials, Fisher *et al*. 2003; carabid beetles, Kotze & O'Hara 2003; plants, Rooney *et al*. 2004; bumblebees, Goulson *et al*. 2005; birds, Jiguet *et al*. 2007; butterflies, Polus *et al*. 2007; bats, Boyles & Storm 2007; coral reef fish, Wilson *et al*. 2008). Specialists are often even considered to be the "great losers" of past and current global changes (McKinney 1997; Devictor *et al*. 2008; Colles *et al*. 2009), and trends in the abundance of these species are used as indicators of unsustainable development at national and international scales (Gregory *et al*. 2005).

Meanwhile, an increasing number of specialization indices have been proposed in the literature. For instance, specialization is inferred indirectly from species distributions and environmental data (Calenge & Basille 2008), or from direct measures of species performances in multiple environments (Kassen 2002). Alternatively, highly detailed measures of dietary specialization have indicated that not only species, but also individuals within a species, are more or less specialized (Bolnick *et al*. 2007). The quantification of ecological specialization is also highly dependent on the data used, the organism studied, and the ecological mechanism of interest (e.g. behavior specialization vs specialization for habitat). Consequently, each publication studying ecological specialization has virtually redefined the concept (Ferry-Graham *et al*. 2002).

The term "specialization" is also now used inconsistently throughout the ecological literature for different biological levels (individual, species, population or community) and measured at very different spatial scales. Although flexibility in the definition of ecological specialization can be helpful, it could also cause confusion that should be clarified for applied purposes. Therefore, clarifying how ecological specialization is measured and, more generally, how the concept of ecological niche is used is still a challenging issue in ecology and conservation (Araújo & Guisan 2006; Kearney 2006; Soberon 2007; Gaston & Fuller 2009).

Concepts of specialist versus generalist species have a long history in theoretical and applied ecology. In the pioneering theoretical literature, ecological specialization was most often synonymous with a limited niche breadth, resulting from evolutionary trade-offs between the ability of species to exploit a range of resources and their capacity to use each one (the "jack-of-all-trades is master of none" hypothesis; MacArthur 1972).

Specialization has also early been outlined as a key concept to predict the adaptive response of populations in heterogeneous and/or fluctuating environments (Levins 1962).

Subsequently, the concept of ecological specialization received several clarifications. In particular, in their seminal review of ecological specialization, Futuyma & Moreno (1988) have emphasized the need to clarify specialization from the classical distinction between the fundamental niche of a species and its realized niche (Hutchinson 1957). Without such a distinction, a species which only uses a subset of available resources because of competitive exclusion will be considered to be specialized, although this species is potentially able to use a broader breadth of resources. Pulliam (2000) further stressed the need to account for dispersal processes when inferring niche characteristics from species distribution: species might be found in unsuitable habitats because of source-sink dynamics or absent from suitable habitats because of dispersal limitation (Thomson *et al*. 1996).

Although these clarifications have increased our understanding of the concept of specialization, a classification of the numerous existing definitions and metrics of ecological specialization is missing. Here, we first highlight the most commonly used definitions of ecological specialization. We then identify why and how specialization should be quantified across various spatial and temporal scales, and from individuals to communities. We further propose a flexible toolbox for measuring ecological specialization depending on the data available and the questions addressed.

Based on this review, we illustrate how using different facets of ecological specialization can motivate timely research directions in several fields of ecology. We particularly explore what can be learnt from different views of the niche in following applied problems: i) the maintenance of species diversity in communities, ii) the causes and consequences of the biotic homogenization process, iii) the identification of the appropriate spatial and temporal scale of different management options, iv) the consequences of species' introduction and v) the importance of focusing on rare or common species in conservation plans.

Finding common ground in widely-used definitions of ecological specialization

Grinnellian versus Eltonian-specialization

Ecological specialization is often defined as the restricted ecological niche breadth (or width) of a given species (Futuyma & Moreno 1988). As such, clarifications of the niche concept have repeatedly emphasized the need to discern *Grinnellian* from *Eltonian* dimensions of the niche (Chase & Leibold 2003; Guisan & Thuiller 2005; Soberon 2007). The Grinnellian niche describes *the response of species to a given set of variables*, here considered as resources (Grinnell 1917), while the Eltonian niche (sometimes called functional or trophic-niche) focuses on *the impact of species in the environment* rather than on its response to particular resources (Elton 1927).

Grinnellian niche can be further extended to Hutchinson's (1957) concept of the ecological niche (which has been the most influential concept) where all biotic and abiotic resources are considered. In this case, the niche is generally defined as a hyper-volume in the multidimensional space of ecological variables, within which a species can maintain a viable population (Hutchinson 1957). Similarly, the Eltonian-niche concept can be extended in an *n*-dimensional functional space, measured as the species position along axes embodying functional attributes, rather than resource variables (Rosenfelt 2002; Mouillot *et al*. 2007). This functional conception of the ecological niche has recently received new theoretical and methodological attention following the growing interest for functional traits in community and applied ecology (McGill *et al*. 2006; Ackerly & Cornwell 2007).

Based on this first distinction between the two classes of ecological niche, the *Grinnellian-specialization* of a species should represent the variance in species' performance across a range of environmental conditions, broadly defined by one or several biotic (and/or abiotic) resources (Fig. 1a). Note that, in practice, Grinnellian-specialization can be defined as the breadth of species requirement using the term "resource" in its largest sense. For instance, habitat-specialization can be a proxy for Grinnellian-specialization if habitat is not only the physical place where the species is found but encompasses conditions that influence species performance in various ways (e.g., species might need specific habitat for reproduction places, predator refuges, or specific food requirements). More generally, Grinnellian specialization reflects the range of each biotic and abiotic factor determining the distribution and abundance of a single species and is most often measured using species' requirements for non-interactive variables (Soberon et al. 2007).

In the Eltonian concept of niche, each species has a particular role in an ecosystem. For instance, according to Elton, there are detritivorous, dispersal, or pollination niches. This functional niche therefore refers to a species position in ecological networks, and is often used in functional ecology and ecosystem ecology. This definition is also closely related to the concept of “guild” or “functional groups” which aggregates species according to their trophic relationships with the biotic environment (e.g., scavengers, grazers, seed eaters). Therefore, the *Eltonian-specialization* refers to the functional position of species in its environment and is measured as the species breadth of functional roles (that we consider a synonym of impact) instead of resource used (Fig. 1b). Elton historically assimilated the niche of a species to its "place in the biotic environment, its relations to food and enemies" (Elton 1927) so that the functional role of the focal species most usually refers to its impacts on other species (e.g., pollination, predation, herbivory). However, abiotic changes generated by the focal species can also be considered (e.g., oxygen generation, carbon dioxide acquisition, mineralization) since they indirectly impact on other species in the ecosystem.

Discerning Grinnellian from Eltonian-specialization in applied ecology is thus essentially a practical mean to emphasize the difference between the breadth of species’ requirements (what the species *needs*) and the breadth of species effects on environment (what the species *does*). This basic distinction does not a priori depend on particular scale or data used and should be relevant to any species. This traditional classification is far from being complete or flawless (see discussion). However, the distinction between Eltonian and Grinnellian specialization sets a meaningful heuristic basis which includes most specialization definitions used with empirical data in applied ecology. Moreover, a similar distinction is used when plant functional traits are used to depict species effects on ecosystems or alternatively species’ responses to environmental disturbance (so-called effect trait versus response trait, Lavorel & Garnier 2002; Garnier *et al*. 2004; Suding *et al*. 2008).

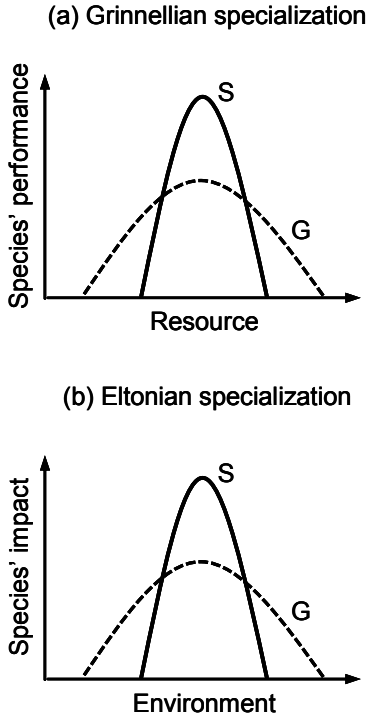


Figure 1: Definition of Grinnellian versus Eltonian specialization: (a) The Grinnellian-specialization of a given species can be described by its variance in performance across a given range of resources. For a given mean performance, the dashed line describes the performance of a generalist species (Generalist, G) and the solid line of a more specialist species (Specialist, S). (b) Eltonian specialization is defined as the variance in the species impact (instead of performance) on the environment. For a given mean impact, the species impact can be distributed through a large breadth of the environment (G) or be more restricted (S).

Fundamental versus realized-specialization

Beyond the distinction between Grinnellian versus Eltonian-niche, the concept of specialization can be further clarified using the classical distinction between the fundamental and realized niche (Hutchinson 1957). Indeed, both Grinnellian and Eltonian-specialization can be usefully considered as an intrinsic species attribute (fundamental), or alternatively, as a contingent property dependent on abiotic and biotic environments (realized). If no distinction is made between realized and fundamental specialization, then disentangling specialization resulting from local adaptation, as opposed to apparent specialization resulting from competitive exclusion and/or source-sink dynamics becomes more difficult (Entling *et al.* 2007).

In practice, the two dimensions (Grinnellian versus Eltonian, and realized versus fundamental) must be seen as the extremes of a continuum. Each dimension emphasize a particular aspect of the ecological niche which often correspond to different objectives and involve different methods. For instance, the analysis of stomach content of the

black-browed albatross (*Thalassarche melanophris*) can be seen as its realized Eltonian-specialization on the coastal food web (Petry *et al.* 2007). Additionaly, inferring the potential breadth of species impact from specific functional traits (e.g., morphology or behaviour) would reflect the fundamental Eltonian-specialization. Investigating the variance in species performance along different resource gradients (or habitats) measured in the field (i.e., including all biotic interactions and dispersal constraint) would reflect a realized Grinnellian-specialization, but measuring species performance across different conditions while controlling for between-species interactions and dispersal constraints, would reflect the fundamental Grinnellian-specialization.

Ecological specialization across spatial, temporal and ecological scales

Spatial and temporal dependence of ecological specialization

Regardless of its particular definition, the great flexibility of the specialization concept has also led to definitions and measurements of specialization at various spatial and temporal scales. The ecological specialization is indeed always implicitly conditioned to a set of resources (or type of impact) considered, and to the spatial and temporal scale at which it is evaluated. For instance, on a continental scale, some insects might be called specialists because they eat plants only within one genus. On the smaller scale of their geographical ranges, the same species could be considered generalists because they feed on numerous species of that genus (Fox & Morrow 1981). Similarly, the realized specialization of a given species measured at a given point in time may only reflect a subsample of what conditions the species can experience during a longer time-period (or a subsample of their functional role. Surprisingly, although this problem of scaling is recurrent and inherent to most ecological patterns and process, ecological specialization is generally treated and quantified as insensitive to differences in temporal and spatial scales. In applied ecology, once specialization is measured (or decided by expert judgment), species are most often simply categorized as specialist or not.

In fact, whether the realized Grinnellian or Eltonian-specialization is found to be constant across locations for a given species mostly depends on four mechanisms which may or may not promote the shift in niche breadth: i) the spatial and temporal fluctuation of environmental conditions and species interactions, ii) local genetic adaptations, iii) phenotypic plasticity of individuals or iv) the sampling design itself if

different environmental conditions are sampled across the locations considered. Specialization may therefore be found to be constant across locations if similar environmental conditions are sampled and if individuals have not experienced different local adaptations (Fig. 2a). Alternatively, the realized specialization may vary across locations if different environmental conditions are sampled in the field and/or if individuals have experienced different local adaptations across these locations (Fig. 2b).

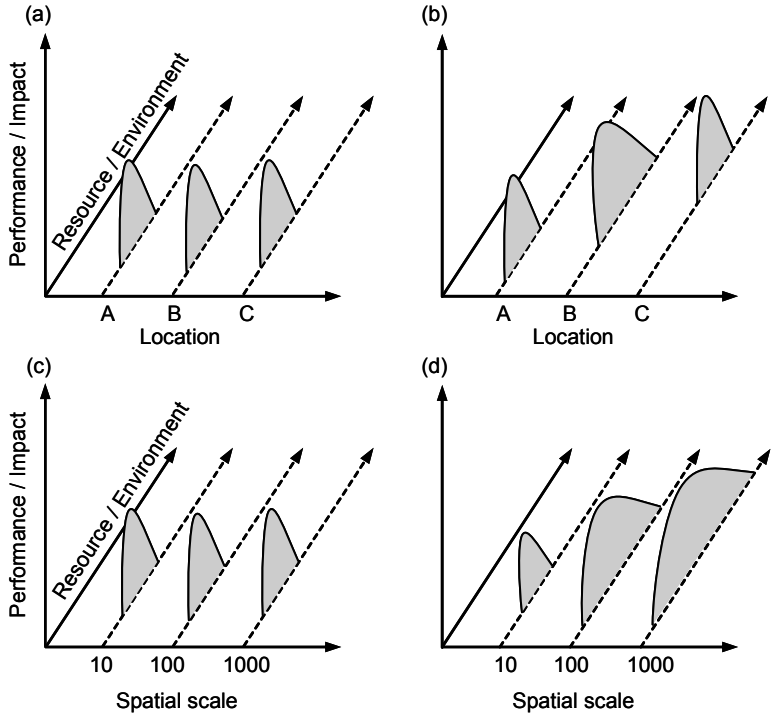


Figure 2: Spatial dependence of specialization: (a) Specialization is often considered as an invariant species-property and to be consistent across locations. (b) Alternatively, species may have different levels of specialization in varying locations. This spatial-dependence of niche-breadth can be induced by true shift in specialization across locations, or by variable resource availability. A spatial-dependence can also be observed when the scale at which specialization is measured increases (c) & (d).

Similarly, a particular species which appears locally specialized for any ecological factor can be found to be equally specialized at larger spatial scales (Fig. 2c). For instance, Krasnov *et al*. (2008) found a scale-invariant pattern in the relationships between local and global host specificity (a measure of niche breadth for parasites). Alternatively, a scale-dependence of ecological specialization is often induced by a sampling effect: increasing the scale of observation increases the number of resources present and used (as well as the breadth of possible functional roles) so that niche

breadth can show a monotonic positive relationship with the scale of observation (Fig. 2d).

The spatial and/or temporal scaling of ecological specialization not only raises methodological issues (e.g., when seeking for unbiased measures of specialization) but also important ecological processes involved in species response to global changes. Indeed, Levins (1962) has early shown that the fate of individuals of more or less specialized species is highly dependent on whether temporal and/or spatial environmental fluctuations are occurring at high frequency (fine-grained) and at low frequency (coarse-grained). This difference in the timing of environmental fluctuations (or in the spacing between environmental conditions) should influence both Grinnellian and Eltonian-specialization. If environmental conditions experienced by individuals are relatively constant during their lifetime (coarse-grain fluctuations), local adaptations will favor individuals with higher level of Grinnellian-specialization (Levins 1968). Similarly, the effects of coarse vs. fine-grained environmental variation can influence different functional traits and the realized Eltonian-specialization of individuals (e.g., variation in water regime on plants, Engelmann & Schlichting 2005).

From individual to community-specialization

The concept of specialization was historically developed as a species attribute (but see Maguire 1973 and Roughgarden 1974), however, it can be interestingly extended to any ecological level (individual, population, species, and community) for applied/management issues (Fig 3). Indeed, some species that are considered as ecological generalists are often heterogeneous collections of specialized individuals (Bolnick *et al*. 2007). This within-species variation of ecological specialization (e.g., the specialization of individuals of a given species) has important conservation implications. For instance, if a species is considered to be specialized to a given resource (assuming that all individuals are equally specialized to this resource), conservation guidelines (e.g., increasing the proportion of a particular resource) could be suitable for some individuals only but negatively affect other individuals not specialized to this particular resource (Bolnick *et al*. 2003).

At the community level, specialization can be estimated as the mean specialization of species present in that community and may be used to derive indicators of conservation interest. For example, the Community Specialization Index (CSI) reflects the relative

abundance of more or less specialized species in a given community (Devictor *et al*. 2008). This indicator was shown to be powerful and robust to reflect community response to spatial and temporal disturbance (Devictor & Robert 2009). Similarly, specialization for communities has been explicitly developed within the context of species interaction networks (Blüthgen *et al*. 2006) or to compare the specialization of food webs and ecosystems (Dunne *et al*. 2002).

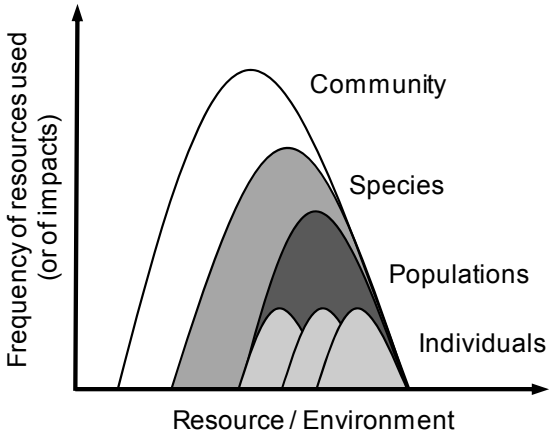


Figure 3: Ecological specialization from individuals to communities. Both Grinnellian and Eltonian-specialization can be usefully defined at any ecological level. A specialized community is composed of a range of more or less specialized species, which are themselves composed of more or less specialized populations, which are finally composed of a range of individuals with differing degrees of specializations.

Scaling up ecological specialization

Obviously, there is not a single appropriate spatial, temporal or ecological scale to measure ecological specialization. Instead, the relevance of using a given spatial, temporal or ecological scale will depend on the objective. In practice, spatial and/or temporal scale-dependence of specialization as well as its variations between ecological levels (from individuals to community) can even be explicitly described. In this respect, Pickett & Bazzaz (1978) suggested that, by analogy with the classical partitioning of regional diversity, ecological niche can usefully be partitioned into local scale (at which interactions among species occur, α-niche) and regional scale (measured across multiple sites, β-niche). Hughes *et al*. (2006) have also proposed that specialization measured at any scale can be partitioned into local (α_S) and regional (γ_S) components. The local and regional specialization can be further related to each other by the between-scale

specialization ($\beta_s = \gamma_s / \alpha_s$) which is influenced by the turnover of resource used by the species across scales (or turnover of species' impact for Eltonian specialization).

This partitioning may be very useful in applied ecology to distinguish local generalists which, in the meanwhile, appear to be specialist at larger scale because they use many resources within habitats, but have a low turnover of resources among habitats (Hughes *et al.* 2000). Interestingly, a similar approach can also be used to scale ecological specialization across ecological levels. Indeed, the total niche breadth measured at any ecological level can be subdivided into a within-level component and a between-level component. In this respect, Roughgarden (1972) proposed to view the total niche width of a population (*TNW*) as the sum of respectively a within and a between individual component (traditionally called *WIC* and *BIC*). This approach was successfully used to assess how much variation in a total apparent specialization (*TNW*) can be explained by variation within or between individuals (i.e., by calculating *WIC*/ *TNW*) (Sargeant 2007).

We suggest that this scaling-up of ecological specialization could be extended to any type of specialization (Eltonian and Grinnellian) and for any ecological level. For instance, for Eltonian-specialization an explicit partitioning of species traits into alpha and beta components can be used (Ackerly & Cornwell 2007). This second type of scaling (i.e., across ecological levels) should also help to investigate variation in specialization in space and time on the long run. For instance, following specific management efforts, tracking both the change in the degree of individuals' specialization and of aggregated entities (population, species) could help to highlight the unsuspected consequences of these efforts (local adaptations, plasticity, niche shifts).

