Structures génétiques et histoires évolutives de polychètes inféodées aux sédiments fins envasés dans l' Atlantique Nord Est : les genres Pectinaria sp. et Owenia sp.

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Structures génétiques et histoires évolutives de polychètes inféodées aux sédiments fins envasés dans l'Atlantique Nord Est: les genres *Pectinaria sp.* et *Owenia sp.*

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PREFACE

Les conséquences du réchauffement climatique se font déjà ressentir au niveau de la biodiversité avec, pour certaines espèces, des expansions de l'aire géographique vers de plus hautes latitudes et/ou altitudes, et un risque d'extinction global ou local plus important bien que pour l'instant difficile à évaluer. Au niveau marin, des modèles prédictifs plus fiables doivent être envisagés pour étudier le degré de connectivité entre populations en fonction de la fragmentation du milieu afin de pouvoir assurer une meilleure gestion de la biodiversité marine. Le devenir de la biodiversité est une préoccupation majeure mais cette considération passe par la compréhension des phénomènes passés qui ont influencé les traits de vie et l'histoire évolutive des espèces. La phase critique du transport larvaire doit aussi être considérée, car 70% des espèces marines possèdent une phase larvaire pélagique qui leur permet d'établir des échanges entre populations dans un environnement potentiellement très dispersif. Les espèces non commerciales à cycle de vie bentho-pélagique vivant en milieu fragmenté sont des modèles de choix pour ce genre d'études, car l'empreinte génétique laissée dans les populations naturelles par les phénomènes passés et présents, peuvent nous permettre d'interpréter les patrons biogéographiques contemporains et de déterminer les risques d'extinction à différentes échelles spatio-temporelles. Il est tout aussi important d'intégrer les données démographiques et génétiques à micro-échelle d'espace et de temps car l'action de certaines forces structurantes à différents stades de vie détermine le niveau de structure génétique dans les populations et donc leur adaptation locale.

L'évolution d'une espèce ne peut être comprise totalement qu'en intégrant les processus évolutifs s'exerçant à toutes les échelles d'espace et de temps, et à cette fin, ceux-ci déterminent le choix des marqueurs moléculaires. Ce travail de thèse s'inscrit dans cette optique et aborde par l'utilisation des polymorphismes enzymatique, nucléotidique (gène

mitochondrial de la Cytochtome Oxidase sous-unité I - mtCOI) et de la taille du marqueur (microsatellites), (1) l'étude des processus de colonisation à macro-échelle évolutive chez les genres *Owenia* et *Pectinaria*, eu égard à l'histoire géologique de l'Atlantique Nord Est (2 articles), (2) l'étude des flux géniques contemporains entre les populations *Pectinaria koreni* à l'échelle de l'Atlantique Nord Est, en confrontant données génétiques avec celles issues de simulations hydrodynamiques de la dispersion larvaire en Manche (1 article), et (3) l'étude des processus jouant sur le fonctionnement spatio-temporel d'une métapopulation locale (échelle micro-évolutive : couplage Baie de Seine orientale/Baie des Veys sur 3 ans : 2 articles).

SOMMAIRE

INTRODUCTION

Le milieu marin est un environnement phylogénétiquement très diversifié, et pourtant, seulement 15% des espèces formellement décrites sont marines (Briggs, 1994). Ses propriétés physiques et le fait que les masses d'eau sont en continuel mouvement, font de cet environnement un milieu particulièrement dispersif. Cependant, il est caractérisé non seulement par des gradients environnementaux (e.g. de luminosité, de salinité, de température et d'oxygène) qui diffèrent selon l'étagement bathymétrique et la position géographique, mais aussi par une forte hétérogénéité au niveau du substrat en environnement côtier (e.g. fragmentation du milieu, qualité et abondance de la nourriture) où les conditions environnementales sont plus variables. Les organismes marins sont en général très féconds (Widdows, 1991), et présentent des tailles efficaces ainsi que des potentiels de dispersion beaucoup plus importants. Ceci offre un potentiel évolutif rapide face à l'hétérogénéité du substrat et la fragmentation des populations marines. Ce potentiel évolutif est d'autant plus important pour les espèces marines sessiles caractérisées par une fertilisation externe des œufs avec un cycle de vie bentho-pélagique comprenant une phase larvaire pélagique pouvant favoriser la colonisation de nouveaux habitats. Pour ces espèces, le maintien des populations et la persistance locale de l'espèce dépend des caractéristiques biologiques intrinsèques (e.g. comportement grégaire des adultes reproducteurs et synchronisme de la ponte), des conditions hydrodynamiques du milieu (e .g. vitesse du courant, présence de fronts et de tourbillons) et de l'impact des différentes forces évolutive (la mutation, la sélection, la dérive génétique et la migration) aux différentes échelles d'espace (de la parcelle à l'aire de distribution de l'espèce) et de temps (de la génération au temps de résilience de l'espèce).