A toolbox for measuring ecological specialization

General principle and widely used approaches

According to the general definitions of ecological specialization (Fig. 1), measuring niche breadth requires to understand i) what environmental conditions individuals can experience (or what is the extent of their impact for Eltonian specialization) and ii) how the performance of individuals (i.e., growth, survival and reproduction) is affected by those environmental conditions (or what is the strength of their impacts). Generally, the practical ways of measuring specialization do not match these theoretical definitions. For example, in the Eltonian view of the niche, each species has a special role. Eugene Odum popularized this view in saying that the niche is the "species' profession" (Odum 1953). But this definition does not provide a clear formal quantification of what should be taken as the actual species' "role" nor of its specificity. Therefore, when functional groups are delineated using this view, or when a species is considered to be specialized in terms of its functional traits relative to other species, Eltonian niche simply describes the degree of "being special". The breadth of impact is then inferred from its "functional distance" to other species, or from the species position in an interacting network, but is hardly quantified directly.

In practice, measuring what environmental conditions individuals are experiencing (or impacting) faces two additional challenges. First, one needs to select a number of meaningful variables most likely to influence species' performances (or to represent species' impacts). Second, the measurement of those variables, along with the quantification of variations in species' performances (or impact), must be unbiased. To overcome these technical constraints, many studies classify species as specialist or not according to arbitrary criteria. When continuous metrics are proposed, they are more or less related to simple approaches reflecting species' needs or species' roles (see Fig. S1, Supporting information). However, in the growing literature of applied and conservation ecology, depending on the question being addressed and on the data available, many other measures of ecological specialization have been proposed at various ecological and spatial scales (see below). We suggest that our review can be turned into a practical and flexible toolbox which encompasses most of the widely-used metrics of ecological specialization.

Fundamental Grinnellian-specialization

To measure fundamental Grinnellian-specialization (Fig. 4a), any measure of niche breadth can be used as soon as the species performances are reflecting robust species-specific characteristics rather than particular species requirements contingent to local and temporal situations. Ideally, Fundamental Grinnellian-specialization should therefore be derived from controlled experiment in which species performances are measured separately across several resources (e.g., common garden experiment, Venail *et al*. 2008) or along a controlled resource gradient (Wright *et al*. 2006). Instead, a priori knowledge on species-specific requirements is often used to produce ad-hoc classification of ecological specialization level (e.g., by attributing an increasing score of specialization from the less to the most specialized species) and considering this specific attribute as a fixed species' characteristic.

Whether or not species distribution models can be used to approximate the fundamental Grinnellian niche at large scale is recurrently debated (Guisan & Thuiller 2005; Kearney 2006; Araújo & Guisan 2006; Soberon *et al*. 2007). On the one hand, the species' fundamental Grinnellian niche could be considered to be more influenced by large-scale bioclimatic variables than between-species interactions (Soberon *et al*. 2007). Hence, to some extent, species distribution models could be used to approximate the fundamental Grinnellian-specialization. On the other hand, large-scale empirical data used in any species distribution model are themselves implicitly shaped by biotic interactions, dispersal limitation and limiting resources (Guisan & Thuiller 2005). As such, they cannot be used to represent the fundamental niche (Kearney 2006). An alternative and emerging research direction for the approximation and the mapping of the fundamental niche is to integrate mechanistic trait-based approaches to species distribution models (e.g., accounting for species-specific physiological constraints or other known aspects of the fundamental niche) (Kearney & Porter 2004).

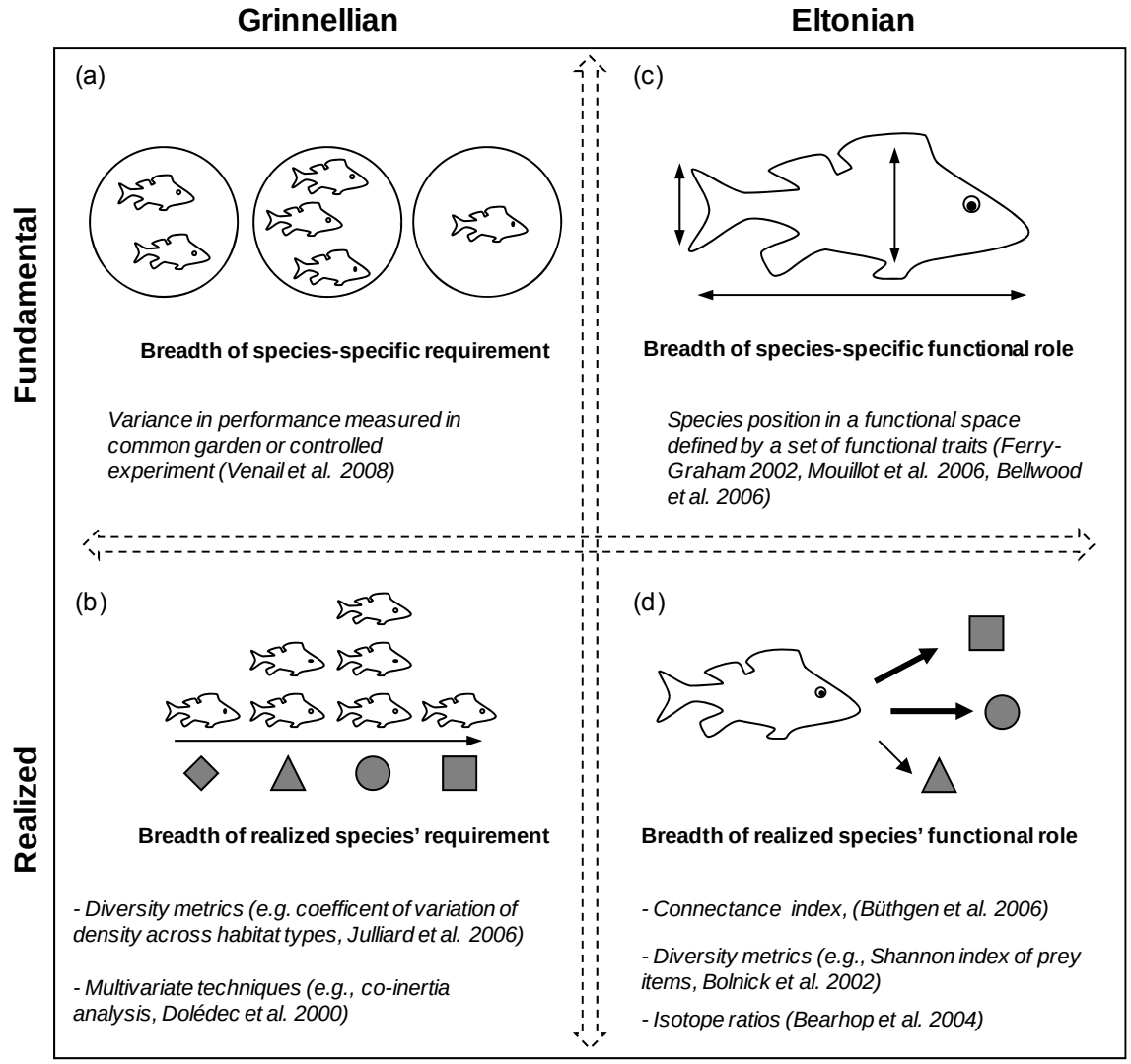


Figure 4: A tool-box for widely used metrics of ecological specialization: Most commonly used metrics of specialization can be positioned in this box according to the type of niche considered (Grinnellian versus Eltonian) and whether the fundamental or the realized specialization is measured. Note that this typology is flexible (i.e. the dashed arrows reflect that specialization metric are more positioned along a continuum rather than belonging to a unique classe). The figure represents a fish for which specialization is measured using different metrics: (a) the variation in species' performance in controlled experiment (three different conditions are materialized by three circles) will reflect its fundamental Grinnellian-specialization; (b) the variation in performance along different resource categories (symbolized by geometrical items) using field data will reflect the realized Grinnellian-specialization; (c) the fundamental Eltonian-specialization would be derived from metrics based on the species-specific functional traits. (d) Finally, the realized Eltonian-specialization will be quantified using the diversity and strength of impact of the species on others (symbolized by geometrical items).

Realized Grinnellian-specialization

The realized Grinnellian-specialization (Fig. 4b) is most often quantified using the diversity of resources used by a species in the field. A single resource can be explicitly considered (breadth of light conditions, food resource) or implicitly encapsulated within discrete categories (e.g. "habitat" classes). Kommonen et al. (2004) have thus quantified butterfly specialization simply using the number of habitat classes where the species occurs. Such measures of specialization implicitly focus on species requirements, but make no distinction between strong interactions and weak or occasional ones and ignore variation in species' performance. To further account for variation in resource used and/or species performance, the most widely used metrics of realized Grinnellian-specialization have incorporated resource frequency (and/or variation in species' abundances or densities, considered as a proxy for performance) in traditional diversity indices (e.g., Shannon, Simpson indices, or coefficient of variation). Using this approach, there are many ways to measure specialization, but all are related to the simplest estimation provided by Levin's niche breadth (Fig. 4a).

These simple metrics have become very popular measures of ecological specialization because they are easy to explain and calculate. However, they are prone to sampling biases and pitfalls, long known to obscure estimates of diversity. One of the well known (although often ignored) problems of measuring specialization using this approach is the integration of resource availability. As early noted by Colwell and Futuyma (1971) this problem simply results from the fact that "the koala is really a generalist on Eucalyptus leaves, while the Virginia opossum is specialized for eating garbage". With most diversity metrics, the use of rare resources is given the same weight as the use of common ones. Therefore, these indices cannot discriminate species with strong shifts in resource preferences from those using resources simply in proportion to their occurrence in the environment (Feinsinger *et al*. 1981). If specialization is quantified relative to what is available, specialization may even decrease with increasing scale, as a smaller fraction of the available resource types are used by a species. In this case, specialization metrics cannot be considered as reflecting species requirements but is more a complex and obscure mixtures between true specialization and resource distribution.

In fact, most simple specialization metrics can be severely biased by the way resources are classified or weighed, and/or if the range, non-linearity, and spacing of the

resource are not appropriately sampled (Colwell & Futuyma 1971). If these problems are not corrected, specialization of species occurring in few samples can be biased (the most classic artifact is that rare species systematically tend to be specialized).

Different approaches have been suggested to deal with these problems which all have their costs, biases and strengths (see Manly *et al*. 2002 for a review). First, diversity indices (e.g., Simpson or Shanon indices) can be adapted to account for resource availability (Hurlbert 1978; Feinsinger *et al*. 1981 and see Smith *et al*. 1982 for a comparison of performance of such modified indices). Second, resource utilization can be defined based on explicit distinction between the raw proportion of a given resource and the proportion of the same resource standardized by its relative availability (so called "electivity", see Winemiller & Pianka 1990 and Hofer *et al*. 2003 for more details).

The realized Grinnellian-specialization metrics could also be derived from species distribution models (reviewed in Guisan & Thuiller 2005) or multivariate analyses (ter Braak 1986; Dolédec *et al*. 2000; Calenge & Basille 2008). Although not being specifically developed to measure specialization, these approaches can make significant contributions to specialization metrics in applied ecology. First, it is relatively simple to extract usual niche characteristics with the use of parametric generalized linear models fitting unimodal response curve (an optimum and a variance around the optimum, Austin 1990). Using this approach, measures of niche breadth can be derived from generalized linear models applied to presence-absence or abundance data.

Second, most of the multivariate analyses such as canonical correspondence analysis (CCA, ter Braak 1986), redundancy analysis (RDA, ter Braak 1987) or co-inertia analysis (Dolédec *et al*. 2000) can be used to analyze, represent and measure niche characteristics. The two characteristics which are easily extracted from these techniques are niche position that measures marginality in species habitat distribution, and niche breadth, that measures amplitude in species habitat distribution and which can be considered as a measure of Grinnellian realized-specialization.

The main advantages of these multivariate approaches from applied perspectives are that they remove colinearity among variables and implicitly search for the most influential combination of variables, and the maximization of species' niche separations. Their drawbacks mostly lie in their underlying hypotheses that are not always met with ecological data. For instance, CCA was originally developed to separate species niches along environmental gradients under the assumption of unimodal response curves. It is

therefore particularly adapted to situations in which limiting factors correspond to large gradients. Similarly, RDA has been used for analyzing linear relationships between species and gradient, and is therefore more adapted to situations where short portions of the responses can be approximated by straight lines. Unlike the former two methods, co-inertia analysis makes no assumption about the shape of species response curves to the environment and gives equal weight to species-rich and species-poor sites (Dolédec *et al*. 2000), making this technique well suited to measure species specialization in many different situations (Calenge & Bastille 2008, Thuiller *et al*. 2005).

Fundamental Eltonian-specialization

The fundamental Eltonian-specialization reflects the intrinsic breadth of species' functional role and is generally measured using its species-specific functional, morphological, and/or behavioural traits (Fig. 4b). In this respect, Bellwood *et al*. (2006) have proposed to measure the "morphological specialization" of labrid fishes from a Principal Component Analysis performed on morphological traits involved in trophic interactions (e.g., body mass, mouth gape, mandibulae muscle mass). The specialization of a focal species is then defined as its functional distance (according to its traits) from the centre of gravity of a species pool (Bellwood *et al*. 2006; Mouillot *et al*. 2007).

In these approaches (Fig. 4c), measuring functional traits generally does not tell whether the traits of interest have any relation to an actual species' performance or what is the real species' impact (Ferry-Graham *et al*. 2002). Alternatively, measuring fundamental Eltonian-specialization can explicitly use specific effect traits directly reflecting the ability of the species to perform a particular impact (e.g., specific leaf area and plant height are highly correlated to competitive strength for light). Note that in any case, measuring fundamental Eltonian-specialization using averaged species characteristics is generally silent on the realized Eltonian-specialization of particular individuals in the field, and is also silent on their actual requirements (Grinnellian specialization).

Realized Eltonian-specialization

The realized Eltonian-specialization of a given species (Fig. 4d) is most often measured as the number of partners or 'links' between the focal species and others (e.g. number of preys, number of pollinator species visiting a flowering plant), or in more

complex connectance metrics (Blüthgen *et al*. 2006; Blüthgen *et al*. 2008). Interestingly, in case of mutualistic interaction network (e.g. pollinators and plants) measuring the specialization of each side of the network, often reveal asymmetric specialization (Bascompte et al. 2003). Accounting for this asymmetry (instead of focusing on the specialization of one side only) can help to better describe whether and how species are affected by disturbance (Ashworth et al. 2004).

When quantifying specialization of interaction networks, the number of interactions between the focal species and others is most often simply counted, ignoring variation in the strength of interactions (Blüthgen *et al*. 2008). Alternatively, as for Grinnellian-specialization, diversity metrics (e.g., Simpson or Shannon diversity index) can be used to include variations in interaction frequency between a focal species and its interacting partners. These indices can also be adapted to account for the availability of the interacting partners (e.g., the availability of preys for a predator, Sargeant 2007).

As most of measured species' impacts result from "snapshots" of interactions between species at a point in time, tracking stable isotopes has also become a common alternative to estimate the realized Eltonian-specialization, providing for time- and space-integration of the trophic ecology of organisms (Bearhop *et al*. 2004). According to this approach, a more Eltonian-generalist species should interact with a larger breadth of species and have more variation in its isotope composition. For instance, Layman *et al*. (2007) have proposed to use the area on the isotope niche space of the grey snapper (*Lutjanus griseus, L.*), using the stable isotope composition δ13C– δ15N measured on individuals as a proxy for (Eltonian) specialization for that species. Yet, these approaches have been questioned as they can produce misleading results if the isotopic concentrations of basal food sources are not incorporated, and data not appropriately standardized before analysis (Hoeinghaus & Zeug 2008).

Note that although Eltonian niche can be ideally defined as the breadth of impact of a focal species on others (or more generally in the environment), most existing approaches of Eltonian niches cover at most a potential of an impact (fundamental or realized), but rarely this impact. While measuring Grinnellian-specialization have benefited from many technical and empirical syudies, more research is needed to quantify real Eltonian niches.

Flexible and integrative metrics

As discussed above, the literature on ecological specialization (applied and theoretical) is structured according to whether specialization is seen as the breadth of species-specific requirements or of species-specific roles. Most widely used metrics of realized specialization directly reflect this distinction. However, we showed that particular metrics can alternatively be used as reflecting Eltonian or Grinnellian specialization. For instance, diversity metrics (e.g. traditional Shanon index) can be used to quantify the diversity of impact or of requirement. Similarly, connectance index can be used to quantify the number and complexity of links between species (i.e., specialization is seen as a specific role) rather than species needs, or, on the contrary, to mirror species requirements in terms of its interacting partners. Therefore, difference between Grinnellian and Eltonian specialization is more objective-dependent than conditioned on the use of a particular metric.

Although specialization is inherently dependent on the variables considered (a species can be specialized for a given resource and generalist for others), most studies are measuring specialization along a unique niche dimension (e.g. specialization along a resource gradient or for habitats). However, one may be interested in integrating the multidimensional nature of the niche. In this case, a principal coordinate analysis (PCoA) can be used. This method allows to position species in a reduced Euclidean space according to a distance matrix describing pairwise distance between species (Legendre & Legendre 1998). Interestingly, this distance matrix can be derived from the Gower distance which can be calculated with all types of variables (qualitative, quantitative, or a combination of both, also allowing missing data). This method can be used for Eltonian specialization (with variables describing species impacts) or Grinnellian specialization (with variables describing species requirements). The specialization of a focal species can then be defined either as its distance from the centre of the PCoA or by the multidimensional volume filled by its individuals (Mouillot et al. 2007, Carnes & Slade 1982). Similarly, the specialization of a community can be estimated by the multidimensional volume filled by its species.

Discussion and implications

Limits in using the traditional niche concepts to measure specialization

Ecological specialization is one of the most versatile concepts in ecology. Given the multi-dimensional and the multi-scale nature of niches, it can, by definition, never be fully quantified (Colwell & Futuyma 1971). Moreover, there are several limits in using the concept of ecological niche in applied ecology. Ecological niche has evolved semantically following the maturation of ecological concepts and paradigms as well as following the increase in statistical tools to measure niche dimensions. Given this complex historical development and its multiple (often conflicting) interpretations, ad-hoc definitions of ecological niche are frequently newly proposed in applied ecology, with no strong connection with ecological theory (Milesi & López de Casenave 2005).

In reviewing how ecological specialization is generally defined and measured, we have highlighted the limits and values of various definitions and metrics of specialization, rather than proposed a unique definition. We also showed that the need for explicit distinctions between Eltonian and Grinnellian specializations is context-dependent. Indeed, whether interaction between a given species and particular resources is considered as a species' requirement or as a species' impact is more a matter of point of view than a clear and objective distinction. Moreover, measuring functional traits can both reflect potential species requirement (i.e. a response trait) and potential species impact (i.e. an effect trait, Lavorel & Garnier 2002). In this context, Chase & Leibold (2003) proposed that ecological niche could alternatively be viewed as an irreducible product of the species-environment interaction resulting from both species' impacts and requirements. This is particularly relevant for species that depend on niche construction, a process whereby organisms, through their impacts on habitats or on other species, modify their own niche (Odling-Smee *et al*. 2003). In this case, as species' impacts will affect species' persistence only if these impacts influence, in turn, species' requirements, the inclusion of impacts as part of the niche is even less needed (Vasquez *et al*. 2005).

Overall, drawing a firm distinction between each concept of niche and selecting one as being better than others seems neither necessary nor useful. In fact, given the great inherent flexibility of the concept of ecological niche, we think that searching for a definitive definition and a unique metric of ecological specialization is probably

meaningless. An alternative approach is to popularize major strengths and weaknesses of using definitions and/or metrics of ecological specialization to allow their best use in applied problems.

Interestingly, in spite of the limitations mentioned above, we showed that most studies have explicitly or implicitly distinguished species' requirements from species' impacts when measuring specialization (with or without explicit reference to Grinnell, Elton or to any conception of the niche) and as a species-specific attribute (i.e., fundamental) or as dependent on particular environmental conditions (i.e., realized). Therefore, the distinction between classical facets of the ecological niche (Eltonian and Grinnellian vs fundamental and realized) still remains a practical heuristic basis which is flexible enough to embrace most concepts and metrics linked to specialization while discriminating different objectives (Fig. 4).

Other useful approaches for measuring ecological specialization

Along the traditional main lines underlined in this review, the concept of specialization and its related metrics could be further defined according to narrower distinctions useful for different applied investigations. For instance, Ferry-Graham *et al*. (2002) proposed to distinguish functional specialization (driven by morphological traits) from behavioural specialization (a species whose behaviour allows capturing specific items among a pool of preys available). This distinction can be used to assess different management efforts targeting species with specific role due to their functional traits or behavior. Following our framework, these two types of specialization can be classified as Eltonian-specialization (the breadth of impacts). Similarly, food-resource specialization and habitat-specialization may further be differentiated as two aspects of Grinnellian-specialization for specific management targets when needed (see e.g., Hughes 2000; Silvertown *et al*. 2006).

Alternatively, specialization can be measured independently of any firm distinction between Grinnellian and Eltonian niche. For instance, Fridley *et al*. (2007) have recently proposed to measure ecological specialization based upon the assumption that generalist species should co-occur with many different species across sites, whereas specialists should co-occur with relatively few species. This attribute can be easily quantified from species co-occurrence patterns. This metric indirectly quantifies the Grinnellian realized specialization as it reflects the species response (in terms of

occurrence) to environmental heterogeneity (which is embodied by the diversity of co-occurring species). This measure also implicitly incorporates each species impact on each other (as co-occurrence patterns are shaped by biotic interactions). As such, this metric also indirectly reflects Eltonian realized specialization (the capacity to interact with few or many species).

More generally, the distinction between realized vs. fundamental and Grinnellian vs. Eltonian niche is far from offering a complete typology of the ecological niche. Other concepts, more or less related to historical developments of the niche concept have also been proposed (e.g., niche construction, Odling-Smee *et al*. 2003; ecological versatility, MacNally 2005).

Moreover, the distinction between fundamental and realized niche have mainly been influenced by the view that interspecific competition was the major driving force of community structure and composition. This limitation has refrained authors using Hutchinson niche from considering other interspecific interactions. Recently, Vasquez (2005) has proposed to refine the Hutchinson niche to differentiate between (i) the multidimensional fundamental niche, corresponding to the environmental space delineated by all biotic and abiotic environmental factors within which the species can maintain viable populations. Only some combinations of these environmental factors are realized at a given point in space and time, which correspond to the realized environmental space (Jackson and Overpeck 2000). Following environmental changes, new combinations of environmental factor can therefore allow species to expand into previously unrealized portions of their fundamental niche. ii) The realized niche is the portion of the realized environmental space in which the populations actually exist in the field. iii) Finally, the portion of this realized environmental space in which individuals are not present (but could survive and reproduce) is the potential niche. The difference between the realized and the potential niche is thus contingent to source-sink dynamics, demographic stochasticity, and dispersal constraints.

In this latter approach, the major difference with classical distinction between fundamental and realized niches is that fundamental specialization incorporates other species and is only conditioned on the ability of species survival. These extensions of traditional definitions have the great advantage to correspond to more realistic situations encountered in applied ecology. Overall, we suggest that any metric of ecological specialization should be accompanied with a well-defined conception of the

niche. Indeed, the use of ecological niche and specialization is not a problem *per se* but confusions often rise from the mismatch between the data used, the theoretical assumptions and the question being addressed.

Management implications and future research directions

Once Grinnellian and Eltonian niche breadth are identified as two different aspects of ecological specialization, one may ask whether Eltonian and Grinnellian specialization can be measured independently. Indeed, these two aspects of ecological specialization are not independent in either their evolutionary origin or their ecological outcomes. To our knowledge, the statistical and biological relationships between Eltonian and Grinnellian specialization have hardly been explored. Investigating whether and how they are related should provide considerable insight into several theoretical or conservation/management issues.

First, the comparison between the Grinnellian and Eltonian niches of two species can be useful to explain the maintenance of species diversity in a given community. Indeed, defining the difference between Grinnellian niches (considered as the distribution of species performances across environments) and the difference between Eltonian niches (using a trait-based definition), share similarities with the contrast between fitness differences and niche differences suggested by Chesson (2000). Following this parallel, stable coexistence of many species within communities could benefit from similar Grinnellian specialization, but differences in Eltonian specialization. For instance, Bellwood *et al*. (2006) found that specialized morphologies of fish do not necessarily equate to specialized diets, and suggested that this trophic versatility explains the origin and maintenance of high biodiversity on coral reefs.

Second, the distinction between Grinnellian versus Eltonian-specialization should help to investigate the so-called *biotic homogenization process*. According to this process, human-induced environmental and climate changes act as non-random filters, selecting species best able to survive within modified ecosystems. Despite the increasing use of the term biotic homogenization in conservation biology, practical means to measure this process are still largely missing and the underlying ecological mechanisms involved remain unclear (Olden 2006). While growing evidence suggests that Grinnellian-generalists tend to replace Grinnellian-specialists following habitat degradation (Devictor *et al*. 2008), whether this process is different when using

Eltonian-specialization is unknown (but see Smart *et al*. 2006). This issue is a matter of considerable conservation interest as the loss of specific functions (more likely performed by Eltonian-specialists) can eventually lead to the loss of ecological interactions and processes (often providing ecological services) with important consequences at the ecosystem level (Kremen 2005).

Third, the consequences of scale-dependence of realized versus fundamental specialization remain poorly understood in conservation biogeography so that species are most often considered to be equally specialized across spatial scales. For instance, macroecological theory predicts that fundamental Grinnellian-generalists should have larger geographical range than Grinnellian-specialists (the so-called Brown hypothesis, Brown 1994). Yet, for a given fundamental Grinnellian-specialization level, geographically rare, and therefore extinction-prone species, are more likely to persist if they tend to be realized-Grinnellian generalists locally (Williams *et al*. 2006). Recognizing the spatial and temporal dimensions of the niche will also help to reframe different applied problems. For instance, observed colonization events of a specialist species can result from new occupations of its potential niche, from true shift in its fundamental niche or from rapid changes in the realized environment. In each case, management implications (e.g., to reinforce or control the population increase) would be different. In particular, management options can shape the available potential niche at local and short-time scales (to increase or limit extensions of the realized niche) whereas halting a natural shift of the fundamental niche would need to consider management in accordance with evolutionary time scales.

Fourth, highlighting similarities and differences between Eltonian and Grinnellian specializations can also play a critical role in species introduction or reinforcement. For instance, well documented damages have followed species' introduction when species' requirements were considered but their impacts not anticipated (e.g., unexpected negative effects of host-specific biocontrol agents can result from ignored indirect impacts of released predators and parasites on other species, Pearson & Callaway 2003).

Finally, acknowledging the difference between Eltonian and Grinnellian specialization can help to formulate explicit objectives of conservation plans. For instance, conservation programs focusing on the protection of few individuals of an endangered species are more related to the Grinnellian specialization (what individuals need to survive) while other programs focusing on ecological services and ecosystem

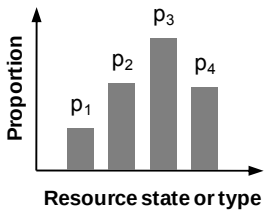
functioning are more interested in Eltonian specialization (what specific functional roles are lost following the depletion of large populations).

Concluding remarks

Although ecological specialization is a conceptual cornerstone in ecology, its definition is highly context dependent and inconsistently used in applied ecology. We have shown in this paper how specialization is intrinsically linked to the niche concept, being nothing more than a measurement of a particular characteristic of the niche (the niche breadth). However, just as the niche is "one of the most confusing, and yet important topics in ecology" (Root 1967), measuring ecological specialization has also created confusion in the literature, mostly due to the increasing diversity in the quantity and type of data available as well as in the scales considered. In integrating the classical Grinnellian/Eltonian and realized/fundamental axes underlying the niche concept, this review should help to place most of applied measures of ecological specialization in their most appropriate empirical and theoretical use. By doing so, one should be more easily able to compare results of different studies and draw consistent conclusions on the impact of global changes on specialist versus generalist species (Colles et al. 2009). Testing explicit predictions based on the different facets of ecological specialization should also open new avenues for the connection between biogeography, evolutionary and functional ecology, one of the most pressing challenges for ecology and conservation.

Supporting Information

(a) Species'needs (Grinnell)



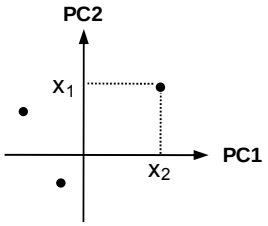
A diversity metric (Simpson, Shannon, coefficient of variation) is often used to measure specialization. All these metrics are more or less related to Levins' metric (1968):

$$\textit{Specialization} = 1/(\Sigma p_i)^2$$

Where p_i is the proportion of resource (or habitat) i used, and summed across the number of resource states.

Such simple measures of niche breadth are biased if species and/or resources are not appropriately sampled (Colwell & Futuyma 1971). Moreover, these measures do not take into account resource availability. Other measures, adjusted for species performance and/or resource availability have been proposed (Hurlbert 1978, Feinsinger *et al.* 1981) with their own strength and weakness (Smith *et al.* 1982).

(b) Species'roles (Elton)



A PCA is performed on functional traits (or processes) of several species. Each dot represents the resulting species' positions in the PCA. Specialization is derived from the distance of species'position to the centroid:

$$\textit{Specialization} = (\Sigma x_i)^{1/2}$$

Where x_i are the coordinates of the species along a subset of relevant selected PCA axes.

The distance of the species' position to the PCA centroid provides a quantitative estimate of functional specialization (Belwood 2006). This approach can be used to measure the functional distance between species (Mouillot *et al.* 2007) and to identify functional groups (Rosenfelt 2002). The selection of axes used is subjective and based on what are considered to be key functions and /or processes.

Two classical approaches to measure Grinnellian and Eltonian specialization: (a) Measures of Grinnellian-specialization are most often related to Levins' metric (Levins 1968). The principle of such approach is to use a fitness profile (abundance is often used as a proxy for fitness) and/or to quantify the proportion of resource used among different resource states (or type) to infer the breadth of species' needs. (b) Conversely, measures of Eltonian-specialization are most often based on functional traits. The principle of such approach is to quantify the relative functional distance between species based on their functional traits to infer their intrinsic functional role in communities or ecosystems.

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Manuscrit 5

Experimental niche evolution shapes the diversity-productivity relationship

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En préparation.

Résumé en Français

L'importance de considérer l'influence des processus adaptatifs sur la forme de la relation entre biodiversité et fonctionnement des écosystèmes est de plus en plus claire. Cette approche souligne que la capacité des espèces à exploiter leur milieu résulte de leur niveau d'adaptation et par conséquent de leur histoire évolutive et donc doit être considérée dans l'étude de la relation entre diversité et fonctionnement. L'évolution peut, selon les conditions de sélection, modifier le niveau de complémentarité entre les espèces et aboutir à différents types de relation.

Dans cette expérience nous avons soumis pendant 64 jours une vingtaine d'espèces bactériennes marines à deux traitements de sélection très contrastés. D'une part elles ont rencontré individuellement un milieu homogène avec une seule source de carbone (chaque espèce dans une seule source de carbone). En présence d'une seule ressource on s'attend à une spécialisation sur celle-ci. D'autre part, elles ont été exposées à un milieu complexe composé d'un mélange de 31 sources de carbone différentes (chaque espèce au mélange de 31 sources de carbone). Ce traitement devrait favoriser l'émergence de stratégies généralistes capables d'exploiter plusieurs ressources.

Comme prévu, la sélection sur le mélange de ressources à sélectionné des espèces avec une niche plus large que la sélection en milieu simple. Dans les deux cas, une relation positive entre richesse spécifique et productivité à été obtenue, indépendamment de l'histoire évolutive des espèces. Cependant, à diversité égale, les généralistes présentaient une plus forte productivité que les ancêtres et les spécialistes.

Egalement, une plus forte complémentarité des niches des spécialistes a résulté en une relation diversité-productivité avec une pente plus forte. L'ajout d'une espèce aurait plus d'effet sur la productivité si celle-ci est une spécialiste que si celle-ci est généraliste. Au final, chez les spécialistes et généralistes, les assemblages de diversité maximale (20 espèces) présentaient le même niveau de productivité.

Nos résultats ont montré qu'alors que chez les espèces ancestrales la relation est basée sur un « *sampling* », chez les espèces évoluées, qu'elles soient spécialistes ou généralistes, c'est leur plus forte complémentarité qui explique la relation positive.

Ces résultats expérimentaux confirment l'importance de considérer l'histoire évolutive des espèces dans l'étude des propriétés écosystémiques.

In preparation

Experimental niche evolution shapes the diversity-productivity relationship

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Abstract

The relationship between biodiversity and ecosystem functioning (BEF) has become a cornerstone of community and ecosystem ecology (Kinzig et al. 2002; Loreau et al. 2002; Naeem et al. 2009), and an important criterion for making decisions in conservation biology and policy planning (Srivastava and Vellend, 2005; Duffy, 2009). It has been recently hypothesized that evolutionary history should influence the BEF relationship because it determines species traits and thus species' ability to exploit resources (Cadotte et al., 2008, 2009). Here, we test this hypothesis by combining experimental evolution with a BEF experiment. We isolated 20 strains from a marine environment and evolved each to be a generalist or specialist strain (Barrett et al., 2005). We then tested the effect of evolutionary history on the strength of the BEF relationship with assemblages of 1 to 20 strains constructed from the specialist, generalist, and ancestral lines (Bell et al., 2005). At equal diversity, assemblages of generalists were more productive on average because of their superior ability to exploit the environmental heterogeneity (Loreau 2001). The slope of the BEF relationship is however stronger for the specialist assemblages because of enhanced niche complementarity. Taking together, these results show the strength of the biodiversity effect on ecosystem functioning depends critically on the legacy of past evolutionary events.

Two fundamental ecological mechanisms are thought to generate positive BEF relationships (Tilman et al. 1997; Loreau 1998, 2000). First, species may occupy complementary ecological niches, for example by feeding on different resources. In communities of functionally complementary species, niches are better filled with increasing diversity, resulting in a positive BEF relationship. Second, the 'selection effect' results in a positive BEF relationship because high-functioning and competitively-dominant species are more likely to be found within species rich communities (i.e. sampling effect). Both mechanisms require a detailed understanding of species' phenotypic traits (Diaz and Cabido, 2001; Diaz et al., 2008; McGill et al. 2006), but there has been virtually no effort within the BEF literature to integrate the basic evolutionary rules governing the distribution of species traits within ecological communities and ecosystem functioning (Venail et al 2008, Cadotte et al., 2008; 2009).

One important trait determining complementarity is the degree of specialization, i.e. the ability of a species to perform well in a limited range of environmental conditions. Species niche width will tend to evolve to match the amount of available environmental variation (Levins 1968). In simple and homogeneous environments, specialized types are expected to evolve, while generalists are expected in a complex environment composed of many resources (Levins 1968; Futuyama & Moreno 1988; Kassen 2002; MacClean, 2005). Because the degree of specialisation of the different species in the community could alter the BEF relationship (Loreau 2001; Thébeault and Loreau, 2003), understanding the BEF relationship must account for both the evolutionary forces driving trait diversity as well as how trait diversity translates into functioning.

By definition, specialists feeding on different resources do not compete with each other, which is to say that they occupy different niches and their effects on ecosystem functioning (here productivity) are additive (i.e. a straight line – Loreau 2001; Fig 1). However, the increased ability to exploit one resource might come with a lower ability to exploit any other, i.e. there is a tradeoff between resource usage intensity (Kassen, 2002; Fig 1a). For any tradeoff shape, evolution toward generalization will affect the BEF relationship (Fig. 1b; Supplementary Material 1). First, ecosystem functioning at low diversity should be lower for specialists because they are inefficient at exploiting environmental heterogeneity. Second, generalization should increase productivity to a

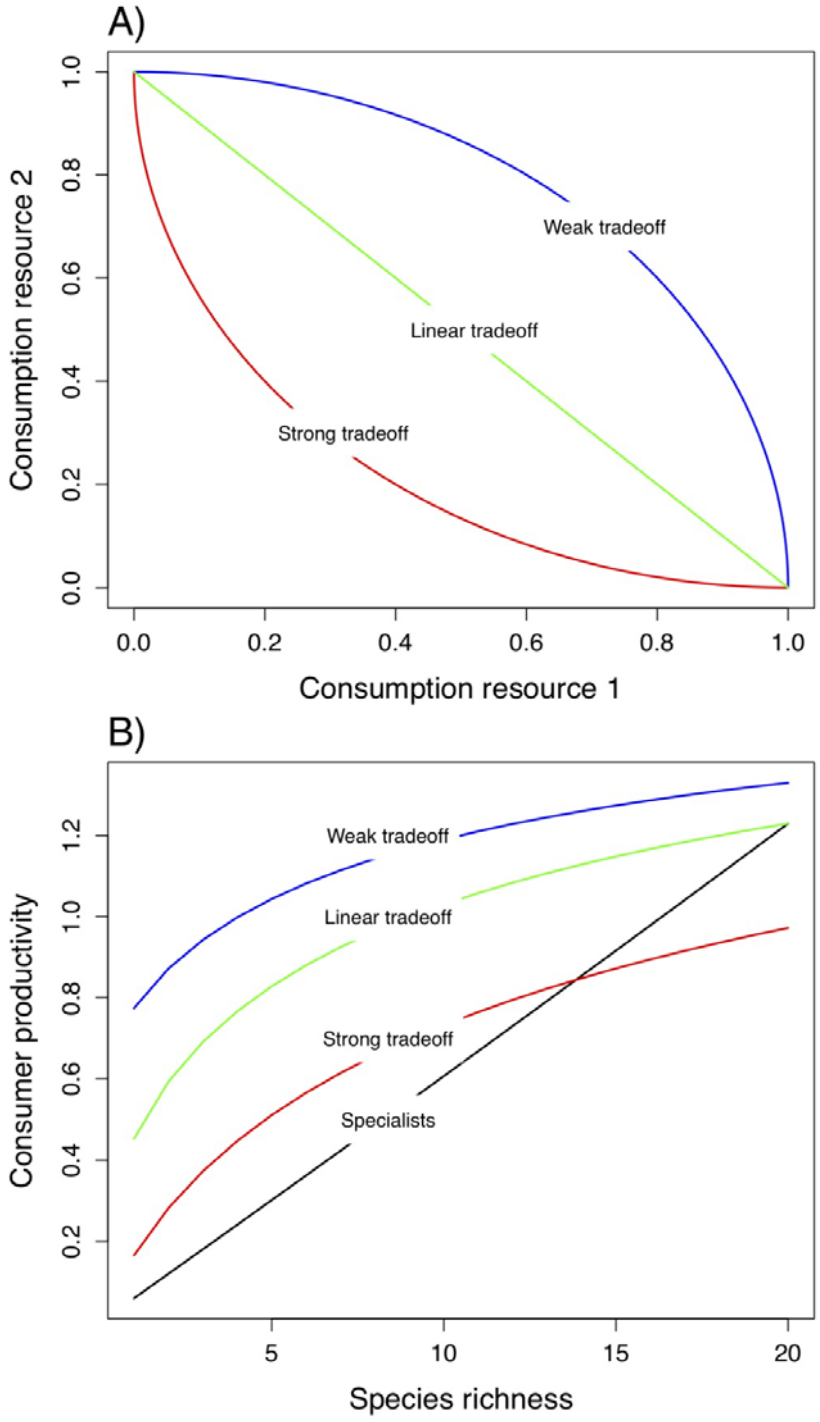


FIGURE 1: We have built a theoretical model that provides us with simple predictions on the effect of niche specialization on the shape of the BEF relationship (full details are given in supplementary material). **a.** Hypothetical relationship between the consumption rates of a generalist species on two resources for different tradeoff functions. The joint performance should follow a straight line if the specie's performance perfectly tradeoff between the different resources. Any deviation to this expectation reflects a relative cost (strong tradeoff) or benefit (weak tradeoff) of generalization. **b.** The BEF relationship will be affected by generalization and the shape of the tradeoff. The figure represents a simulation experiments with 20 resources (R_i) and up to as many consumer species (C_j). The dynamics of this system is given by this simple chemostat model:

$$\frac{dR_i}{dt} = eR_0 - eR_i - \sum_{j=1}^{N} \alpha_{ij} R_i C_j$$

$$\frac{dC_j}{dt} = \sum_{i=1}^{S} \alpha_{ij} R_i C_j - mC_j - eC_j$$

where e is the dilution rate, R_0 is the resource concentration in the inflow (for simplicity we suppose all resources have the same concentration in the inflow), α_{ij} is the per capita consumption rate of the resource i by consumer j and m is the mortality rate. Details on the model and analytical results are given in the Supplementary material. The ecosystem productivity at equilibrium is equivalent to the total resource consumption and is given by $\Phi = \sum_{i}^{N} \sum_{j}^{S} \alpha_{ij} R_i C_j$. In our simulation parameters are $R_0 = 1$, $e = 0.1$, $m = 0.1$. We specified for the specialist the consumption rate for its preferential resource is $\alpha_{i=j} = 1$ and $\alpha_{i \neq j} = 0$ for the alternate resources. For the generalists, we specified the performance on the preferential resource is $\alpha_{i=j} = 0.6$ and an equal partitioning of the consumptions rates between alternate resources that is summing to 0.4 for the linear tradeoff, 0.1 for the strong tradeoff and to 0.55 for the weak tradeoff. We simulated communities of 1 to 20 species for specialists and generalists with linear, weak and strong tradeoffs. The generalization has three effects on the BEF relationship: it influences the productivity at low diversity (intercept), the magnitude of the diversity effect (the slope) and the shape (non-linear).