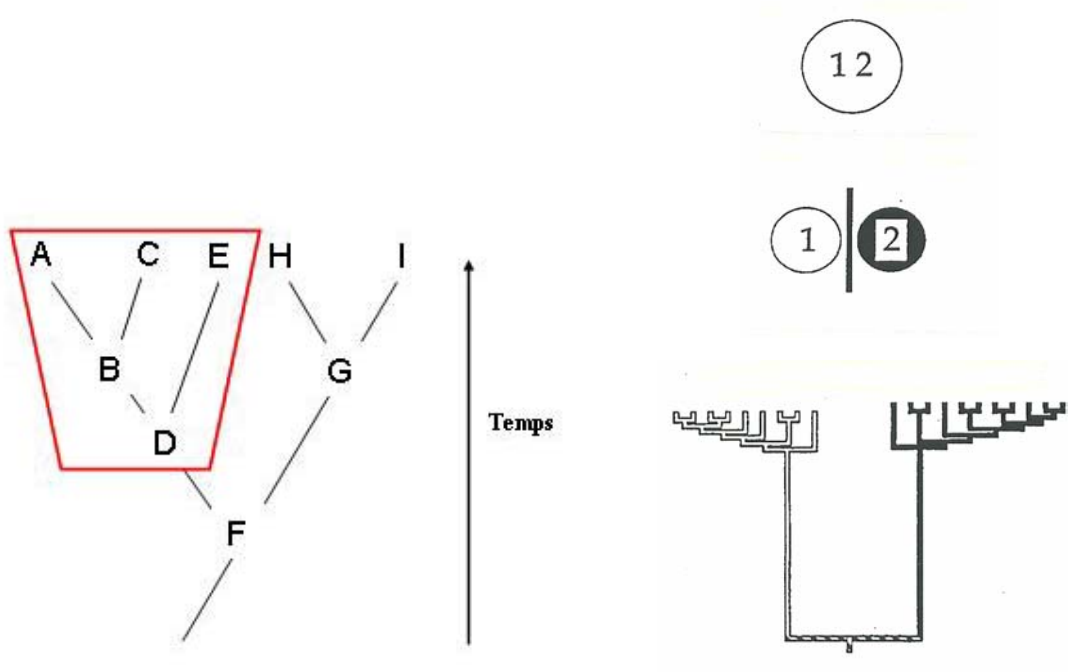
Au regard des phénomènes historiques qui ont marqué l'évolution des espèces marines, l'application d'outils moléculaires a permis de remettre en question les critères d'identification des espèces. Nous commencerons tout d'abord par apprécier l'évolution du

concept d'espèce en fonction des nouvelles méthodes d'analyse en génétique, et nous rappellerons aussi les grands modes de spéciation. Nous aborderons dans un second temps l'importance des échelles spatio-temporelles d'observation dans l'étude du vivant avec des exemples pris en milieu marin, et les concepts théoriques liés à ces échelles d'observation. Finalement, nous décrirons les caractéristiques de notre zone d'étude, l'Atlantique Nord Est, de même que celles de nos modèles biologiques, les polychètes tubicoles inféodées aux sédiments fins-envasés.

I. LE CONCEPT D'ESPECE

Le concept initial, celui de l'espèce biologique (Biological Species Concept ; Mayr, 1942), intègre l'idée qu'une espèce (ici sexuée) est un système génétique fermé, c'est-à-dire un groupe d'individus partageant les mêmes caractéristiques morphologiques et génétiques avec les mêmes chances de se reproduire au sein du groupe (Mayr, 1942). De fait, il implique des mécanismes d'isolement reproductif qui tendent à protéger l'intégrité des génomes entre espèces. Pourtant, de l'application d'outils moléculaires à des fins phylogénétiques, c'est-à-dire à l'étude des relations ancestrales entre espèces et entre individus d'une espèce, il ressort que les différences observées au niveau morphologique ne reflètent pas toujours les différences observées au niveau génétique. Bien que ceci puisse aussi être le reflet d'une mauvaise caractérisation des critères morphologiques utilisés en taxonomie, les espèces marines sont en général dotées d'une considérable plasticité physiologique et morphologique en réponse au milieu.

Le concept de l'espèce phylogénétique (Phylogenetic Species Concept ; Eldredge & Cracraft, 1980 ; Cracraft, 1983) ou de lignée évolutive, quant-à-lui, met l'accent sur l'indépendance évolutive des pools génétiques sans tenir compte de la compatibilité reproductrice. Ainsi, au sein même d'une espèce définie à partir de critères morphologiques, des sous-unités génétiques distinctes, peuvent exister même si l'isolement reproductif n'est



(A) (B)

Figure 1. (A) Exemple de regroupement monophylétique, ici en rouge (i.e. un regroupement de lignées évolutives qui comprent un ancêtre commun et toutes les lignées descendantes). (B) groupement réciproquement monophylétique, c'est-à-dire partageant un ancêtre commun plus récent entre eux qu'avec toute autre lignée ou groupe. Dans l'exemple ci-dessus, un évènement vicariant intervient dans la mise en place d'une barrière au flux génique entre deux populations qui divergent alors pour donner deux lignées évolutives.

que partiel. Ce concept considère l'espèce comme une Unité Evolutive Significative (UES), à savoir des individus montrant des groupements réciproquement monophylétiques (impliquant un ancêtre commun, voir Figure 1) pour les allèles de l'ADN mitochondrial, ainsi qu'une divergence significative des fréquences alléliques aux gènes nucléaires faisant état d'une coupure du flux génique (Moritz, 1994). Ces UES peuvent aussi être qualifiées d'espèces morphologiquement cryptiques, si aucune vraie différence morphologique n'apparaît (voir Palumbi, 1996, Burton, 1998; Dawson, 2001; Knowlton, 2000 sur l'importance des espèces cryptiques en milieu marin). Il faut noter que certaines espèces précédemment suspectées d'avoir deux formes larvaires (la poécilogonie) ont par la suite été reconnues comme espèces cryptiques par groupements monophylétiques distincts. Pourtant, la polychète *Streblospio benedictii* qui présente des groupements réciproquement monophylétiques est considérée par Schulze *et al*. (2000) comme une véritable espèce poécilogonique, les deux formes larvaires lécitotrophe et planctotrophe présentant des haplotypes mitochondriaux identiques.

Des groupements réciproquement monophylétiques ont été observés chez nombre d'espèces d'invertébrés marins, à partir d'études employant des marqueurs enzymatiques et l'ADN mitochondrial (e .g. *Capitella capitata*, Grassle & Grassle, 1976 ; *Crassostrea virginica*, Reeb & Avise, 1990; *Chtamalus sp.* Palumbi & Benzie, 1991 ; *Tigriopus californicus*, Burton, 1998; *Linkia laevigata*, Williams & Benzie, 1998; *Streblospio sp.*, Schulze *et al*, 2000; *Perna canaliculus*, Apte & Gardner, 2002 ; *Hydrobia* sp. ; Wilke & Pfenninger, 2002). Ces lignées apparaissent avoir évolué majoritairement par spéciation allopatrique et effet de vicariance, c'est-à-dire de fragmentation de l'habitat au cours des temps géologiques (Dobzhansky, 1940; Mayr, 1942). Pourtant, des effets sélectifs à petite échelle peuvent aussi induire ou venir renforcer un isolement reproductif et amener la formation d'espèces par spéciation sympatrique (Maynard Smith, 1966 ; Bush, 1975).