greater extent at low diversity than at high diversity, and thus reduce the strength of the BEF relationship (Fig. 1a; Supplementary Material 1). Generalization also increases niche overlap and thus should produce a nonlinear saturating BEF relationship (Fig 1b). Specifically, we predict that the ecosystem functioning will be higher for generalists at low diversity and the strength of the BEF relationship (the slope) will be reduced in communities made of generalists.

We tested these predictions by experimentally evolving niche specialization and conducting BEF experiments (as in Bell et al. 2005). Briefly, we promoted the evolution of generalist and specialist strategies from 20 ancestral bacterial strains that had been isolated from a marine environment. The strains were grown in two different environments consisting either of a single resource (carbon substrate) or of a heterogeneous mixture of 31 resources. Strains were serially transferred to fresh media every 48 hours for 32 transfers, allowing evolutionary adaptations (Buckling et al., 2009). Strains grown in the heterogeneous environments (hereafter called 'generalists') tended to have a higher performance on a wide array of substrates compared to strains that evolved in a homogenous environment consisting of a single resource (hereafter

called ‘specialists’) (see below). We could then construct assemblages consisting of species that originated from the same ancestral strain but with different evolutionary histories.

We conducted a BEF experiment following the experimental evolution of the bacteria for both evolutionary scenarios (generalist, specialist) and with the ancestral strains. We used productivity as our measure of ecosystem functioning, which was approximated by the light absorbance of each community after 48 and 72 hours (Venail et al. 2008). The selection period resulted in a substantially increased productivity at all levels of species richness after 48 and 72 hours for both specialists and generalist assemblages (Fig. 2).

The productivity significantly increased with the log of species richness ($F_{1,1506}$ = 291,2 , $P < 0.001$ and $F_{1,1506} = 179.2$, $P < 0.001$ for 48 and 72 hours respectively). The productivity in monocultures differed significantly between treatments ($F_{2,1506} = 1751.9$, $P < 0.001$ and $F_{2,1506} = 2309.3$, $P < 0.001$ respectively), with ancestors performing the worst, followed by specialists and the generalists performing best. The slope (strength) of the BEF relationship also differed significantly between treatments ($F_{2,1506} = 16.2$, $P < 0.001$ and $F_{1,1506} = 8.8$, $P < 0.001$ respectively), being stronger for specialists. The effect on the intercept and the slope resulted in similar productivity for the specialists and the generalists at high species richness. The productivity of ancestors was however always lower than both evolved treatments for every level of species richness. The difference of the slope of the BEF relationship between specialists and generalists was even stronger after 72 hours, a result similar to what is observed for diversity-ecosystem functioning experiments conducted with plant (Tilman et al., 2001; Cardinale et al., 2007) and marine communities (Stachowicz et al. 2008).

We investigated whether the difference in the shapes of the BEF relationship resulted from specialization by conducting assays of each strain grown on each of the individual 31 carbon sources and estimating their niche width. We recorded for each strain the number of substrates it was able to exploit. Strains cultured on single substrates adapted to fewer substrates than strains cultured on mixed media (Table 1 and Fig. 3).

TABLE 1: The effect of experimental evolution on specialization. Niche specialization is assessed from performance assays of each individual strain on the 31 carbon sources. A strain is considered able to exploit a substrate when its absorbance at 590 nm after 48 hours is larger than the 95% distribution of the blanks.

	Ancestors	Specialists	Generalists
Nb. of substrates used per species	4.00 ± 0.69	4.80 ± 0.51	10.75 ± 1.49
Nb. of substrates used by the 20 species community	16	14	29
Productivity of monocultures on the mixed media	0.42 ± 0.01	0.53 ± 0.01	0.61 ± 0.01
Average maximum productivity for each substrate	0.82 ± 0.05	0.84 ± 0.06	1.03 ± 0.08

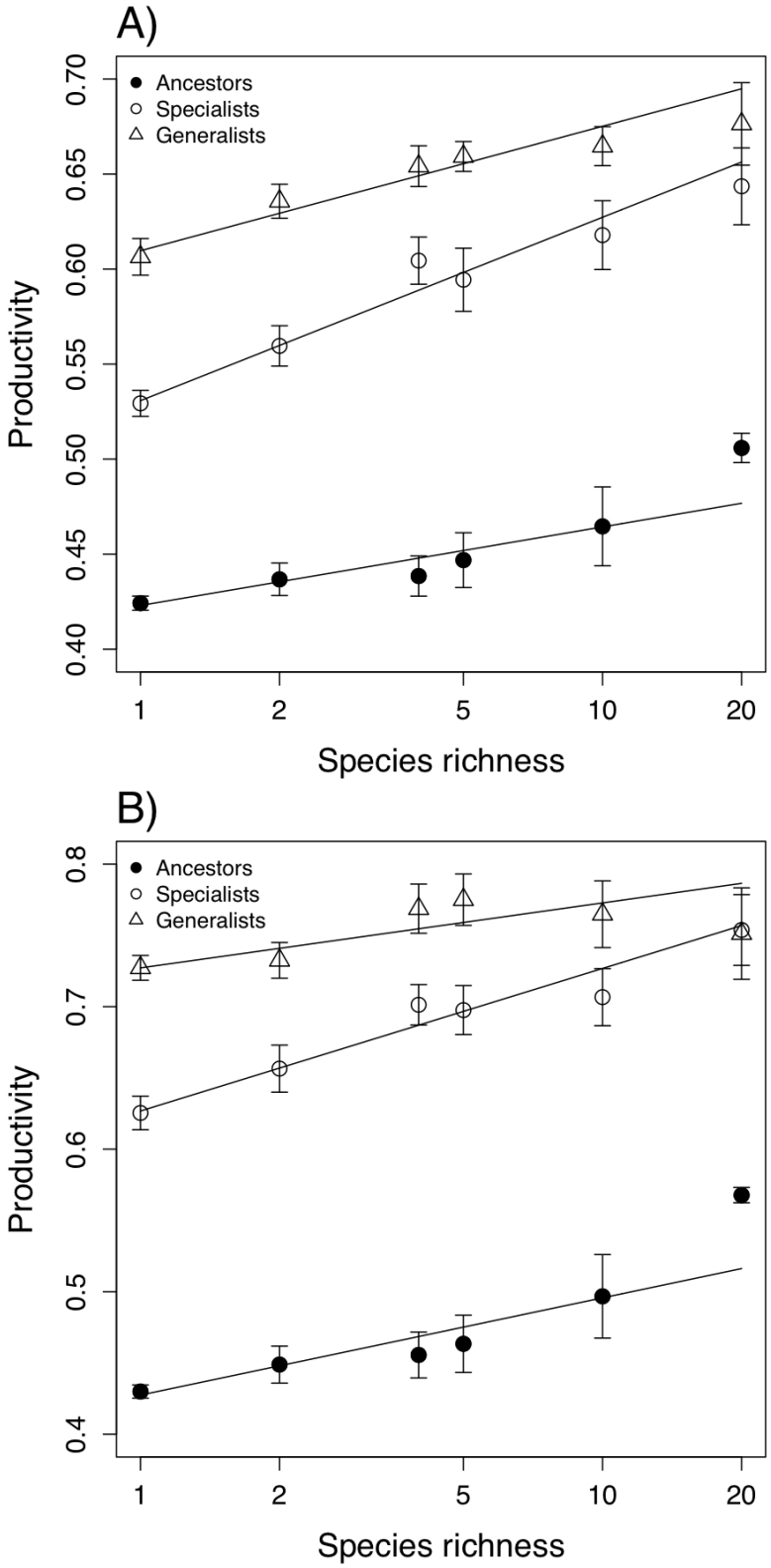


FIGURE 2: Evolutionary treatments affect the shape of the biodiversity-productivity relationships. The productivity is measured as the absorbance at 590nm after A) 48 hours (corresponding to the conditions experienced during the selection experiment) and B) 72 hours. Lines depict the results of the ANCOVA model.

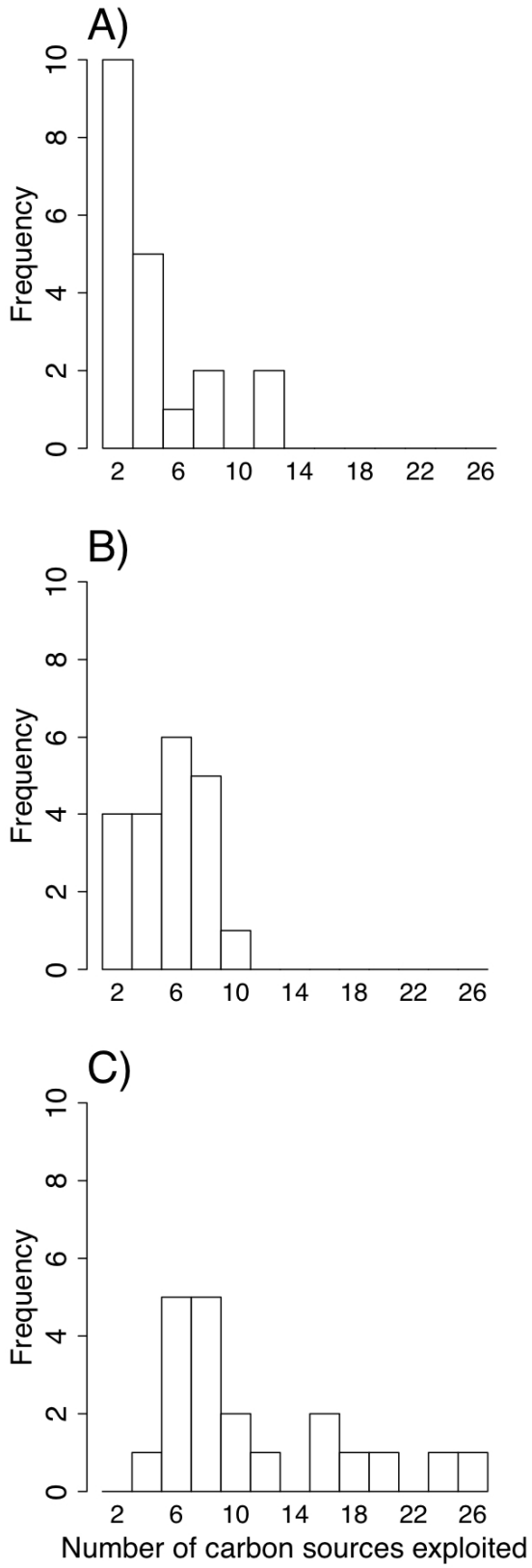


FIGURE 3: Frequency distributions of the number of carbon sources used by each strain, showing niche specialization for each of the evolutionary treatments (see Table 1 for the assessment of niche specialization and comparison between treatments). **a.** Ancestors; **b.** Specialists; **c.** Generalists.

The generalists were able on average to exploit a larger number of carbon sources (10.75 ± 1.49 SE out of the 31 substrates) than the specialists (4.80 ± 0.51 SE; t-test for paired samples, t_{19} = 3.53, P = 0.002). This ability to exploit more carbon substrates is reflected on the performance of the strains when growing on the complex media. The average performance of generalists growing alone on the complex media was higher than the one of specialists (0.61 ± 0.01 SE versus 0.53 ± 0.01 SE respectively; Fig 2, statistics of the ANCOVA above). The average performance of the ancestors was much lower than specialists and generalists (0.42 ± 0.01 SE), most likely because these strains were not yet selected to grow on this medium.

Most strains cultured on the complex media adapted to several substrates without a reduction of their performance on each substrate (Table 1). The maximal performance recorded for each substrate was significantly higher for generalists than the maximal performance for specialized strains (Table 1, t-test for paired samples, t_{30} = 2.95, P = 0.006), which suggests there was no tradeoff. Other studies have also shown that the concurrent adaptation to multiple resources does not always limit the capability to exploit each resource individually (Barrett et al., 2005 and references therein). In fact, mutations increasing fitness on a given resource can even sometimes increase fitness on other resources (i.e. synclinal selection: Bell 1990, MacLean and Bell 2002; MacLean et al., 2004; Jasmin and Kassen 2007), preventing the occurrence of tradeoffs.

In addition to enhanced complementarity, a better performance of the generalists on each carbon source might thus contribute to the difference in the BEF relationships between evolutionary treatments (Fig. 2). We calculated a niche diversity index (NDI) for every assemblage of the specialist and generalist treatments based on the ability of each species to exploit the different substrates. The NDI is the number of different substrates a community is able to exploit based on the assays of individual species (Salles et al., 2009, see Methods). Given that generalists are more productive on each carbon source, we expect then that for a given NDI the productivity will be larger for the generalist assemblages. We found a highly significant positive relationship between the community NDI and productivity ($F_{1,1004}$ = 424.9 , P < 0.001, Fig. 4) with significantly different intercepts between evolutionary treatments. Our experimental evolutionary treatments had two different roles in the BEF relationship, as it determined species complementarity and maximal productivity at equivalent complementarity.

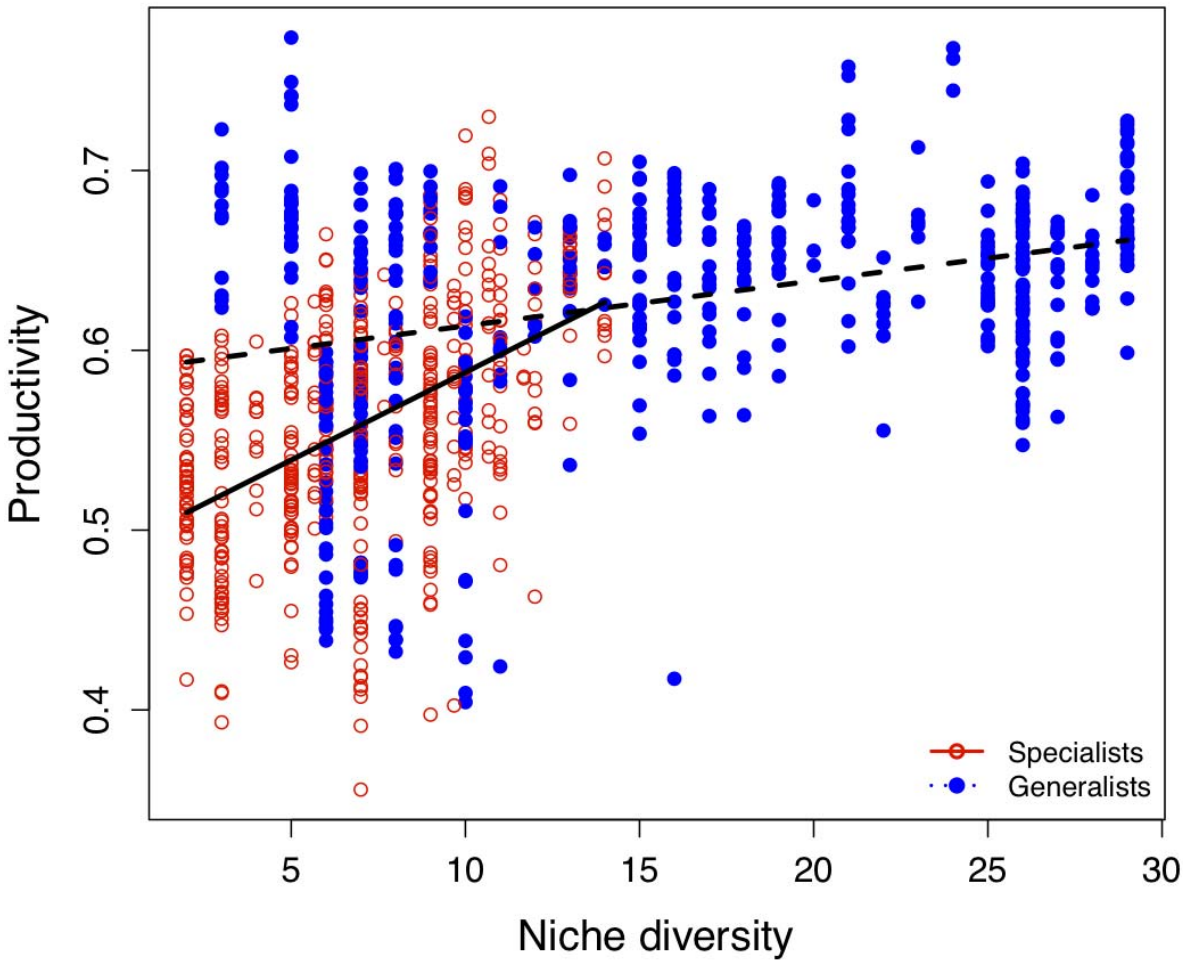


FIGURE 4: The relationship between niche diversity (NDI) and ecosystem functioning. The niche diversity index (NDI) is the total number of carbon substrates a community is able to exploit, assessed from the individual ability of each strain to exploit the carbon substrates and the community composition. Lines depict the results of the ANCOVA model.

The ancestral strains had not previously encountered the experimental conditions, and so it is unsurprising that the intercept of the BEF relationship was greatly reduced as a result of their maladaptation (Fig. 2). Nonetheless, there is still a significant positive BEF relationship, although the slope of the relationship is relatively shallow. Since each species was equally represented in the experiment, it is possible to estimate the degree to which they are associated with higher- or lower- than average levels of functioning (Bell et al. 2009). We found that the inferred species contributions were dominated by a single ancestral strain, whereas they were distributed more equitably in the specialist and the generalist treatments (Supplementary Material 3). The data therefore provide evidence that there are at least two effects of evolutionary history on BEF. First, the BEF

will be stronger for communities of specialists because of enhanced complementarity. Second, if most species are maladapted, few species are able to contribute to functioning and sampling effects dominate. Such a mechanism might be particularly important for ecosystem functioning in variable environments, where species are far from their optimal fitness peaks (Yachi and Loreau, 1999).

In this study we have deliberately evolved independent lineages of specialists and generalists and kept these groups distinct when we performed the BEF experiments. This separation was necessary to compare assemblages of species originating from the same ancestral strain but with different evolutionary histories. It is likely however than in nature species will diversify in complex assemblages of specialists and generalist species depending on the ecological context, particularly the grain of the environmental heterogeneity and the community composition (Abrams 2006). These will combine with physiological constrains to shape tradeoffs in the usage of different resources (Levins 1968, Kassen 2002). We see our experiment as a first step setting the extreme cases of BEF relationship we should expect under different evolutionary scenarios. We suggest more sophisticated approaches are required that link the competitive and environmental contexts to understand how the evolutionary history will move the BEF relationship between the extreme case scenarios described here.

Recent meta-analyses have chosen to highlight consistencies among BEF experiments by demonstrating that many BEF relationships are positive and saturating (Cardinale et al., 2006; Balnavera et al., 2006). Despite these consistencies, an enormous amount of variation remains unexplained. Even within bacterial microcosm experiments, a variety of relationships have been observed and different ecological mechanisms have been inferred (Hodgson et al. 2002, Wohl et al., 2004, Bell et al., 2005; Jian. 2007; see Bell et al. 2009). The experiments described here shed new light on the BEF relationship by demonstrating its malleability under different evolutionary scenarios. Not only can evolutionary pressures alter the shape of the BEF relationship, it might also affect the ecological mechanisms underlying it. We believe investigations should now turn to the understanding the evolutionary pressures that maintain niche diversification in natural communities, along with the tradeoffs involved. Evolutionary theory provides predictions on how species will evolve specialist or generalist strategies when confronted with simple and complex environments and the challenge will be to

include this knowledge within our current understanding on the BEF relationship.

METHODS SUMMARY

We isolated 31 phage-free strains from coastal seawater sampled off the Bay of Blanes, Spain (40°40'N- 2°50'E) on September 20th-21st, 2007. Each strain was individually submitted to a selection period on different single carbon substrates of an Ecoplate ® (with three replicate lines per treatment) to obtain specialists and to a selection period on a highly heterogeneous complex media made from the mix of the 31 carbon substrates of the Ecoplate to obtain generalists. We transferred the strains to a fresh media every 48 h during 64 days of incubation at 20°C. After the selection period, we kept 20 strains among the ones that persisted and conducted a biodiversity-ecosystem functioning experiment. We assembled microcosms with diversity of 1, 2, 4, 5, 10 and 20 strains for each of the three treatments of experimental evolution (ancestors, specialists and generalists), with four different assemblage histories replicated three times, for a total of 1512 microcosms. We measured the light absorbance at 590 nm after 48 and 72 hours to approximate cell density (reported values are corrected by removing the average value of the blanks). We also conducted assays for each strain (three replicates per strain) by incubating every strain on the 31 carbon substrates for 48 h at 20°C. The assays were used to quantify the generality of the individual strains and the niche diversity in the assemblages. The niche diversity index (NDI) is the total number of carbon substrates a community is able to exploit, assessed from the individual ability of each strain to exploit the carbon substrates and the community composition.

METHODS

Bacterial isolation from agar plates.

A bank of strains was obtained from coastal seawater sampled at the Bay of Blanes, Spain (40°40'N- 2°50'E) on September 20th-21st, 2007. Five replicates of 100µl seawater samples were spread on Marine Agar plates (Beckson and Dickinson Difco™ Marine Agar 2216; autoclaved 20 min at 121°C) and let grown for 5 days at 12°C (i.e. *in*

situ temperature at sampling time). Ninety five colonies with distinct morphotypes were isolated throughout 4 weeks, clean streaked three times, and frozen in glycerol at -80°C.

All the 92 bacterial strains were tested for production of prophage (i.e. containing an inducible viral genome) upon treatment with and without (control) the inducing agent mitomycin C at 1µg ml^{-1} final concentration (Weinbauer and Suttle, 1996). Incubations were carried out in 96-well microplates for 24 hours. The growth kinetics of each strain was obtained by inoculating 10 µl of overnight cultures in 200µl Marine Broth culture media (Zobell). Cultures were allowed to grow in the dark at 20°C for 48h. Changes in cell density were measured by light absorbance reading set up at 590 nm wavelength (using a FLUOstar Optima spectrophotometer, BMG) every 15 minutes. We calculated the difference of the absorbance at the stationary phase between control and treated samples, and found a bi-modal distribution (no effect and strong effect of mitomycin C) with a threshold corresponding to a 30% growth reduction by mitomycin C. We define a strain containing cells with an inducible viral genome as a strain with a growth reduction larger than this threshold. Strains under this threshold were considered as "phage-free". We randomly selected 31 strains for the selection experiment.

We also verified the 92 strains were phylogenetically distinct. A single colony of each strain was picked and dissolved in 10µl TE (10nM Tris, 1mMEDTA, pH 8.0) buffer, heated for 5 min at 95°C and centrifuged briefly. One µl of the supernatant was used as the PCR template for 16S rDNA gene amplification. The PCR reaction buffer (25 µl total volume) contained 200 µM of each deoxynucleoside triphosphates in 10 mM Tris-HCl (pH 9.0 at room temperature), 50 mM KCl, 1.5mM $MgCl_2$, 0.2 µM of primers 27F and 1492R (Lane 1991) and ~ 2.5 units of puReTaq polymerase as included in the illustra puReTaq Ready-T0-Go PCR beads kit (GE Healthcare). PCR thermal cycling program was as follows: 95°C for 2 min; 30 cycles of 95°C for 30 s, 50°C for 30 s, and 72°C for 45 s; and 72°C for 7 min. The PCR products were finally sent to AGOWA Sequencing Corporation for unidirectional sequencing using primers 27F and 519R (Lane 1991).

The quality of the sequences was controlled by removing traces of the sequencing primers by using PHRED (Ewing and Green 1998) with a base-calling score of $n \geq 20$. Ambiguous base calls (i.e., "N"s) at the ends of the sequences were trimmed away. All sequences were analyzed using the programs Mallard (Ashelford et al. 2006) and

CHECK_CHIMERA from the Ribosomal Database Project (Maidak et al. 1999). Neither program detected any chimeras. Sequences were aligned using the multiple-alignment program ClustalW (Chenna et al. 2003). Phylogenetic relationships were inferred by the maximum-parsimony and neighbor-joining analyses using PAUP*4.0b10 (Swofford 2002).

Selection experiment:

BIOLOG ® Ecoplate contains 31 different carbon sources (plus one blank) belonging to different chemical families. In addition to the carbon source, each well contains a tetrazolium dye (5 cyano-2,3 ditolyl tetrazolium chloride), which is reduced to a violet-fluorescent formazan molecule when the carbon source is oxidized. Color development is measured spectrophotometrically (with a FLUOstar Optima BMG) at 590nm and is used as a proxy of bacterial production.

Each of the 31 strains was used to found three replicates for each selection treatment. For the specialist treatment, each strain was attributed at random to one of the 31 carbon substrates. The Ecoplates used for the specialist treatment where prepared 2 hours before each transfer with the addition of 140 µl of M9 minimal salts (NH_4Cl, 0.1gl^{-1}; $Na_2\ HPO_4$, 6 gl^{-1}; KH_2PO_4, 3gl^{-1}; NaCl, 0.5 gl^{-1}) with salinity adjusted to 3.56% by the addition of NaCl to match salinity of the environment they were sampled. For the generalists treatment, we obtained a complex media by mixing the 31 carbon substrates. The Ecoplates were prepared as for the specialists and after 30 min the content of the Ecoplate (except the blanks) was transferred into a sterile flask, mixed with an orbital shaker and redistributed in a 96 well sterile microplate.

Each colony was initially grown for 24h at 20°C in 0.5 ml of marine broth (MB) media under constant orbital shaking. 10 µl of this solution was then used to inoculate each well of the Ecoplates of the two selection treatments. We intentionally did not washed the cells of the MB media in order to transfer a slight quantity of this media to assure the survival of the strains. Preliminary trials shown most of the strains were not initially surviving in the Ecoplates without the MB. Consequently, for the first seven transfers we added a minimum quantity of MB, declining from 7% volume to 0% regularly at each transfer. The strains were incubated 48 hours in the dark at 20°C in

humid chambers. Strains were serially transferred to maintain maximal growth rate and renew the substrate. A transfer consisted of inoculating every well from a new plate with 10 µl of the corresponding previous well. Before each transfer, new Ecoplates were prepared as described above. The mixed media was prepared every 10 transfer and stocked at 4°C. The selection experiment ran for 32 transfers (several hundreds generations). At the 32th transfer, the content of all wells of each microplate were mixed with 50% glycerol and frozen at -80°C.

We measured light absorbance after conducting the selection experiment. Several strains or replicates went extinct during the experiment, mostly for the specialist treatment. Therefore we selected the most productive replicate among the selection lines that survived the specialist treatment, and the corresponding selection line of the generalist treatment and of the ancestors. We selected 20 selection lines out of the 31 that were subject to the selection experiment.

Biodiversity-ecosystem functioning experiment:

We assembled random combinations of species at 6 levels of species richness for each treatment (ancestors, evolved specialists and generalists). We used an experimental design that enabled separation of the effects of species richness and species composition (Bell et al., 2005; 2009). The experimental design is a set of $20/s$ microcosms, each with s species present. Within this set, the microcosms are constructed by sampling the 20 species without replacement. The construction of a system of microcosms is carried out independently four times. Therefore, for any given s, the number of microcosms considered is 3 X 4 X $20/s$. We chose the level s to be every factor of 20 (s = 1, 2, 4, 5, 10, 20). Each system is replicated three times, for a total of 1512 microcosms.

Bacterial communities were assembled in 96 wells - 1ml sterile microplates. Each strain was initially grown for 24 h at 20°C in 6 ml of marine broth (MB) media under constant orbital shaking in humid chambers. The cultures were centrifuged (5 min at 3 500 r.p.m) and washed by eliminating the MB and adding 6 ml of the M9 minimal salts with buffered salinity. Because the strains had different productivities in the MB, we first measured cell density by flow cytometry (as describe in Bouvier et al. 2007) to later

adjust the cell densities at the inoculation in the BEF experiment. We adjusted the cell density at a concentration of 0.5 X 10^{-6} cells ml^{-1} with buffered M9. The bacteria were left in starvation for two hours and then we completed the assemblages by distributing 20 µl (40 for the monocultures) in the appropriate wells of the microplates. Once the assemblages completed, 10 µl of each community was transferred in microplates containing 140 µl of the mixed media (the cultures were initiated with 0.5 X 10^{-4} cells). The cultures were incubated at 20°C in humid chambers for 72 hours. Light absorbance at 590 nm was measured at 48 and 72 hours.

Assays.

Assays were conducted to measure individual strain performances on each carbon substrate at the end of the experiment. Before the assays, frozen mixtures from the end of the selection period were reconditioned in 6 ml of MB media for 24 h at 20°C in humid chambers under constant orbital shaking. The cultures were centrifuged (5 min at 3 500 r.p.m) and washed by elimination of the MB and addition of 6 ml of the M9 minimal salts with buffered salinity. The cultures were left in starvation for two hours. The Ecoplates were prepared with 120 µl of the buffered M9 solution. The Ecoplates were incubated with 30 µl of cultures (preliminary analysis showed this quantity was necessary to obtain a signal from the ancestors). Each strain was replicated three times. The cultures were incubated at 20°C in humid chambers. Light absorbance at 590 nm was measured after 48h.

Statistical analyses.

The selection treatment (three levels) and species richness (log-transformed) were entered in an Analysis of Covariance (ANCOVA) of the bacterial productivity. The dependent variable was the light absorbance at 590 nm and we analyzed the effects of the experimental treatments after 48 and 72 hours. The results of the assays were averaged over the three replicates. A strain was considered able to exploit a carbon substrate when the light absorbance was larger than the 95% of the distribution of the blanks. We calculated for each community a niche diversity index (NDI): the total number of carbon substrates a community is able to exploit, based on the community

composition and the individual ability of each strain to exploit the carbon substrates. A second ANCOVA was conducted (excluding the ancestors) considering the niche diversity index instead of species richness. We also fitted a linear model describing the effect of species richness and species identity on ecosystem functioning without requiring knowledge of the contribution of individual species to ecosystem functioning in mixture (see Bell et al., 2009 for details of this methodology). The model has for independent variables the effect of linear richness, non-linear richness, individual species effects, the species pool and the effect of composition. The model returns species-specific coefficients that could be interpreted as the contribution of individual species to ecosystem functioning relative to the average species.

SUPPLEMENTARY MATERIAL 1.

Theoretical predictions for the effect of niche specialization on the BEF relationship. We are interested by the effect of niche specialization on three properties of the BEF relationship: 1) the intercept (the productivity of monocultures); 2) the maximum productivity (when the community is saturated) and 3) the slope of the relationship (its shape). Loreau (2001) has shown the importance of niche specialization, here our focus is on different shapes of the tradeoff in resource usage intensity.

The model

To answer this question, consider a simple chemostat model (DeAngelis, 1992) for a heterogeneous environment containing N different resources R_i and s consumer species C_j. This model is more appropriate for bacteria than a consumer-resource model of the type predator-prey, as there is no density-dependence in the growth rate of the resource. We describe this system with the set of differential equations:

Eq. 1

$$\frac{dR_i}{dt} = eR_0 - eR_i - \sum_{j=1}^{N} \alpha_{ij} R_i C_j$$

$$\frac{dC_j}{dt} = \sum_{i=1}^{S} \alpha_{ij} R_i C_j - mC_j - eC_j$$

Where e is the dilution rate, R_0 is the resource concentration in the inflow (for simplicity we suppose all resources have the same concentration in the inflow), α_{ij} is the per capita consumption rate of the resource i by consumer j (linear functional response) and m is the mortality rate. We suppose there is no preferential selection of resources, i.e. a species consumes resources in function of their respective abundances. The resources are also equally converted into consumer biomass, and the density of consumers is proportional to the amount of resource consumed. The ecosystem productivity at equilibrium is simply the total flow of resources to the species compartment (Loreau, 1998) and is given by:

Eq. 2 $$\Phi = \sum_{i}^{N} \sum_{j}^{S} \alpha_{ij} R_i C_j .$$

Effect of generalization on intercept of the BEF relationship

It was shown in Fig. 1b that the intercept of the BEF relationship is higher for the simulated assemblages of generalists than for the specialists. This would mean that in single species assemblages, generalists have higher productivity than specialists, even when the trade off is strong. This results seems counter intuitive because the presence of a strong trade off means that generalists are reducing more the consumption rate of their preferred resource than they increase their consumption rate of their alternative resource. To understand this result one must keep in mind that productivity is a function consumption rates, population sizes and resource concentration. Consequently, with species generalization the gain in productivity obtained by increasing the consumption rate of an alternative resource does not necessarily equals the lost in productivity consequent to less intense consumption of the preferred resource.

To illustrate this, let consider a simple situation with two resources and one consumer species. Suppose the consumption rate of the resource 1 by the specialist is $\alpha_{11} = 1$, and because it is a specialist, the consumption of the alternate resource is $\alpha_{12} = 0$. Now we want to know what happens if generalization increases by a small amount. That is to say that the consumption rate of the alternate resource increase by a small amount d_2, leading to consumption rate $\alpha_{12}^{'} = \alpha_{12} + d_2 = d_2$ (denoted by a ‘), with a cost d_1 on the

consumption rate of the first resource, which becomes $\alpha_{11}^{'} = \alpha_{11} - d_1$. We have a linear tradeoff when $d_1 = d_2$ (Fig. 1a), a weak tradeoff when $d_1 < d_2$ and a strong tradeoff when $d_1 > d_2$.

Now, consider the limit in the vicinity of perfect specialization, i.e. where $d_1 \to 0$ and $d_2 \to 0$. In this situation, the concentration of the resource 1 (denoted by a hat) tends to $\hat{R}_1 \to \frac{m+e}{\alpha_{11}}$ and the concentration of the second resource tends to $\hat{R}_2 \to R_0$ (because this resource is not exploited). In this limit, $\hat{R}_1 << \hat{R}_2$ because resource consumption reduces R_1 concentration considerably. Consequently, the gain in productivity associated with a small generalization ($\approx d_2\hat{R}_2$) will be much larger than an equivalent lost in productivity associated with reduce consumption of the preferred resource ($\approx d_1\hat{R}_1$). This explains why the productivity increases much faster with a small increase in the consumption of alternate resource (even with a strong tradeoff) than it reduces with an equivalent decrease in the consumption of the preferred resource (Fig. S1.1 and Eq. 2).

It is interesting to understand what king of tradeoff would lead to either a gain or a lost in productivity monoculture with increasing generalization. We note the productivity of a specialist is Φ_{spec} and of a generalist is Φ_{gen}. The consequence of generalization on productivity is given by the difference between the productivity of generalist and specialist, thus $\Delta\Phi = \Phi_{gen} - \Phi_{spec}$. By definition, there is no effect of generalization on productivity when $\Delta\Phi = 0$, there is a gain for positive values and a reduction for negative values. We seek to understand the tradeoff between consumption rates of resources 1 and 2 (denoted α_{11} and α_{12} respectively) distinguishing between a positive and a negative effect of generalization on productivity in monoculture. We do so by solving the equation $\Delta\Phi = 0$ for α_{11}, noting the consumption rate of the resource 1 by a specialist is α_{spec}, leading to:

Eq. 3
$$\alpha_{12}^{*} = \frac{\alpha_{spec}(\alpha_{spec} - \alpha_{11})(m+e)^2}{(\alpha_{spec} - \alpha_{11})(m+e)^2 + \alpha_{spec}^2 \alpha_{11} R_0^2}$$

The equation S1.3 gives the tradeoff between consumption rates of the two resources necessary to keep constant productivity with increasing generalization. This tradeoff is non-linear and strong (downward curve – Fig. 1A). We illustrated this tradeoff at Figure

S1.1. The figure shows the primary productivity of monoculture for different combinations of consumption rates. The curved black line represents the parameter combination leading to a productivity equivalent to the one of a specialist species with $\alpha_{spec} = 1$ and the straight dotted line a linear tradeoff. A strong reduction in the consumption rate of the preferred resource is necessary to balance the gains in productivity consequent to generalization ($d_1 >> d_2$). Any parameter combination below this lines means that generalization has a negative effect on productivity (compared to specialization). The figure shows that generalization tends to increase productivity at low diversity. There is also a strong gain in productivity with a linear tradeoff. Although a negative effect of generalization on productivity is possible, we conclude that generalization in most cases will increase productivity at low diversity.

Effect of generalization on the slope of the BEF relationship

It is much easier to understand the effect of generalization on the productivity in mixtures. Consider as an example a simple two consumers - two resources system. In this simple situation, the slope of the BEF relationship is simply the difference between the productivity in mixture and the productivity in monoculture.