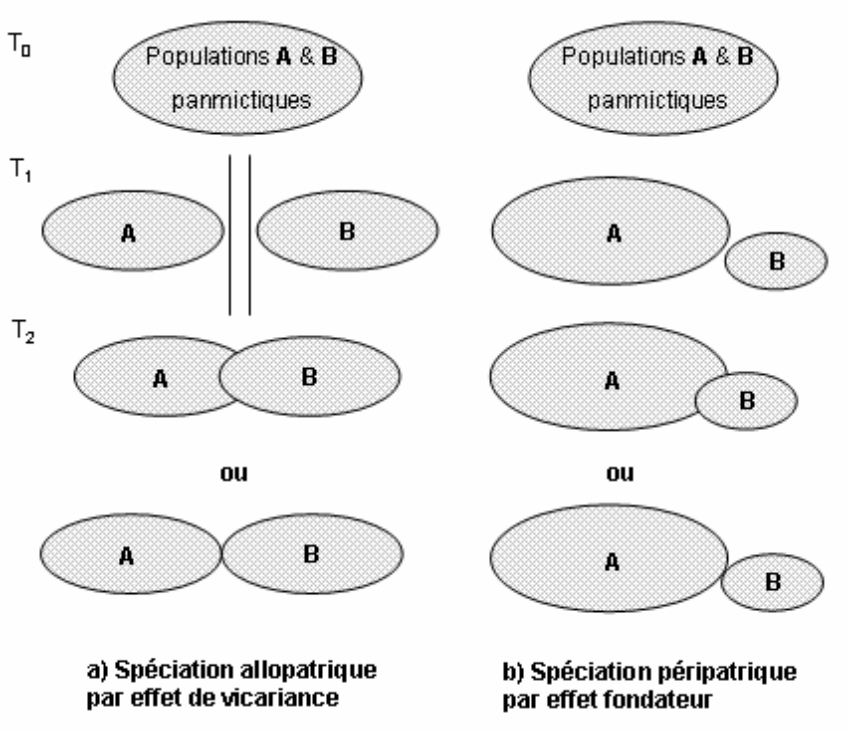


Figure 2. Les types de spéciation allopatrique. Au temps T0, les populations A et B partagent le même pool génétique. Au temps T1, il y a soit divergence par la mise en place d'une barrière physique entre les deux populations (effet vicariant), soit par un effet fondateur (isolement péripatrique). Au temps T2, la barrière peut disparaître (a) ou il peut y avoir une expansion de l'aire géographique (b). Dans tous les cas, un changement de l'environnement peut amener les populations A et B soit à se remettre en contact dans une même région (A et B seront alors des espèces sympatriques dans la même zone géographique), soit les espèces peuvent se retrouver en situation parapatrique, c'est-à-dire dans des aires adjacentes mais sans recouvrement réel, si les pressions de sélection sont hétérogènes.

I.1. Les grands modes de spéciation

En l'absence de migration, les autres forces évolutives (la sélection, la mutation et la dérive génétique) peuvent agir différemment entre populations d'une même espèce et amener celles-ci à diverger rapidement. Cette divergence est en général due à la mise en place de barrières pré-zygotiques souvent liées à l'éloignement géographique ou à des barrières physiques à la dispersion, par exemple la création d'un isthme provoquant la modification des courants marins (Waters & Roy, 2003), mais également à des modifications de l'environnement ou du comportement reproducteur des individus lorsque ceux-ci s'approprient de nouvelles niches écologiques et/ou n'exploitent pas les mêmes ressources au même moment (Schlutter, 2000). L'isolement pré-zygotique peut suffire dans le cas d'espèces vivant en strict allopatrie (e.g. incompatibilité des systèmes de reconnaissance gamétique par réarrangements chromosomiques). Pourtant, si l'isolement reproductif n'est que partiel, l'intégrité des génomes parentaux peut être maintenue par un mécanisme d'isolement post-zygotique ayant pour conséquence la mise en place d'une barrière au flux génique entre les isolats remis en contact (Bierne, 2001). On peut donc distinguer les modes de spéciation liés à la mise en place de barrières physiques (spéciation allopatrique et péripatrique) de ceux, liés à la mise en place de barrières génétiques au sein d'un groupe d'individus issu d'une même localité géographique (spéciation sympatrique et parapatrique), surtout dans les zones de remise en contact où les phénomènes d'hybridation sont courants.

I.1.1. Spéciation allopatrique et péripatrique

La spéciation allopatrique par effet de vicariance (Figure 2), c'est-à-dire l'apparition de peuplements frères composés d'espèces jumelles par simple fragmentation de l'aire géographique des espèces au cours des temps géologiques, est considérée comme la plus fréquemment rencontrée dans la nature car elle est très souvent validée par des études comparatives (Barraclough & Nee, 2001). Elle implique la mise en place d'une barrière

physique entre deux (ou plusieurs) sous-populations qui évoluent alors séparément par dérive génétique et/ou par adaptation locale, induisant une différenciation. Par la suite, les populations isolées peuvent subir un isolement reproductif pouvant aboutir soit à la spéciation soit au maintien de l'intégrité des génomes parentaux même si l'isolement reproductif n'est que partiel dans la zone de chevauchement des populations différenciées.

Dans le cas d'une spéciation péripatrique (Figure 2), le processus est essentiellement le même mais nécessite un effet fondateur et l'action de la dérive génétique comme principale force évolutive. La sélection peut également intervenir dans ce mode de spéciation pour renforcer les différences génétiques entre les populations en fixant plus rapidement certains allèles avantagés (Turelli *et al*., 2001). Nous n'entrerons pas dans le débat concernant l'importance des pressions de sélection et de dérive dans les processus de spéciation allopatrique, mais des différences au niveau de la niche écologique (tel le choix d'habitat) peuvent conduire à une sélection écologique contre les hybrides lors de la remise en contact de populations préalablement isolées (Schluter, 2000).