Now consider that for this simple system, species are symmetric, which means that they both have similar performance on alternative resources ($\alpha_{11} = \alpha_{22}$ and $\alpha_{12} = \alpha_{21}$) and similar death rates. Their coexistence is assured because they still prefer one resource over the other ($\alpha_{11} > \alpha_{12}$). We solve Eq. 1 at equilibrium to find the productivity in mixture and obtain:

Eq. 4 $$\Phi_{mix} = \frac{2e(R_0[\alpha_1 + \alpha_2] - m)}{\alpha_1 + \alpha_2}$$

The first result coming from this equation is that with a linear tradeoff, there is no effect of generalization on productivity in mixtures. In this situation, the sum of the consumption rates on alternative resources is constant (ie $\alpha_{11} + \alpha_{12} = \alpha_{spec}$), and consequently the productivity depends on average consumption rates, the mortality rate and the flows of the chemostat (determined by R_0 and e). We illustrated the effect of different combinations of the consumption rates at Fig S1.2. The figure shows that with a weak tradeoff, the productivity increases with generality, while the productivity decreases with productivity for a strong tradeoff.

The effect of generalization on the productivity in mixture is however much weaker than the effect on the productivity in monoculture. Consequently, the slope of the BEF is much stronger for specialists than for generalists, no matter what is the shape of the tradeoff (Fig. S1.3).

FIGURE S1.1. The effect of specialization on ecosystem functioning in a monoculture with two resources. The dynamics are described by the system of equations Eq. 1. The productivity is illustrated as a function of the consumption rate of the two resources. Perfect generalists are found along the 1:1 line (when they eat at equal rates the two resources) while perfect specialists are found along the axes (when one of the consumption rates is equal to 0). The dotted line indicates a linear tradeoff (see Fig. 1b for weak and strong tradeoffs). The solid line illustrates the tradeoff between consumption rates along which there is no effect of generalization on productivity in monoculture (the consumption rate of the specialist is set to $\alpha_{spec} = 1$). Generalization tends to increase productivity in monocultures relative to specialist species, and thus a strong tradeoff is necessary to keep productivity constant. The species could not maintain in the white region. Other parameters are $R_0 = 1$, m = 0.1 and e = 0.1.

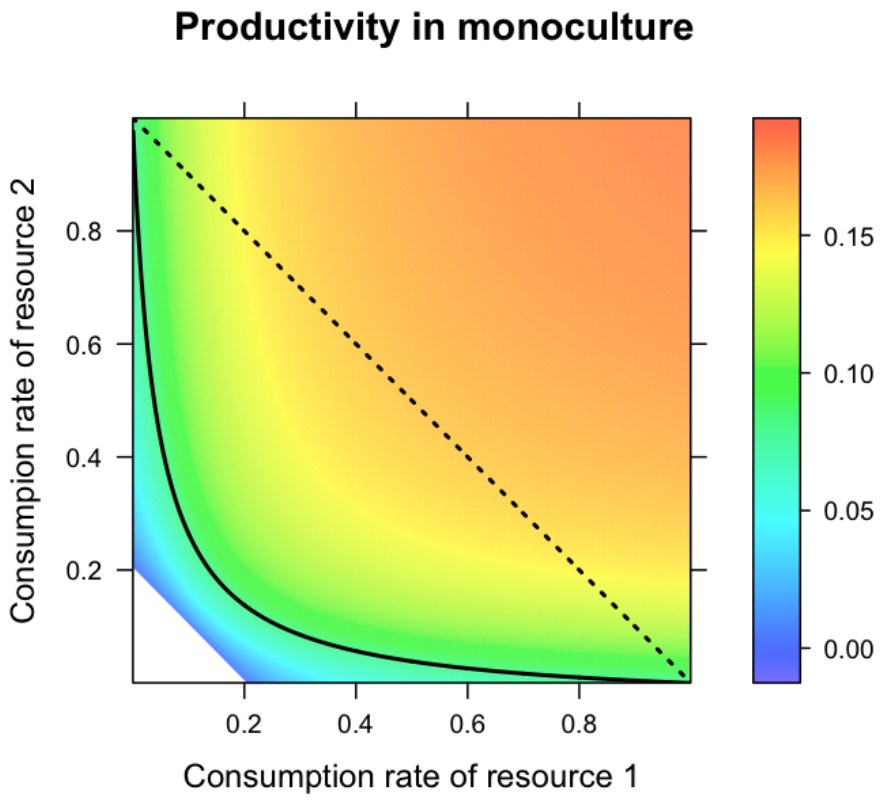


FIGURE S1.2. The effect of specialization on productivity in a two species mixture with two resources. The species are symmetric, i.e. they both consume alternate resources at similar rates and have similar mortality rates. Specialists are found along the axes and perfect generalists along a 1:1 relationship. The productivity in mixtures is not influenced by generalization when the tradeoff is linear. A weak tradeoff (upward curve) will increase productivity (see also Fig 1b) and a strong tradeoff (downward curve) will reduce productivity. Parameters are as for Figure S1.1.

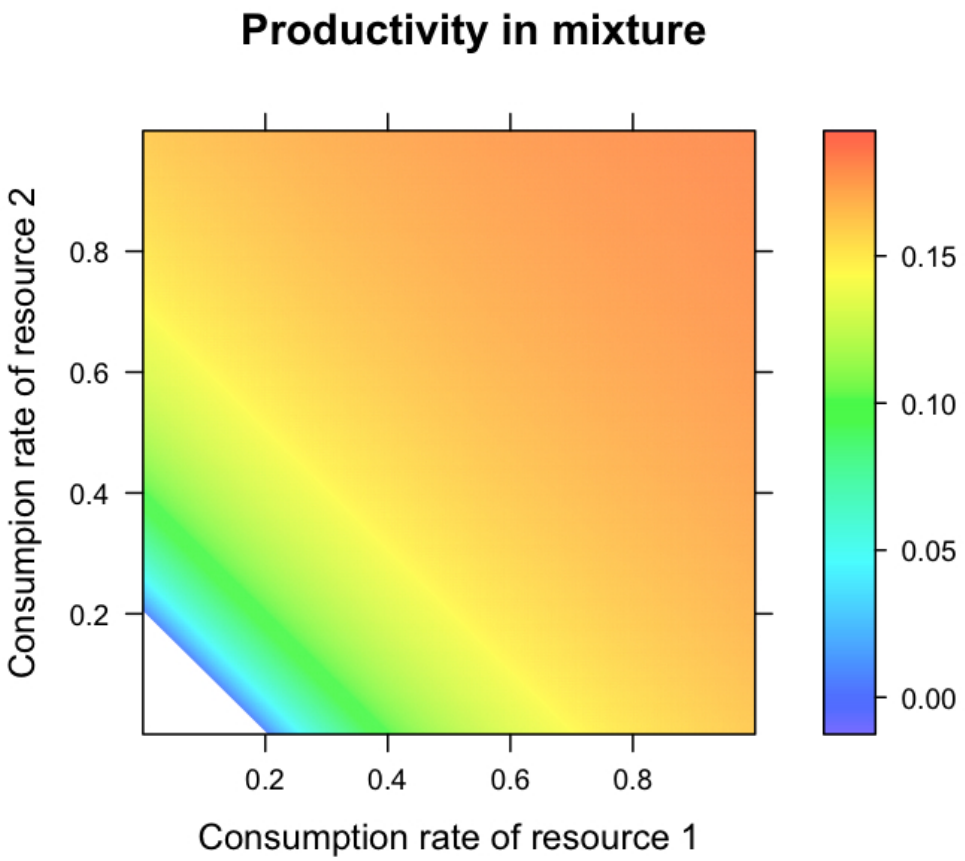
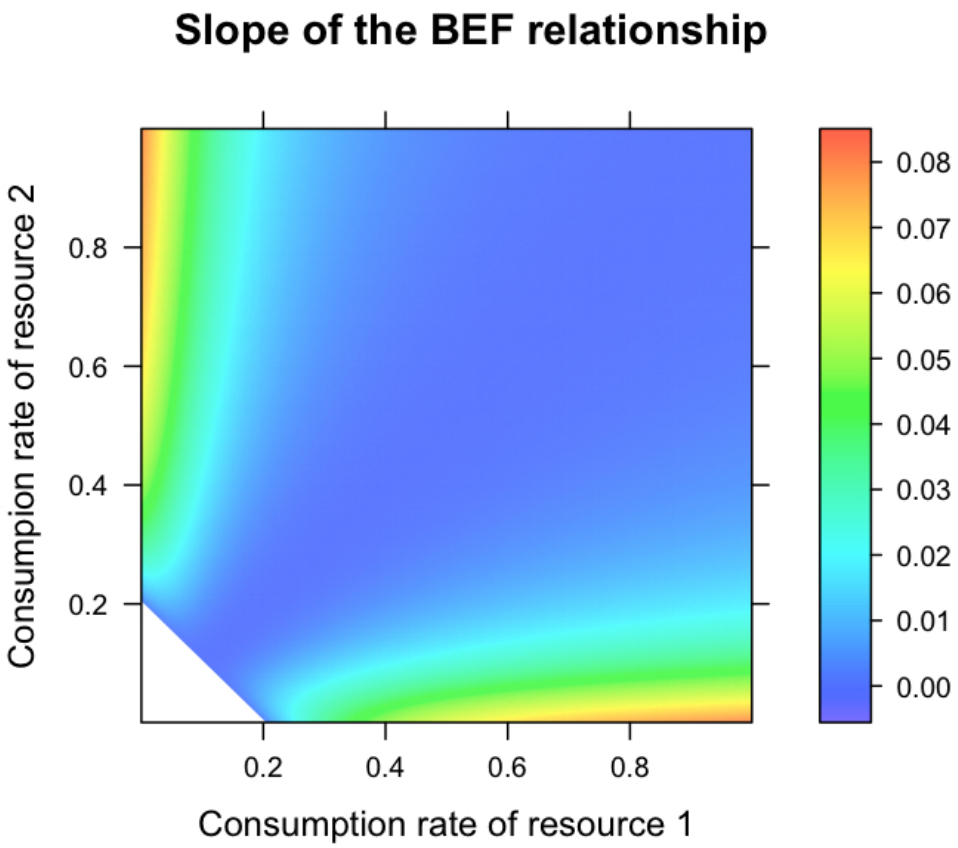


FIGURE S1.3. The effect of specialization on the slope of the BEF relationship for two species mixtures. Specialists are found along the axes and perfect generalists along a 1:1 relationship. The slope of the BEF relationship for this two species system is the difference between productivity in mixture and in monoculture. The slope of the BEF relationship is maximal for specialist species and minimal for generalist species. Parameters are as for Figure S1.1.



SUPPLEMENTARY MATERIAL 2.

Summary ANOVA table for the linear models partitioning the variance between species and community effects. For details on this analysis, see Bell et al. (2009).

ANCESTORS

Factor	df	SS	MS	F	p
Richness - linear	1	0.111	0.111	100.7	<0.001
Richness - nonlinear	4	0.006	0.002	1.4	0.283
Species effect	19	0.534	0.028	532.9	<0.001
Error species effect	18	0.020	0.001	3.6	<0.001
Error nonlinear effect	123	0.123	0.001	3.3	<0.001
Residuals	291	0.090	0.000		

SPECIALISTS

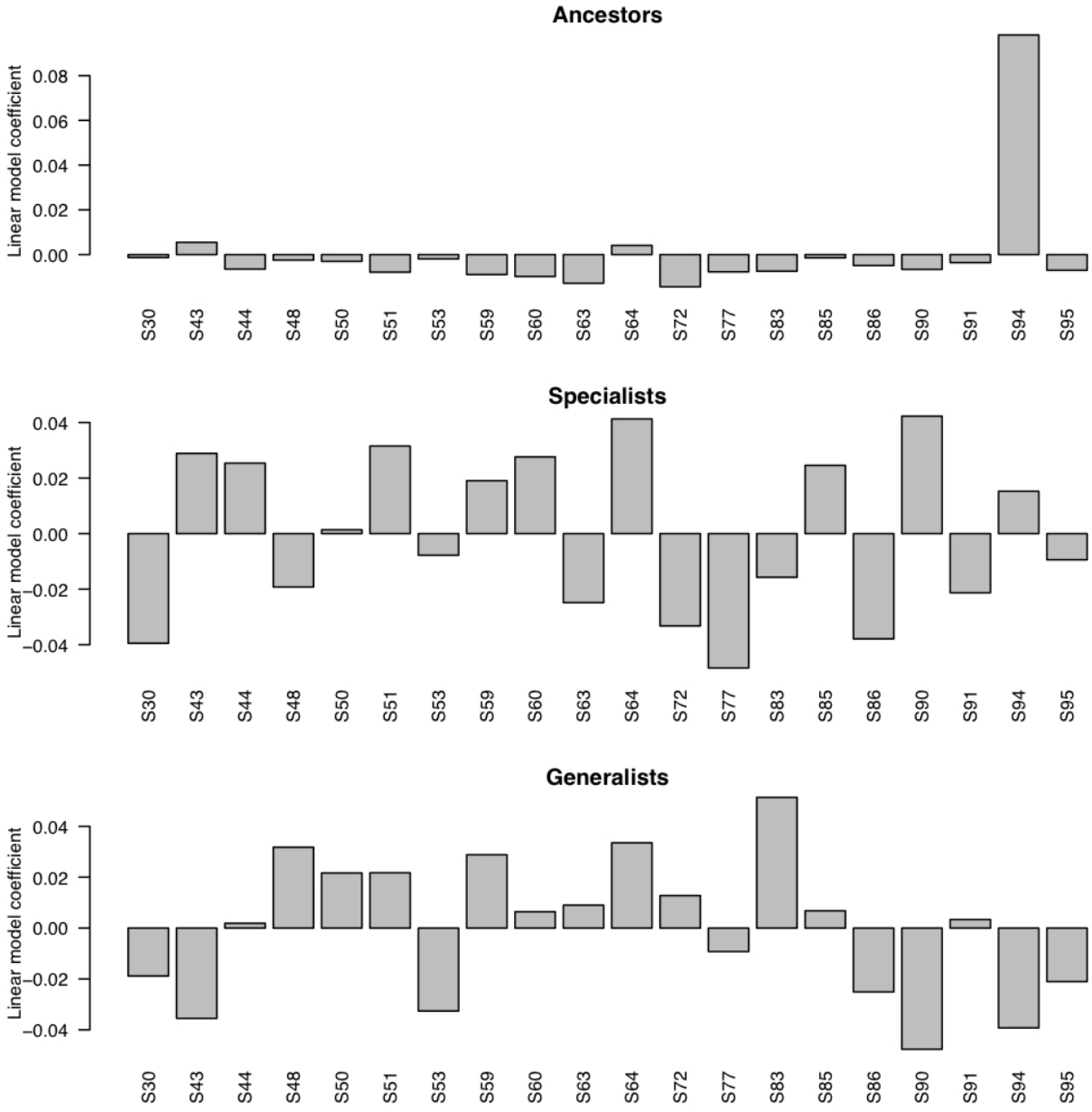
Factor	df	SS	MS	F	p
Richness - linear	1	0.377	0.377	110.7	<0.001
Richness - nonlinear	4	0.187	0.047	13.8	<0.001
Species effect	19	0.701	0.037	182.9	<0.001
Error species effect	18	0.061	0.003	3.9	<0.001
Error nonlinear effect	123	0.472	0.004	4.4	<0.001
Residuals	291	0.251	0.001		

GENERALISTS

Factor	df	SS	MS	F	p
Richness - linear	1	0.152	0.152	59.5	<0.001
Richness - nonlinear	4	0.115	0.029	11.3	<0.001
Species effect	19	0.481	0.025	55.2	<0.001
Error species effect	18	0.046	0.003	2.9	<0.001
Error nonlinear effect	123	1.073	0.009	9.8	<0.001
Residuals	291	0.258	0.001		

SUPPLEMENTARY MATERIAL 3.

Species-specific effects in the diversity-productivity linear models (see also Supplementary material 2). A positive coefficient means that a species is contributing more to productivity than the average species, while a negative coefficient means it is contributing less than average. For details on this analysis, see Bell et al. (2009).



References (to be done)

III- CONCLUSION GENERALE

Une approche expérimentale de la biodiversité.

Ce travail de thèse apporte quelques éléments nouveaux à la compréhension du **rôle de l'hétérogénéité environnementale spatiale et temporelle dans l'émergence et le maintien de la diversité.** A l'exception du travail de revue sur la spécialisation auquel j'ai participé (*manuscrit 4*), cette thèse propose des approches expérimentales sur la biodiversité.

Trois études ici décrites illustrent le rôle de la **dispersion** dans le processus de radiation adaptative en milieu **spatialement hétérogène** dans des métacommunautés bactériennes (*manuscrits 1, 2 et annexe*). Une étude explore l'importance du **grain** environnemental dans l'émergence et maintien de la diversité en milieu **temporellement fluctuant** (*manuscrit 3*). Deux études montrent l'importance de considérer le processus **adaptatif** dans l'étude de la **relation entre diversité biologique et le fonctionnement des écosystèmes** (*manuscrit 1 et 5*). Deux travaux montrent l'importance de prendre en compte les **mécanismes de coexistence** pour comprendre la **relation entre diversité et fonctionnement des systèmes biologiques** (*manuscrits 1 et 2*). Finalement, central pour la compréhension de l'émergence et le maintien de la diversité biologique, le concept de **spécialisation écologique** y a aussi largement été utilisé (*manuscrit 4*).

Voici quelques messages simples qui se détachent de ces travaux ; ou en anglais, les « *take home messages* ».

Les microorganismes comme modèle d'étude.

De nombreuses études suggèrent que les microorganismes sont un outil fondamental dans l'étude de la biodiversité (Feldgarden et al. 2003, Jessup et al. 2004, Cadotte et al. 2005, Gillespie et Emerson 2007, Buckling et al. 2009). En réalité les études en microcosmes offrent la possibilité d'intégrer les processus écologiques (comme la dispersion) et évolutifs (comme l'adaptation) dans un même système expérimental pour mieux comprendre la dynamique des systèmes naturels. Avec des protocoles expérimentaux simples, ils apportent beaucoup à la compréhension de certains processus qui résultent très difficiles à étudier sur le terrain. Ils permettent de tester les hypothèses théoriques et génèrent des nouvelles directions de recherche. Ce sont bien sur des systèmes très simplifiés mais ils permettent d'identifier les mécanismes susceptibles d'influencer la diversité des systèmes naturels.

Dans cette étude j'ai privilégié l'utilisation de *Pseudomonas fluorescens* SBW25, une bactérie ubiquiste isolée sur une feuille de betterave il y a plus de 20 ans. Un millilitre de culture bactérienne peut contenir jusqu'à 100 000 000 cellules, une nouvelle génération peut émerger en seulement quelques heures et elle est très facile à cultiver.

Je suis certain que dans l'avenir, les microcosmes vont encore générer des réponses à des nombreuses questions tant en écologie comme en évolution. Il est question d'utiliser les microorganismes pour explorer entre autres le processus de coévolution (par exemple Buckling et Rainey 2002, Morgan et al. 2005) et l'évolution de la coopération (par exemple West et Buckling 2002, Brockhurst et al. 2006).

1. Rôle de la dispersion dans une radiation adaptative en milieu spatialement hétérogène.

Une des questions à laquelle nous avons répondu dans cette thèse et qui illustre le gros potentiel des microorganismes pour combiner processus écologiques et évolutifs est celui du rôle de la dispersion dans la diversification dans milieu spatialement hétérogène. Dans le cas des organismes asexués, tels que les bactéries que nous avons utilisées, la principale source de variabilité est la mutation. En conditions naturelles, le potentiel adaptatif des populations bactériennes est souvent limité par un manque de variabilité génétique (Barton et Partridge 2000). En conditions de laboratoire, par contre, la haute densité de leurs populations assure une fréquence élevée d'apparition de mutations avantageuses ce qui favorise l'étude de l'évolution en action (Dykhuizen 1990, De Visser et Rozen 2005).

Trois travaux inclus dans cette thèse ont utilisé une approche en métacommunautés pour explorer expérimentalement le rôle de la dispersion sur la diversité en milieu spatialement hétérogène (*manuscrits 1, 2 et 3*). Ces travaux ont abordé différents aspects d'une même radiation adaptative à partir d'un clone unique de départ avec la bactérie *Pseudomonas fluorescens SBW25* et après ~500 générations d'évolution, permettant d'étudier non seulement les conditions pour le maintien de la diversité (problématique historiquement écologique) mais surtout celles pour son émergence (problématique historiquement évolutive).

a. Diversité régionale

Le rôle de la dispersion (flux génique) dans le processus adaptatif en milieu hétérogène a été central dans de nombreux débats (Räsänen et Hendry 2008 pour une revue) permettant de le qualifier de double effet. Il est qualifié à la fois

comme force « créatrice » ou comme force « contraignante » du processus adaptatif (Slatkin 1987). Ce sont surtout les études qui démontrent une relation négative entre flux génique et diversification adaptative dans des populations naturelles qui argumentent en faveur de cette dernière hypothèse (Langerhans et al. 2003, Hendry et al. 2002, Rice et Hostert 1993, Cuevas et al. 2003). En même temps, de nombreuses études ont aussi soulignées l'importance de la dispersion comme « catalyseur » de la réponse adaptative (Slatkin 1987, Gomulkiewiz et al. 1999, Morgan et al. 2005, Perron et al. 2007).

Le *manuscrit 1* illustre ces deux « facettes » de la dispersion dans une radiation en milieu hétérogène. Sous l'effet de la sélection divergente, des niveaux de dispersion intermédiaires (entre 1 et 10% de migrants injectés toutes les 10 générations) ont accéléré le processus de radiation. Le résultat est une diversité régionale maximale pour des niveaux de dispersion intermédiaires. Ce résultat diffère un peu par rapport aux attendus théoriques qui prédisent une diversification maximale pour des communautés fermées (0% dispersion) et à l'équilibre (Mouquet et Loreau 2003). Nous envisageons ce pic de diversité pour un niveau de dispersion intermédiaire comme une étape transitoire dans le processus de diversification en milieu hétérogène. Il illustre donc l'aspect dynamique de la diversité en milieu hétérogène. Ce type de dynamique serait très difficile à mettre en évidence dans les systèmes naturels.

Dans cette expérience, la coexistence de plusieurs génotypes est possible à l'échelle régionale par une partition spatiale des niches. On parle alors de radiation écologique (Schluter 2001). Cette étude a servi entre autres pour mettre en évidence l'importance de considérer non seulement les conséquences démographiques de la dispersion mais aussi ses conséquences évolutives afin de mieux comprendre la structure de la diversité.

b. Diversité locale

L'approche génétique de la diversité stipule que celle-ci résulte d'un équilibre entre sélection, dérive, mutation, recombinaison et flux génique (Hartl 2000). Alors qu'en général les deux premières se chargent d'éroder la variabilité au sein d'une population, la mutation, la recombinaison et le flux génique génèrent constamment des nouveaux variants (De Visser et Rozen 2005).

L'approche écologique spatiale de la diversité souligne également un rôle de la dispersion comme source de diversité à l'échelle locale (Hanski 1999, Leibold et al. 2004). La perspective « *mass effect* » (Shmida et Wilson 1985, Loreau et Mouquet 1999) suggère que les espèces seraient maintenues dans des habitats défavorables grâce à un apport contant d'individus par dispersion.

Ces deux approches de la diversité, génétique et spécifique, prédisent une augmentation de la diversité locale avec la dispersion. Mais suggèrent également que trop de dispersion peut aboutir à une homogénéisation et une réduction de la diversité (Mouquet et Loreau 2002).

Le *manuscrit 2* explore l'effet de la dispersion sur la diversité locale. En accord avec les prédictions, les communautés avec un niveau de dispersion intermédiaires présentaient la plus forte diversité. En même temps, la fréquence de variants mal adaptés a augmentée linéairement avec le niveau de dispersion. Dans une métacommunauté de type « sources-puits » l'immigration est une condition nécessaire pour le maintien de la diversité (m*ass-effect*). Avec une trop forte dispersion, la métacommunauté s'est homogénéisée, ce qui s'est traduit par une réduction de la diversité locale.

c. Patron d'adaptation locale

L'adaptation locale peut être définie comme une performance relative supérieure des variant locaux par rapport à des variants venus de l'extérieur

(Kawecki et Ebert 2004). La mise en évidence d'un patron d'adaptation locale a souvent été présentée comme la preuve de l'action de la sélection dans un environnement spatialement structuré (Slatkin 1987, Bell 1990). Les approches théoriques soulignent la dispersion comme un mécanisme s'opposant à l'adaptation locale car il favorise l'arrivée de migrants mal adaptés, produisant une réduction des performances des populations comparé à celle des populations fermées (*migration load,* Lenormand 2002).

Dans le *manuscrit annexe* j'ai exploré l'effet du niveau de dispersion sur le patron d'adaptation locale. Bien qu'encore préliminaires, ces résultats semblent montrer un effet de la dispersion sur le patron d'adaptation locale (Morgan et al. 2005). Ils suggèrent que la fréquence d'évolution de scénarios d'adaptation locale et la magnitude de l'adaptation seraient supérieures pour des niveaux de dispersion intermédiaires que pour des communautés fermées ou connectées par trop de dispersion. Ce résultat confirmerait les résultats précédents (*manuscrit 1*) sur le rôle adaptatif de la dispersion et un maximum de diversification pour des niveaux intermédiaires de dispersion. Cette étude semble également suggérer qu'un patron d'adaptation local n'est pas un résultat obligé de l'évolution en milieu hétérogène. Des contraintes telles que le temps d'évolution, le contraste des environnements peuvent limiter sa mise en place. Nous plaidons pour plus d'expériences en évolution expérimentale qui manipulent directement la dispersion.

2. Conditions pour la diversification en milieu temporellement fluctuant.

Les études empiriques sur le rôle de l'hétérogénéité temporelle comme moteur de diversification ont généré des résultats variables (Riddle et al. 1986, Grover 1988, Scheiner et Yampolsky 1998, Litchman 1998, Kassen et Bell 1998,

Flöder et al. 2002, Kassen 2002 pour une revue, Suiter et al. 2003, Buckling et al. 2007).

Basés sur les prédictions théoriques, il semblerait que ce manque de consensus s'explique par le grand nombre de facteurs qui déterminent l'émergence de diversité dans des telles conditions (Levins 1968). Parmi ces facteurs, le grain environnemental et la forme du compromis évolutifs sont très importants. Seul un compromis évolutif fort et un grain intermédiaire permettraient l'émergence et le maintien de la diversité.

Le *manuscrit 3* explore l'émergence de la diversité et de la spécialisation dans un environnement temporellement fluctuant en utilisant différents niveaux de fréquence des variations (grain). Afin de contourner la difficulté de connaître la forme du compromis évolutif entre deux traits, cette étude utilise 20 paires de conditions environnementales différentes. Nous espérions ainsi inclure une diversité de formes de compromis évolutif et générer un résultat général sur le rôle du grain. Après ~300 générations d'évolution, les plus forts niveaux de diversité ont été obtenus pour des grains intermédiaires. Le niveau de spécialisation a augmenté avec le grain alors que des fluctuations fréquentes dans les conditions environnementales ont favorisé l'émergence de généralistes. Le pic de diversité pour des grains intermédiaires semble résulter d'une coexistence de stratégies intermédiaires. Cette étude signale la rareté des conditions environnementales favorisant une spécialisation réciproque. Cette dernière ne serait possible qu'en présence d'un compromis évolutif fort et en absence de contraintes pour l'adaptation (variabilité génétique et temps suffisant).

3. Rôle de l'évolution dans la relation entre diversité biologique et fonctionnement des écosystèmes.

Un effet positif de la biodiversité sur le fonctionnement des écosystèmes est un argument fort en faveur de sa conservation (Schwartz et al. 2000, Srivastava and Vellend 2005). Les approches écologiques qui défendent cette hypothèse sont nombreuses (Hooper et al. 2005 pour une revue). Cependant, une limitation de ces approches est le fait de considérer les espèces comme des entités figées. Les limites d'une telle approche sur la biodiversité qui n'inclut pas les processus évolutifs semblent évidents. Dans deux des travaux présentés dans cette thèse (*manuscrits 1 et 5*) nous abordons l'importance d'inclure le processus adaptatif pour comprendre la relation entre diversité et fonctionnement.

Le premier résultat (*manuscrit 1*) a été la mise en évidence par évolution d'une relation positive entre diversité et productivité à l'échelle régionale. En partant d'un clone unique, la manipulation du niveau de dispersion entre des communautés bactériennes a crée, après ~500 générations, un gradient de diversité régional. Une plus forte complémentarité (Tilman et al 1997, Loreau et al. 2001) entres les espèces à l'échelle régionale a eu comme conséquence une meilleure exploitation des ressources disponibles et donc une plus forte productivité.

Le *manuscrit 5* décrit une expérience dans laquelle nous manipulons les conditions environnementales pour favoriser l'émergence, par adaptation, de stratégies d'exploitation différentes. Un milieu simple a favorisé la spécialisation écologique alors qu'un milieu complexe a favorisé l'émergence de généralistes (Barrett et al. 2005). La construction d'assemblages de diversité croissante pour chaque stratégie a montré que bien que les généralistes sont en moyenne plus productifs au moment d'exploiter un mélange de 31 ressources, les spécialistes sont plus complémentaires.

Nous sommes dans une période dans laquelle les données phylogénétiques sont de plus en plus disponibles ce qui permet d'ajouter une nouvelle dimension à l'écologie des communautés (par exemple Cavender-Bares et al. 2009). Ces travaux sont en train de révéler l'énorme importance de l'évolution dans le processus d'assemblage des communautés ainsi que dans l'importance de l'histoire évolutive des communautés dans l'étude des conséquences de la biodiversité (Cadotte et al. 2008, 2009). Notre étude confirme les hypothèses de ces études en montrant comment l'évolution peut modifier le patron de diversification.

4. Importance des mécanismes de coexistence dans la relation entre diversité biologique et fonctionnement des écosystèmes.

L'approche spatiale de la diversité (métacommunautés) propose une grande quantité de mécanismes de coexistence que selon l'échelle considérée (local ou régionale) permet de prédire une variabilité dans le patron de la relation entre diversité et fonctionnement des écosystèmes (Mouquet et Loreau 2003, Cardinale et al. 2004, Gross et Cardinale 2007). Dans une métacommunauté du type « source-puits », la coexistence locale se base sur l'arrivée constante de migrants qui son maladaptés localement (Pulliam 1988). A l'échelle régionale la coexistence s'explique par une spécialisation sur les différentes niches disponibles. Par conséquent, une relation positive entre diversité et fonctionnement n'est prévue qu'à l'échelle régionale où la complémentarité peut s'exprimer (Mouquet et al. 2002).

Les manuscrits *1 et 2* confirment l'importance de considérer les mécanismes de diversification pour comprendre la forme de la relation entre diversité et le fonctionnement des écosystèmes. Dans ces expériences,

l'hétérogénéité se situe à l'échelle régionale (plaque Biolog). C'est donc à cette échelle qu'a eu lieu la diversification des niches écologiques aboutissant par complémentarité à un effet positif de la diversité sur la productivité. L'hétérogénéité spatiale ne suffit pas pour expliquer l'effet positif de la diversité sur la productivité. Elle doit être couplée à une complémentarité entres les espèces (Weis et al. 2008) qui garantit que les ressources soient exploitées par les espèces mieux adaptées (Mouquet et al. 2002). Au niveau local, c'est la dispersion qui garantit une plus forte diversité. Par conséquent, à cette échelle une plus forte diversité n'a pas entraîné une plus forte productivité locale (Amarasekare et Nisbet 2001, Loreau et al. 2003, Mouquet et Loreau 2003).

Récemment l'étude des traits a été signalée comme primordiale pour comprendre la relation entre diversité et fonctionnement (Hillebrand et Matthiessen 2009). Si le trait considéré est important pour la coexistence des espèces est en même temps il est important pour la fonction, on s'attend à une relation positive entre diversité et fonction. Par contre si les traits qui déterminent la coexistence ne sont pas importants pour dans les fonctions des espèces on ne s'attend pas à avoir de relation entre diversité et fonction.

5. Causes et conséquences de la diversité.

Cette étude présente une double approche de la diversité en milieu hétérogène. D'abord, elle présente les « causes » de la diversité (Tilman 2000), en explorant les conditions pour son émergence et pour son maintient. Nous nous sommes appuyés simultanément sur des concepts issus de la biologie évolutive et de l'écologie des communautés pour confirmer le rôle de l'hétérogénéité environnementale comme facteur de diversification. Nos avons démontré que la dispersion intermédiaire dans un environnement spatialement structuré et un

grain intermédiaire dans un environnement temporellement fluctuant favorisent la diversité biologique.

En deuxième temps, nous avons exploré les « conséquences » de la diversité (Tilman 2000) ; en regardant la relation entre celle-ci et la productivité des communautés bactériennes. Le rôle de la diversité sur le fonctionnement des systèmes biologiques dépend de l'histoire adaptative des communautés ainsi que des conditions environnementales qui déterminent la coexistent des espèces.

6. Perspectives

Ce travail ouvre de nouvelles pistes de recherche. Par exemple il reste à approfondir d'un point de vue expérimental certains aspects de l'émergence de la diversité en milieu spatialement hétérogène : je pense par exemple au rôle du type de sélection (*hard* ou *soft*) et à la fréquence des habitats. Le modèle expérimental *Pseudomonas fluorescens* me semble un candidat idéal pour répondre à ces questions. On pourrait générer une sélection de type « *soft* » en contrôlant au moment des transferts le nombre d'individus de chaque patch qui vont engendrer la génération suivante. Ceci serait en principe possible en mesurant la densité cellulaire de chaque population avant chaque transfert (par cytométrie pour plus de sensibilité) et en ajustant selon ces valeurs le volume de culture transféré. Différentes fréquences d'habitats peuvent être générées facilement en utilisant des microcosmes contenant chacun une source de carbone différente et en faisant varier leurs volumes. On pourrait ensuite mesurer la diversité à l'intérieur des populations en isolant des génotypes individuellement.

Je pense aussi à effectuer une étude plus approfondie sur l'émergence des compromis évolutifs et à la dynamique de leurs formes. Evidement, ces études ne seraient que les étapes préliminaires avant de créer des protocoles

expérimentaux plus complexes en faisant varier plusieurs facteurs au même temps (dispersion, type de sélection, fréquence d'habitats…). Je pense à deux façons de procéder. La première serait en utilisant une banque de génotypes spécialistes (très performants dans une ressource mais mauvais dans d'autres) et les soumettre à une période de sélection aussi longue que possible dans les environnements dans lesquels ils ne performent pas bien. On pourrait ainsi suivre la dynamique de l'adaptation dans le temps et tracer la forme du compromis. Une deuxième possibilité serait de soumettre des populations bactériennes pendant une longue période de temps à un environnement contenant deux sources de carbone simultanément. On s'attend à l'émergence de génotypes généralistes si le compromis est faible. Deux spécialistes devraient émerger si le compromis est fort. Parallèlement, on devrait spécialiser des populations sur les deux environnements indépendamment. Au final on replace les différents génotypes évolues selon leur performances dans les deux environnements. Entre autres, Barrett et al. (2005) et Jasmin et Kassen (2007) ont déjà utilisé ce type d'approche expérimentale pour étudier le patron de spécialisation en milieu hétérogène en utilisant *Pseudomonas fluorescens*. Ces études ont mis en évidence une grande variabilité dans les résultats ce qui suggère une grande variabilité dans les formes des compromis évolutifs. Souvent des généralistes intermédiaires évoluent dans les mélanges et la présence de sélection synclinale (réponse corrélée positive) semble limiter leur spécialisation. Je ne suis pas au courant de l'existence d'expériences qui explorent la dynamique temporelle de la forme des compromis évolutifs.

Les résultats des études sur la biodiversité en microcosmes sont indispensables pour la compréhension de systèmes naturels plus complexes et ils ont permis d'établir un grand nombre d'idées pour la compréhension de la diversité. Cependant, il est difficile de transférer ces résultats à des situations naturelles (Hillebrand et Matthiessen 2009). Une première étape qui me semble essentielle pour continuer serait d'ajouter plus de complexité à ces expériences. Par exemple par l'inclusion de plusieurs niveaux trophiques. Ces aspects sont

actuellement en train d'être explorés surtout avec des systèmes hôte-parasite (Morgan et al. 2005, Hoeksema et Forde 2008, Buckling et al. 2009). Ces études ont montré certaines déviations par rapport à des systèmes plus simples et ouvrent des possibilités pour l'étude du processus de coévolution. Par exemple Morgan et collaborateurs (2005) utilisant *Pseudomonas fluorescens* SBW25 et son phage spécifique Φ2 ont montré comment la dispersion des parasites favorise leur adaptation locale alors que la dispersion des hôtes n'a eu aucun effet.

Dans l'étude de l'émergence de la diversité en milieu temporellement fluctuant j'ai exploré le rôle du grain environnemental avec des fluctuations périodiques. Des approches théoriques suggèrent qu'en plus du grain, le patron des fluctuations joue également un rôle décisif dans l'émergence de la diversité (Caswell et Cohen 1995, Gonzalez et Holt 2002). Les fluctuations cycliques seraient capables de maintenir plus de diversité que les fluctuations aléatoires (Hedrick 1974, Scheiner 1993). Parmi les fluctuations aléatoires, le niveau d'auto corrélation (couleur du bruit selon Caswell et Cohen 1995) pourrait aussi jouer un rôle important. Une faible fréquence des variations (bruit *rouge*) semblerait être une contrainte pour la diversification (Massin et Gonzalez 2006). Il serait intéressant de faire de nouvelles expériences pour tester ces hypothèses. L'idéal serait de contraster deux traitements : Périodique vs. Aléatoire pour différents niveaux de grain moyen. Basés sur les résultats su *manuscrit 4*, cette nouvelle expérience inclurait uniquement les paires de sources de carbone qui semblent favoriser la diversification et trois grains environnementaux moyens : 2, 4 et 8.

ANNEXE

Manuscrit en preparation

The influence of dispersal on local adaptation in experimental bacterial populations

P. A. Venail, N. Mouquet, R.C. MacLean and O. Kaltz.

Résultats préliminaires, manuscrit en préparation.

Les résultats présentés ici sont issus de l'expérience d'évolution décrite dans las manuscrits 1 et 2. En quelques mots il s'agit de la radiation adaptative de la bactérie *Pseudomonas fluorescens* SBW25 pendant ~500 générations dans un environnement spatialement structuré composé de 95 sources de carbone différentes. Nous avons contrôle la diversification en manipulant le niveau de dispersion entres les communautés.

L'objectif principal de cette étude est d'étudier l'effet du niveau de dispersion lors de la diversification en milieu hétérogène sur le patron d'adaptation locale. Nous définissons l'adaptation locale comme une performance moyenne supérieure des génotypes locaux relative à celle des génotypes issus d'autres localités. Cette approche de l'adaptation locale correspond à ce qui a été définit comme : Local vs. Visitant.

Nous proposons deux mesures de l'adaptation locale. La première est la *magnitude* qui correspond à la différence entre la performance du génotype local et la performance du génotype étranger. La deuxième est la fréquence d'adaptation réciproque qui détermine la présence d'une magnitude d'adaptation locale positive sur deux ressources simultanément.