I.1.2. Spéciation parapatrique et sympatrique

Ces modes de spéciation s'appliquent en général en l'absence d'isolement géographique mais en présence de barrières génétiques apparues suite à des modifications de l'habitat ou de la niche écologique, du comportement reproducteur (e.g. décalage de la période de ponte conduisant à un isolement des populations dans le temps) ou du génome (e.g. polyploidie ou réarrangements chromosomiques).

Dans le cas d'une spéciation parapatrique, l'isolement reproductif apparaît entre populations adjacentes échangeant des migrants le long d'une zone de contact. L'isolement par la distance nécessaire à une spéciation parapatrique dépend en général de la mise en place d'un gradient de sélection agissant sur l degré de différenciation des populations. Si ce gradient est étroit alors la divergence entre les traits/gènes conférant l'isolement reproductif

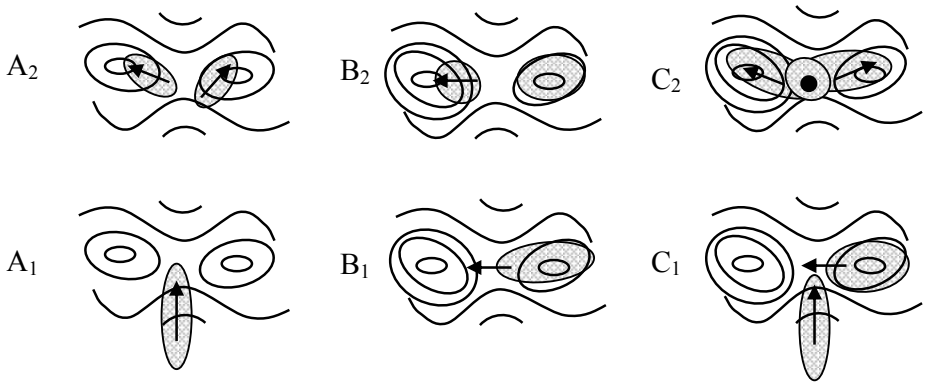


Figure 3. Divergence des populations en fonction d'un « paysage sélectif ». Les pics adaptatifs se trouvent au sommet des « collines », la mal-adaptation est représentée par la « vallée » séparant les collines. Les flèches noires indiquent les directions évolutives (la sélection quelle soit directionnelle, stabilisante ou divergente, dépendra des paramètres environnementaux, biologiques et des interactions écologiques). (A) population colonisatrice initiale avançant vers le haut d'une colline sélective se scinde en deux pour occuper deux pics adaptatifs. (B) population ancestrale occupant un pic adaptatif donne naissance à une population jumelle qui évolue vers un second pic adaptatif. (C) Deux populations, l'une occupant un pic adaptatif, l'autre avançant le long de ce pic se rencontrent et s'hybrident. La valeur sélective intermédiaire des hybrides peut alors induire la mise en place d'un gradient populationnel entre deux pics adaptatifs. Redessiné d'après Simpson (1953) et des scénarios évolutifs de Seehausen (2004) et Schlutter (2001).

peut apparaître à de très petites échelles géographiques. Ces processus sont souvent révélés par l'existence de clines de fréquences alléliques étroits en relation avec des zones d'hybridation. Ces clines sont maintenus par adaptation/spécialisation à différents environnements (Garcia-Ramos & Kirkpatrick, 1997) par exemple, en fonction des zones de transition biogéographique, ou par de la sélection contre les hybrides (Gavrilets, 1997) si les individus hétérozygotes (hybrides) pour certains gènes sont désavantagés par rapport aux homozygotes (non hybrides). Dans ce cas, la zone d'hybridation peut évoluer en une barrière interspécifique par renforcement des différences (phénotypiques ou alléliques) interspécifiques.

La spéciation sympatrique implique la mise en place de barrières reproductrices au sein même d'une population initialement panmictique (l'union des gamètes au hasard), la sélection divergente/disruptive pouvant favoriser la valeur sélective (fitness) de deux optimums phénotypiques au dépend des intermédiaires par des processus de « renforcement » de caractères divergents entre ces optimums. Bien que la sélection au profit des hybrides soit sensée être un phénomène rare (Turelli *et al*., 2001), de grandes modifications environnementales dans la zone d'hybridation, peuvent entraîner le déclin des espèces parentales et favoriser la valeur sélective des hybrides (voir des exemples en milieu terrestre : Grant & Grant, 1992 ; Campbell *et* al., 1997 ; Wang *et* al., 1997 ; Sclutter, 2000). Selon Seehausen (2004), l'hybridation semblerait être assez commune lors de la colonisation de nouveaux habitats et prédisposerait les populations colonisatrices à une diversification adaptative rapide en réponse à de fortes pressions de sélection divergente/disruptive pouvant conduire à une accélération du taux de mutation et/ou à des isolements de type parapatrique, sympatrique ou même allopatrique (Figure 3). Néanmoins, pour que cette forme de spéciation se produise, il faut une forte corrélation génétique entre les traits liés à l'adaptation locale et ceux conférant l'isolement reproductif (Felsenstein, 1981).

II. IMPORTANCE DES ECHELLES D'OBSERVATION

Toute population possède une organisation spatiale et donc l'échelle spatiale à laquelle l'échantillonnage sera effectué aura une grande importance selon les questions posées : l'échantillonnage pourra être local (micro-échelle, dans le cas d'une population composée de parcelles), régional (méso-échelle, dans le cas d'une métapopulation régionale de populations locales) ou global (macro-échelle, dans le cas d'une lignée évolutive composée de métapopulations régionales). L'évolution des unités spatiales en terme de différenciation ou d'homogénéité génétique ne s'effectuera pas bien évidemment aux mêmes échelles de temps. Les principaux mécanismes évolutifs ne joueront donc pas toujours de la même manière selon l'échelle de temps et d'espace et il est crucial d'identifier l'importance respective de ces différentes forces évolutives (dérive, sélection, mutation et migration) dans la structuration, l'évolution et la distribution des populations marines. Comprendre le devenir des écosystèmes marins face aux pressions anthropiques revient donc à aborder non seulement l'histoire évolutive des organismes et l'impact des barrières actuelles et historiques sur les patrons biogéographiques, mais aussi de comprendre les mécanismes régulant l'organisation spatio-temporelle d'une population à l'échelle régionale et locale, c'est-à-dire les processus biologiques assurant le fonctionnement des populations et leur expansion/ limitation dans l'espace.