Une analyse préliminaire des résultats semble indiquer que la magnitude de l'adaptation locale ainsi que la fréquence du patron d'adaptation locale réciproque sont maximales pour des niveaux de dispersion intermédiaires.

In preparation

The influence of dispersal on local adaptation in experimental bacterial populations

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Abstract

[To be done]

INTRODUCTION

Selection in spatially heterogeneous environments is predicted to favor ecological diversification (Slatkin 1987, Lenormand 2002). Consequently, given enough time, local individuals may be selectively superior to any immigrant, resulting in a geographical pattern of local adaptation, where performance of resident genotypes in their local (sympatric) environment is superior to that the performance of non-residents in foreign (allopatric) environments (Kawecki and Ebert 2004). The evolution of specific adaptations to local conditions may result in the evolution of ecological specialization and the emergence of genotype-by-environment interactions for fitness (G x E, Bell 1990). By consequent, patterns of local adaptation and G x E interactions for fitness in

spatially heterogeneous environments have very often been assumed as evidence for the action of divergent natural selection.

Natural selection has several limitations (Barton and Partridge 2000) and local adaptation is not an obligated result of selection in a spatially heterogeneous environment (Kawecki and Ebert 2004). A major limitation to natural selection, very common in small and marginal populations, is the unavailability of genetic variability (Antonovics 1976). This limitation is circumvented mainly by recurrent mutation, recombination and gene flow (i.e. dispersal, Kawecki 2000). Dispersal may promote adaptive diversification by introducing genetic variation or the spread of advantageous alleles (Slatkin 1987, Gomulkiewics et al. 1999, Forde et al. 2004). Paradoxically, gene flow may also limit the adaptation to local conditions as maladapted variants enter to the population (Levins 1968, Slatkin 1987, Lenormand 2002). The consequence is a reduction in mean fitness of populations because of immigration (i.e. migration load). Consequently, low dispersal may enhance local adaptation while higher rates may be detrimental (Gomulkiewicz et al. 1999, Kawecki and Ebert 2004). Given the potentially opposing effects of gene flow on local dynamics of selection (beneficial at low levels, detrimental at higher), we would expect non-linear relationships between levels of dispersal/gene flow and the strength of local adaptation. That is, local adaptation should peak at some intermediate level of gene flow.

Empirical evidence for positive (Greishar and Koskella 2007), negative (Brown et al. 2001, Bolnick and Nosil 2007) and null correlations (Gonzalo –Turpin and Hazard 2009) between dispersal and local adaptation are available. Several studies in natural populations (Hendry and Taylor 2004, Räsänen and Hendry for a review) revealed negative associations between gene flow and diversification and have been used to infer the importance of gene flow (i.e. dispersal) as a mechanisms preventing diversification. Direct manipulations of dispersal may provide clear evidence of the expected contrasting effects of gene flow but remain scarce (Räsänen and Hendry 2008).

[A review of experiments is required]

Experiments with microorganisms are an interesting tool for testing ecological and evolutionary predictions on the outcome of selection in heterogeneous

environments (Buckling et al. 2009). Here we experimentally explored the effect of four different dispersal rates on the pattern of *LA* in evolving metacommunities of the bacteria *Pseudomonas fluorescens SBW25*. We allowed bacteria to diversify from a single clone in coarse spatially heterogeneous environments for ~500 generations. We define local adaptation as a higher performance of a genotype in the environment from which it has been sampled relative to the performance of any foreign genotype in the same environment (i.e. local vs. foreign). We argue this aspect of local adaptation is the most relevant to study the impact of dispersal in the theory of adaptation in heterogeneous environments (Kawecki and Ebert 2004). Our results corroborate the contrasting effects of dispersal on local adaptation. However, in average only one fifth of the studied cases revealed scenarios of reciprocal local adaptation.

2. MATERIAL AND METHODS

(a) Study organism.

Pseudomonas fluorescens SBW25 is a ubiquitous bacterium with big diversification potential that has been used during the last two decades as a model system for the study the emergence of diversity in heterogeneous environments (Buckling et al. 2009). Bacteria rapidly adapt in microcosms, allowing the direct study of evolutionary processes (MacLean et al. 2004; Barrett et al. 2005; Venail et al. 2008). We started this experiment from a single clone of *P. fluorescens* SBW25, previously adapted for ~900 generations to a complex environment with eight different carbon substrates (Barrett et al. 2005).

(b) Selection experiment.

One clone of the bacterium *Pseudomonas fluorescens* SBW25 adaptively diversified for 40 days (~500 generations) in a spatially heterogeneous environment while the amount dispersal among patches was experimentally controlled (Venail *et al.* 2008). This ancestral strain has previously evolved for ~900 generations in a mixture of 8 carbon substrates (Barrett *et al.* 2005). As a highly heterogeneous environment we

used the Biolog GN2® microplates (i.e. Biologs) composed of 95 independent wells with a capacity of 150µl. Each well contains a single carbon substrate and tetrazolium violet, a dye which turns violet as the substrate is used by bacteria. Our initial strain was triplicated and each clone used to inoculate four different Biologs to get exposed during 40 days to 4 different dispersal levels among patches: 0%, 1%, 10% and 100% dispersal. During this selection period we serially transferred the bacteria to new Biologs every 24 hours (i.e. batch culture). At each transfer we allowed dispersal of bacteria between patches so the culture used to inoculate each new well of a Biolog contained a controlled percentage of a pool of immigrants derived from the mixture of the 95 patches. Transfers were performed by using a pin replicator (Boekel 96 pin/well model #140500). After forty days of selection we stored at -80°C the content of each well after adding 60 µl of 50:50 glycerol/M9 salts solution.

(b) Assays.

Ten evolving populations from different carbon sources were randomly sampled among the 95 available in the Biolog system. For each replicate of each dispersal level (10 wells x 4 dispersal levels x 3 replicates = 120 communities in total) we randomly sampled five genotypes after streaking out the content of the well in KB agar Petri dishes, for a total of 600 evolved genotypes sampled. The performance of each genotype was individually assessed in the 10 selected carbon sources of the Biolog GN2 microplates. Because of technical reason we assayed them on 8 blocks (i.e. days; 75 genotypes per day) using a randomized block design. In preparation for the assays, each genotype was amplified for 24h in 1 ml of M9KB media at 28°C under constant orbital shaking (200 r.p.m) and stored at -80°C in a 30% glycerol solution. Then, one ml of culture was unfrozen and washed (centrifuge 3 min at 7200*g*, discard the liquid media and addition of 1ml of M9 minimal salts). A 25 ml glass vial with M9 minimal salts were inoculated with 125 µl of the washed solution, starved for two hours and stored at 28°C before inoculation into the Biolog microplates (140µl per well). We scored absorbance at 590 nm for each genotype in each carbon source after 24h of incubation in the dark at 28°C in humid chambers. In each block we also included 5 replicates of the ancestral clone day (same protocol) to control for any "day effect". Absorbances were corrected by the blank well which contains no carbon source.

(c) Measuring Local Adaptation.

Magnitude of Local Adaptation

We define a local genotype as a genotype assayed in the carbon substrate from which it has been sampled. A foreign genotype is a genotype assayed in any other of the 9 carbon substrates. We define the *magnitude of local adaptation* as the difference in performance between a local genotype and a foreign genotype (*L-F*). Thus, the performance of each local genotype was compared against the mean performance of the 5 foreign genotypes from each of the other 9 evolved populations.

Analysis of types of reaction norms

For each couple of genotypes and for each couple of carbon sources we scored if both values of *L-F* where positive (Fig. 1; POS-POS, Leimu and Fischer 2008), both negative (NEG-NEG) of one positive and one negative (POS-NEG). A POS-POS scenario reflects a case of crossing reaction norms where both genotypes are locally adapted (fitter than foreign). A NEG-NEG case also reveals crossing reaction norms but this time with both local genotypes maladapted (less fit than foreign). Both POS-NEG and NEG-POS reflect non crossing reaction norms and suggest one genotype in fitter than the other in both environments. Only a POS-POS scenario reveals a case of reciprocal *local adaptation* (Kawecki and Ebert 2004). We calculated the frequencies of each of these cases for each dispersal level.

(d) Statistical analysis.

Before any analysis on the effect of dispersal on the pattern of local adaptation, we tested for an eventual effect in the absorbance measurements made on different. We contrasted the eight sessions of the five replicated assays of the ancestral strain. We used a multiple factor mixed effects ANOVA to test for dispersal (fixed, n = 4) and carbon source (random, n = 10) effects on the magnitude of local adaptation. We also included a replicate(dispersal) nested factor. We used a two-way ANOVA and multiple

comparisons to test the effect of dispersal on the frequency of each adaptation scenario (POS-POS, POS-NEG, NEG-NEG). We used R and JMP5 software for statistical analyses.

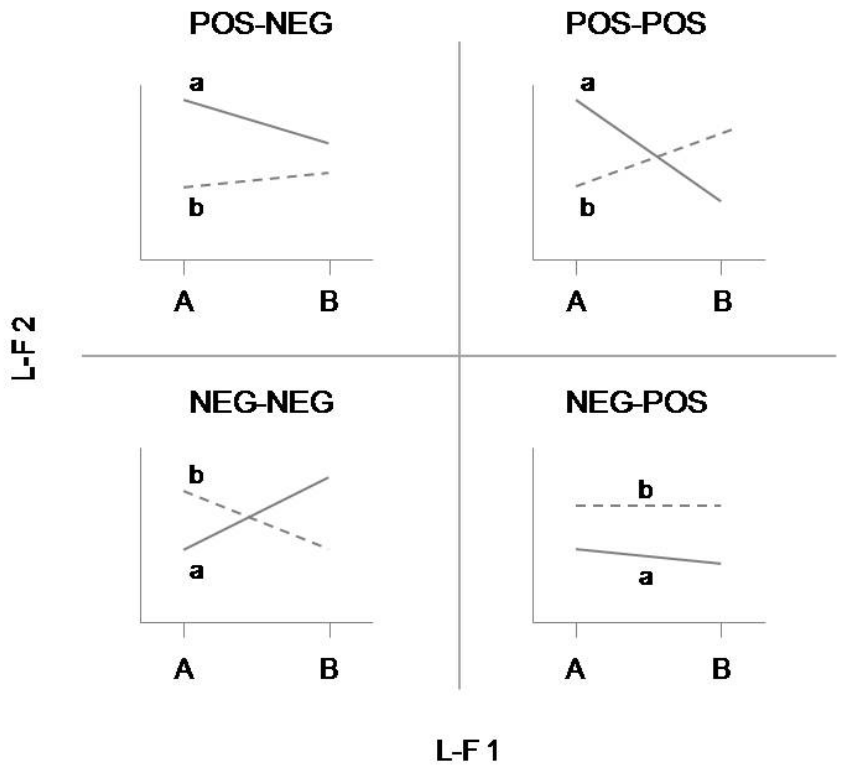


Figure 1: Four different patterns of local adaptation of two genotypes (*a, b*) tested over two environments (*A, B*). Genotype *a* is local in *A* while *b* is local in *B*. POS-NEG: *a* is locally adapted (local – foreign *L-F* positive) while *b* is maladapted in B (local – foreign *L-F* negative). POS-POS: both genotypes are locally adapted. NEG-NEG: both genotypes are maladapted.

3. PRELIMINARY RESULTS

We did not observe any "block-effect" on the replicated absorbance measurements of the ancestral strain ($F_{7,39}$ = 0.7, P = 0.67), allowing all the assayed genotypes (n = 600) to be pooled for a global analysis of the effect of dispersal on *LA*.

Effect of dispersal on the magnitude of local adaptation.

The ANOVA (Table 1) did not reveal a general effect of dispersal on the magnitude of local adaptation. Carbon source effect and the interaction component are also significant. In

one carbon source (A, Figure 2) dispersal was detrimental for local adaptation while in two other sources (I and J, Figure 2) gene flow was beneficial for local adaptation. Dispersal had no effect on the magnitude of *LA* in the other 7 carbon sources tested. The study of the distribution of the magnitude of local adaptation revealed that the mean magnitude of local adaptation was positive for the 1% and 10% treatments (Table 2) while for the 0% and 100% dispersal the mean magnitude of *LA* was not different from 0.

Maximal frequency of reciprocal local adaptation at intermediate dispersal levels.

The overall mean proportion of the POS-POS, POS-NEG and NEG-NEG scenarios were 0.2, 0.62 and 0.18 (Figure 4). Dispersal had an effect on the frequency of the POS-POS and POS-NEG scenarios (Table 3). The frequency of reciprocal *LA* (i.e. POS-POS) was higher in the 1 and 10% dispersal treatments relative to the 0% dispersal treatment (Figure 4) while the frequency of non-reciprocal *LA* (i.e. POS-NEG) was lower in the 1 and 10% dispersal treatments relative to the 0% dispersal treatment.

Table 1: Results of the full factorial mixed ANOVA test on the effect of dispersal (fixed factor) and the carbon source (random factor) on the *magnitude of local adaptation.*

Effect	D.F.	F	p-value
Dispersal	3	0.5379	0.6579
Carbon Source	9	14.003	**< 0.0001**
Dispersal x Carbon Source	27	3.9668	**< 0.0001**
Replicate(Dispersal)	8	3.6968	0.8915
Error	120		

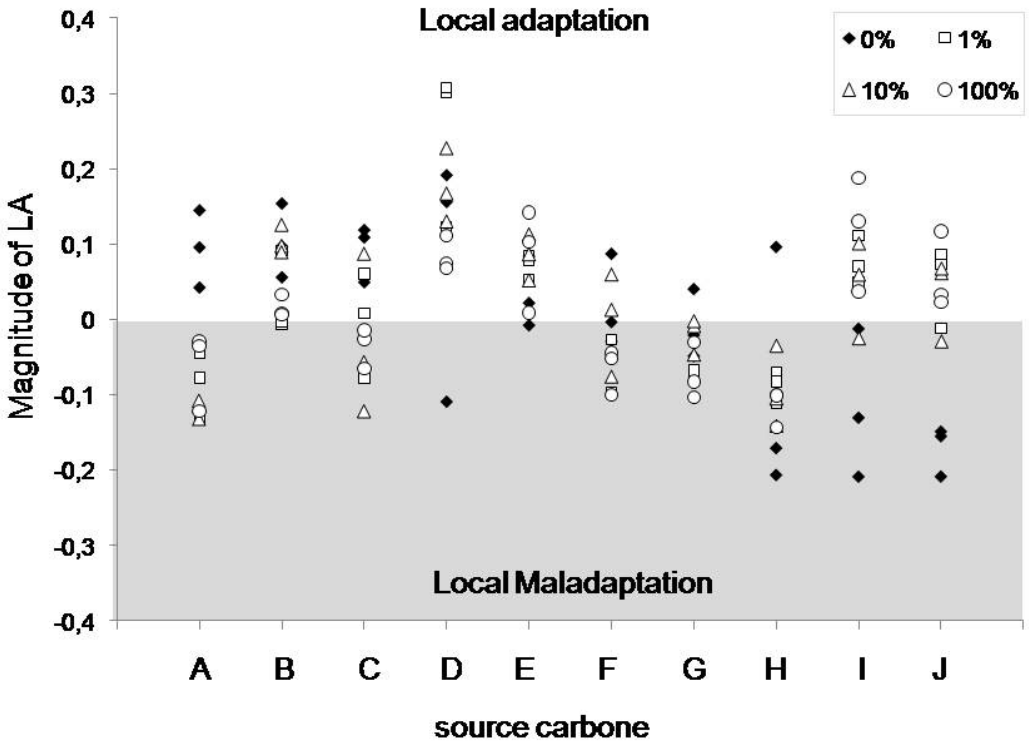


Figure 2: Effect of dispersal on the average magnitude of local adaptation (local – foreign performance) for the 10 carbons sources (A to J). Shaded area represents negative magnitudes of local adaptation (maladaptations).

Table 2: Average *magnitude of local adaptation* (*+/- 95% C.I*) for each dispersal treatment.

Dispersal level	Average *(+/- 95% C.I.*)
0%	0.003306 (*-0.0016; 0.00821)*
1%	0.016717 (*0.0011386; 0.022048*)
10%	0.017741 (*0.012234; 0.023249*)
100%	0.001509 (*-0.00294; 0.005956*)

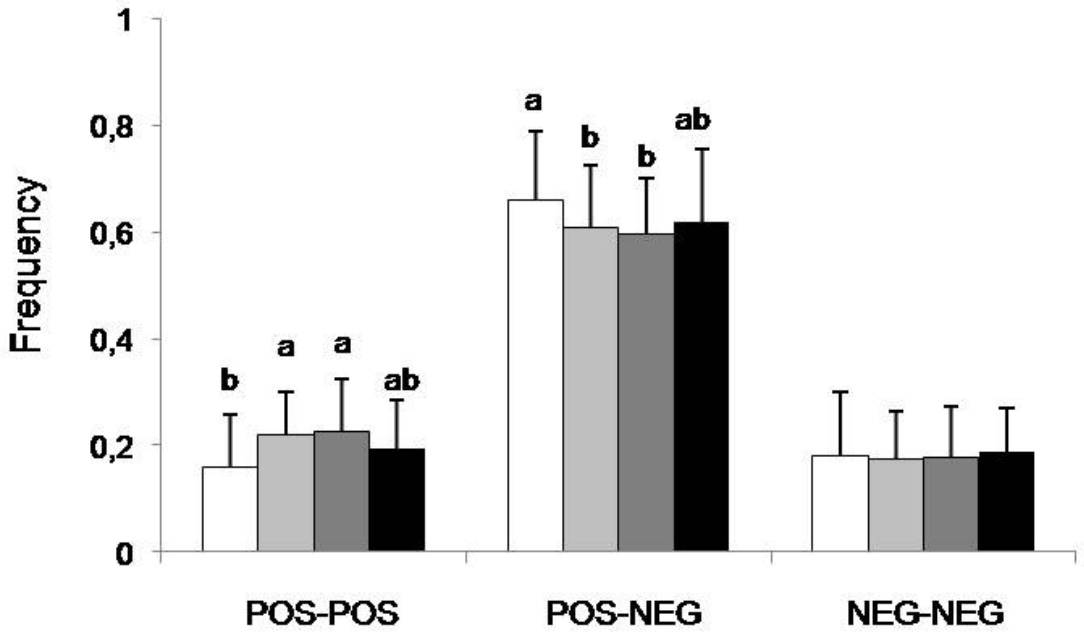


Figure 4: Mean frequency of the POS-POS, POS-NEG and NEG-NEG scenarios for different dispersal levels. Bars represent mean value (+/- S.E). 0% (white), 1% (light grey); 10% (dark grey) and 100% dispersal (black).

Table 3: Results of the two full factorial mixed ANOVA test on the effect of dispersal (fixed factor) and the environmental pair (random factor) on the frequency of the different scenarios of local adaptation.

POS-POS

Effect	S.S.	D.F.	F	p-value
Dispersal	0.3465	3	7.9419	**< 0.0001**
Couple	2.2449	44	3.5077	**< 0.0001**
Dispersal x Couple	2.3513	132	1.2247	0.0735
Residual	5.2363	360		

POS-NEG

Effect	S.S.	D.F.	F	p-value
Dispersal	0.3094	3	7.2756	**< 0.0001**
Couple	4.8125	44	7.7162	**< 0.0001**
Dispersal x Couple	3.0977	132	1.6556	**< 0.0001**
Residual	5.1029	360		

NEG-NEG

Effect	S.S.	D.F.	F	p-value
Dispersal	0.017	3	0.5467	0.6507
Couple	2.3605	44	5.1673	**< 0.0001**
Dispersal x Couple	2.7183	132	1.9835	**< 0.0001**
Residual	3.7376	360		

DISCUSSION

[To be done]

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