II. 1. Observation des structures à macro-échelle

Ici, nous faisons référence à la notion de biogéographie marine dont les concepts fondamentaux reposent sur une interaction étroite entre (1) l'évolution des espèces sur l'aire de répartition par modification progressive de la composition génétique des populations, (2) les phénomènes d'extinctions d'une ou d'une partie des populations, ou la disparition d'une espèce sur cette même aire de répartition et (3) les processus dispersifs qui assurent échanges et mouvements des populations à partir de leur point d'origine. La distribution géographique

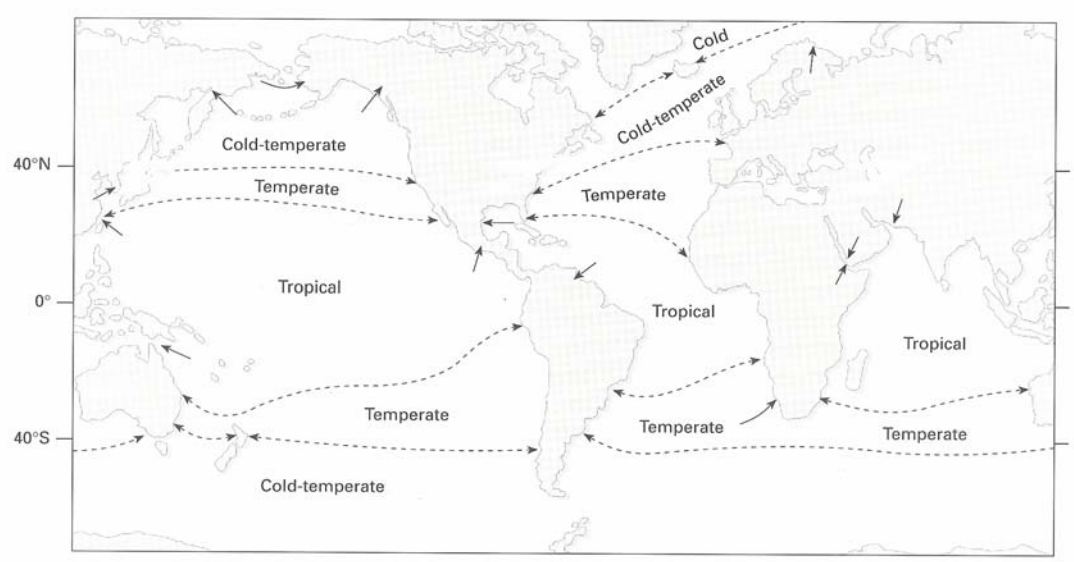


Figure 4. Zones de transition biogéographique décrites en fonction du régime thermique des masses d'eau et des gradients faunistiques (tiré de Cox & Moore, 2000).

d'une espèce (ou lignée évolutive) dépendra donc (1) de la niche écologique ancestrale de la lignée (Wiens & Donoghue, 2004), (2) des facteurs biotiques et abiotiques limitant la dispersion vers d'autres régions, (3) du potentiel d'évolution de la niche écologique selon sa position géographique et (4) du temps écoulé depuis l'apparition de la lignée.

Les propriétés particulières de l'environnement marin font que la délimitation des aires de distribution géographique des espèces marines est beaucoup moins évidente qu'au niveau terrestre. Les océans et les masses d'eau sont interconnectés et en mouvement continuel, les gradients environnementaux et faunistiques sont très progressifs, et le couplage entre l'océan et l'atmosphère influence la direction et la force des courants de surface qui, à leur tour influencent le transport larvaire et donc la distribution des individus d'une espèce. Dans l'environnement côtier, les distributions géographiques des espèces sont pour la plupart très fragmentées à cause d'une plus grande variabilité environnementale, d'une action anthropique plus marquée et de la modification géodynamique du trait de côte au cours des temps géologiques (voir Dinter, 2001). Il existe néanmoins une importante similitude des patrons faunistiques avec l'environnement terrestre lorsque que l'on considère les zones biogéographiques côtières selon la latitude à l'échelle planétaire (Figure 4), car ces patrons dépendent principalement de l'influence de l'irradiation solaire et des courants marins. Les limites/frontières biogéographiques ont donc été décrites en fonction du régime thermique des masses d'eau et des gradients faunistiques (Cox & Moore, 2000).

II.1.1. Les zones de transition biogéographique

Ces limites biogéographiques en milieu marin constituent des zones de transition d'une importante complexité écologique du fait des chevauchements d'espèces en limite d'aire (Holt & Keitt, 2000). Ces limites favorisent l'isolement reproductif, maintiennent des zones d'hybridation ou favorisent la co-existence d'espèces cryptiques. Ces zones de transition peuvent également être de véritables barrières ayant pour action de filtrer certaines espèces

selon leur potentiel dispersif et reproductif, leur plasticité environnementale et leur potentiel de survie en milieu fragmenté. Il faut noter que les changements observés dans la composition des communautés marines entre provinces biogéographiques sont également observés au niveau de la composition génétique de certaines espèces largement distribuées de part et d'autre de ces zones de transition. Ces points de rupture phylogéographiques ont été déjà étudiés : aux Etats Unis, le cap Canaveral en Floride, zone de transition entre provinces tropicale et tempérée chaude (côte Est, Reeb & Avise, 1990); Point Conception entre provinces californienne et orégonienne (côte Ouest, Burton, 1998 ; Dawson, 2001); en Australie, le détroit de Bass, entre provinces péronienne et maugéenne (Waters & Roy, 2003 ; Waters *et al*., 2004) ; en Nouvelle Zélande, le détroit de Cook (Apte & Gardner, 2002) ; les îles de l'Indo- Pacifique (Barber *et al*., 2000, 2002), zone de chevauchement entre trois grandes provinces biogéographiques; le front Almeria-Oran, entre l'Atlantique et la Méditerranée (Borsa *et al*., 1997).

Il découle de ces études que les forts niveaux de structure génétique observés entre provinces biogéographiques qui coïncident avec des zones de discontinuités hydrodynamiques (upwelling ; tourbillons ; fronts océaniques) aient été créés principalement par vicariance sous l'action de phénomènes géologiques et hydrodynamiques. L'aire de distribution peut se repositionner ultérieurement en fonction des frontières biogéographiques qui sont autant de zones de contact secondaires privilégiées où la dispersion est réduite (effet « puit de larves ») et où les zones d'hybridation peuvent venir se caler. Pourtant l'importance respective de la sélection et de l'hydrodynamisme dans le maintien de ces zones de transition n'est pas toujours claire. De plus, d'après Burton (1998), des ruptures phylogéographiques qui coïncident avec des zones de transition biogéographique peuvent aussi être dues, non pas à une barrière hydrodynamique ou biogéographique, mais à un différentiel dans le degré de prédation, à un taux de mutation élevé, à des extinctions fréquentes, à des systèmes

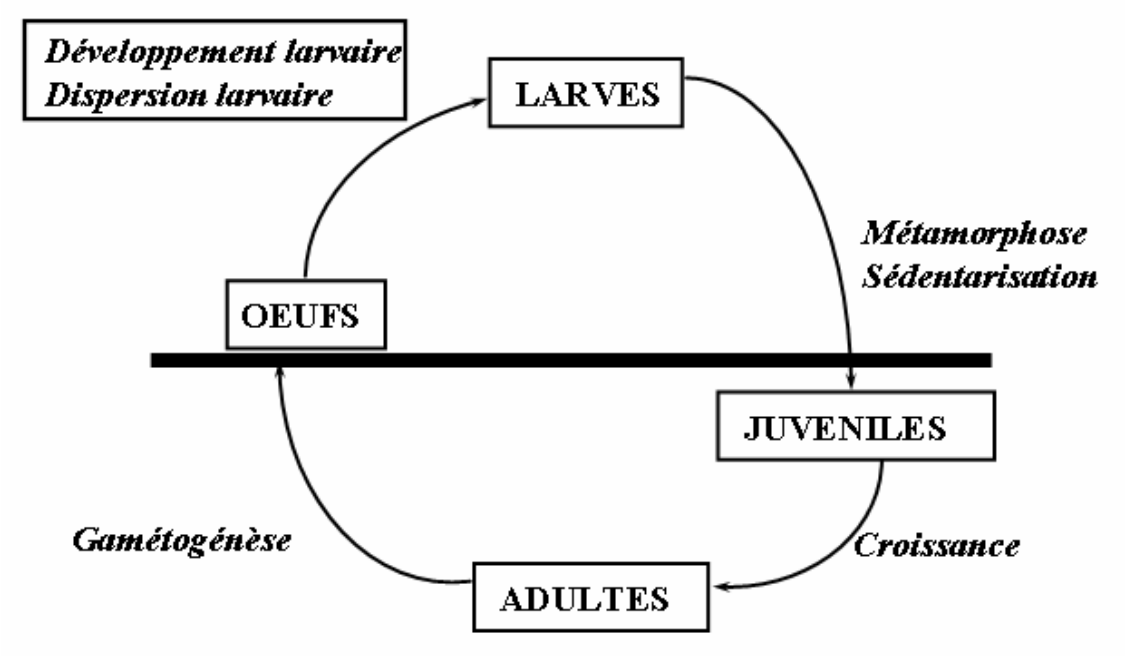


Figure 5. Représentation schématique du cycle de vie bentho-pélagique (d'après Bachelet, 1990).

d'incompatibilité entre populations, ou dans certains cas (par exemple chez les bivalves) à des maladaptations générées par des parasites (Hare, comm. pers.).

II.2. Observation des structures à méso-échelle

A l'échelle d'une lignée évolutive, la structure génétique des populations dépend majoritairement de l'importance relative de la migration, la dérive génétique et la sélection. Dans une population fermée, selon la théorie de la neutralité (Kimura, 1968), le degré de polymorphisme de l'espèce est déterminé par la taille efficace de la population et par le taux de mutation (Kimura & Crow, 1964 ; Nei, 1987). Dépendante de la taille des populations, la dérive génétique aura plus ou moins d'effet sur la fixation des allèles et la diminution de l'hétérozygotie. Par exemple, dans une population de petite taille, les fréquences alléliques peuvent largement fluctuer d'une génération à l'autre sous le seul effet de la dérive génétique. La migration apparaît donc comme un stade critique dans la structuration des populations d'invertébrés marins benthiques, car elle permet d'une part, les échanges d'individus entre populations plus ou moins isolées et, d'autre part, d'éviter la compétition pour la ressource entre adultes et juvéniles, les extinctions locales privilégiant la colonisation de nouveaux territoires. Selon le mode dispersif et les facteurs régulant la phase pélagique, certaines espèces présentent un potentiel de colonisation important tandis que d'autres ne pourront se maintenir que très localement, avec des populations plus ou moins stables au cours du temps. Néanmoins, Palmer & Strathmann (1981) indiquent qu'au-delà d'une certaine distance, les bénéfices d'un stade larvaire de longue durée n'augmentent plus, la perte en larves par diffusion étant trop forte pour le maintien des populations adultes.

II.2.1. Le rôle de la phase larvaire

Soixante dix pour cent des espèces marines benthiques présentent un cycle de vie bentho-pélagique (Figure 5) comprenant deux phases benthique (la phase de croissance et la phase de

maturité sexuelle après laquelle l'adulte se reproduit) et une phase larvaire pélagique (Thorson, 1946), cette dernière pouvant durer de 1-2 jours à plusieurs mois. Chez ces espèces, la répartition et le maintien des populations benthiques sont gouvernés par quatre facteurs : (1) les processus hydrodynamiques tels que les courants d'advection et la diffusion turbulente qui agissent sur la concentration du nuage larvaire, la dissémination ou la rétention des propagules, (2) les propriétés biologiques intrinsèques à l'espèce (succès reproducteur, périodes de ponte, mode de développement larvaire, nutrition et durée de vie larvaire), (3) les interactions larve/plancton et notamment le comportement migratoire des larves qui régule partiellement leur distribution verticale (migration tidale, diurnale ou ontogénique), et (4) la phase de recrutement (choix du substrat, compétition pour l'espace, interactions larves/ adultes). D'après la théorie du ''supply-side ecology'' (Underwood & Fairwheather, 1989; Grosberg & Levitan, 1992), la variabilité spatio-temporelle du nombre d'individus dans les populations des espèces à cycle bentho-pélagique est, initialement dépendante de l'apport en larves qui, lui même est dépendant de l'hydrodynamisme local. Ce dernier influence le recrutement de deux façons principales, soit à partir de processus de rétention larvaire au niveau de la population adulte (auto-recrutement massif sur le site), soit en favorisant le retour des larves vers la population adulte après une phase de dispersion (effet phylopatrique impliquant un auto-recrutement faible passé au crible de la dispersion). Le comportement migratoire des larves pourrait représenter un moyen efficace dans l'utilisation des courants soit dans la colonisation de nouveaux habitats, soit en favorisant la rétention larvaire (i. e. larves revenant à la pointe du flot pour retrouver des habitats favorables; Thiébaut, 1996). Ce processus migratoire est d'ailleurs utilisé par le crabe *Callinectes sapidus* pour conclure son cycle larvaire: après un transport initial vers le large par les eaux de surface, la larve peut ré-envahir les estuaires au stade mégalope (Mc Millen-Jackson *et al*, 1994). Ainsi, malgré sa vie larvaire de un à deux mois, *C. sapidus* montre une nette différenciation génétique de ses populations sur des distances de 300 km (Kordos & Burton, 1993).

Encadré 1. Modèles de colonisation et leurs attendus génétiques

Pannell & Charlesworth (2000) discutent des deux modèles principaux de colonisation (le « migrant pool » et le « propagule pool » ; Slatkin, 1977), dont les conséquences génétiques diffèrent en fonction du modèle théorique utilisé et des taux d'extinction et de migration (Slatkin 1977, Whitlock & McCauley, 1990).

Si l'on considère un modèle de migration en îles avec une colonisation se faisant à partir d'un pool de migrants tirés au hasard à partir de l'ensemble des sous-populations, les phénomènes d'extinctions/ recolonisations tendent à réduire la différenciation et à produire une augmentation de la diversité génétique dans les populations nouvellement établies, si et seulement si, le nombre d'individus fondateurs (colonisant les habitats vierges) est le double de ceux échangés entre populations pré-établies (Wade & McCauley, 1988 ; McCauley, 1991).

Dans le cas d'un modèle « propagule pool » où le pool de colonisateurs arrivant en un site est issu des populations les plus proches, les extinctions répétées peuvent d'une part, produire une augmentation du niveau de différenciation génétique (Wade & McCauley, 1988 ; Whitlock & McCauley, 1990), et d'autre part, conduire à une réduction de la diversité génétique entre populations nouvellement établies (Pannell & Charlesworth, 2000). Bien sur, ces conséquences dépendent de la structure même de la métapopulation (Dias, 1996 ; Harrison & Hastings, 1996 ; Hanski & Gilpin, 1997).

Pour certaines espèces à faible durée de vie larvaire, l'action des fronts océaniques peut venir renforcer le niveau de structure génétique à méso-échelle en causant une rupture du flux génique et un isolement par la distance détectable de part et d'autre de cette barrière physique, si celle-ci persiste. Les fronts océaniques constituent en effet des pièges à larves efficaces qui peuvent conduire à la perte de la plupart de celles-ci pour la population, ces dernières ne pouvant atteindre les habitats favorables à la fin de la phase larvaire. Outre les fronts océaniques, les patrons locaux et globaux de déplacement des masses d'eau, la topographie du fond et la bathymétrie, l'influence des fronts d'estuaires et des barrières climatiques entre provinces biogéographiques peuvent réduire la dispersion des larves pélagiques et ainsi favoriser la différenciation génétique entre populations. A l'échelle d'un groupe de populations interconnectées, retenons que selon la durée de la phase larvaire et de la distribution spatiale des populations, les modèles de migration seront différents (e.g. modèle en îles, Wright, 1931 ; modèle d'isolement par la distance, Wright, 1943). De plus, l'amplitude des phénomènes d'extinctions et de recolonisations peut être une puissante source de flux génique agissant en fonction du mode de migration et de colonisation (i.e. « propagule pool », « migrant pool », voir Encadré 1). Ces structures peuvent alors évoluer dans le temps selon le degré de connexion larvaire influençant la dynamique propre à chaque population.

II.2.2. Le concept de métapopulation en milieu marin

Ces dynamiques ont été définies par le concept de « métapopulation » initialement développé par Levins (1969) pour décrire « une population de populations » structurée spatialement et qui persiste, malgré l'extinction locale des sous-populations qui la composent, à partir du réseau d'échanges qui relie ces sous-populations entre-elles.

Cette idée de réseau de populations de taille finie et d'interactions entre populations locales avait déjà été développée par Wright (1940) ainsi que par Andrewartha & Birch (1954). Le concept dérive également de la théorie dynamique de la biogéographie insulaire

(MacArthur & Wilson, 1967), et, repose notamment sur le fait que dans un milieu fragmenté, la migration a un effet sur la dynamique propre de chaque population locale, ce qui inclue la possibilité d'un rétablissement de la population après extinction (le « rescue effect »). Ce concept a été principalement documenté et utilisé en milieu terrestre, sans pouvoir toujours définir les conditions qui permettent à la plupart des populations terrestres de constituer une métapopulation : (1) indépendance des dynamiques locales; (2) risque d'extinction dans les populations locales ; (3) populations reliées entre elles par la migration ; et (4) migrants pouvant re-coloniser les habitats vierges.

En environnement marin, Botsford *et al*. (1994) définissent la métapopulation comme un nombre de sous-populations d'adultes reliées entre elles par la phase larvaire, l'environnement physique régulant la dynamique de la métapopulation de par son influence sur la distribution du panache larvaire et donc du recrutement des juvéniles. Pourtant même en relâchant certaines des conditions du modèle de Levins (e.g. extinction-recolonisations), des biais à l'application du modèle peuvent apparaître en environnement marin. Le premier biais vient de la difficulté d'échantillonner le milieu marin notamment à micro-échelle spatiale (i.e. détermination des parcelles) et le second, de délimiter les « populations » locales d'une espèce en terme de métapopulation (Smedbol *et al*., 2002 ; Grimm *et al*., 2003), et ce d'autant plus que les populations sont généralement ouvertes en terme de migration.

Pourtant, le concept lui-même s'applique assez bien aux invertébrés marins sessiles possédant un cycle de vie bentho-pélagique (noter les similitudes entre la larve et le pollen en matière de « dispersion passive »), les populations régionales pouvant persister malgré des phases d'extinction locales. Pour ces espèces, les conditions de fonctionnement de la métapopulation peuvent être applicable à différentes échelles (métapopulation régionale composée de sous-populations, populations locales elles-mêmes composées d'agrégats d'individus se reproduisant préférentiellement entre eux). Potentiellement, n'importe quel groupe de populations structuré spatialement peut présenter une structure en métapopulation,

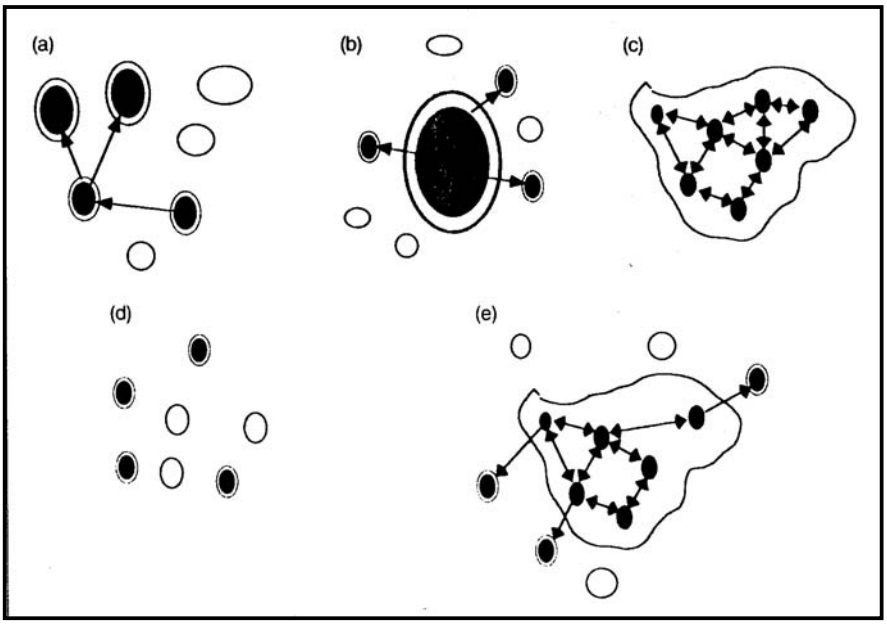


Figure 6. Les différents types de métapopulation. (a) type « classique » (Levins) ; (b) type « continent-îles » ou « source-puits » ; (c) type « population fragmentée » ; (d) *meta*population en déséquilibre ; (e) cas intermédiaire combinant (a) et (d). Cercles remplis : habitats occupés ; symboles non remplis : habitats vierges ; flèches : dispersion ; lignes noires entourant les cercles remplis : limite de distribution des populations locales. D'après Harrison & Hastings (1996).

aussi transitoire soit elle, par exemple, si les risques d'extinction augmentent. Les questions relatives au fonctionnement en « métapopulation » (*sensu stricto* population de populations) dans l'environnement marin peuvent se poser quelque soit le degré de connectivité, d'extinction ou de recolonisation des populations d'une espèce.

Les modèles théoriques écologiques ou génétiques se sont multipliés et complexifiés depuis le modèle original proposé par Levins (voir pour une revue de la terminologie et des modèles l'article de Hanski & Simberloff, 1997, cf. Figure 6 pour les différent modèles). Nous retiendrons dans le cadre de cette thèse, le modèle continent-îles et surtout sa variante, le modèle source-puits (Harrison, 1991; Hanski, 1996, 1999; Gaggiotti, 1996). Ces modèles nous intéressent particulièrement dans le cas des invertébrés à cycle bentho-pélagique possédant de fortes capacités dispersives.

Le modèle en continent-îles (mainland-island) est caractérisé par (1) un flux de propagules en provenance d'une large population stabilisée (le continent = source), vers de petites populations (les îles ou puits) qui n'échangent pas ou peu entre elles, (2) une taille et/ou une longévité inégale entre populations locales et (3) un risque d'extinction négligeable pour la population continent (i.e. la source de migrants). Par conséquent, les populations insulaires sont uniquement maintenues dans le temps par la migration en provenance du ‘‘continent’’. Les caractéristiques génétiques d'une telle métapopulation peuvent être considérées comme « stables » à l'échelle de la durée de vie de la population source. A long terme, la variabilité génétique totale est préservée et tout changement des fréquences alléliques dans les populations de petite taille est contrebalancé par les flux géniques en provenance du ‘’continent’’.

Le modèle source-puits quant-à-lui, peut être décrit de différentes façons. Selon Pulliam (1988), la population source se définit comme une population stable dans le temps qui alimente plus ou moins régulièrement des population-puits marginales dans lesquelles le taux de croissance est négatif. La métapopulation source-puits est dans ce cas caractérisée par une

asymétrie de flux pouvant mélanger plusieurs sources, ce qui aurait pour conséquence une plus forte adaptation à l'habitat source qu'à l'habitat puit, bien qu'en théorie, il puisse y avoir des phénomènes d'inversion source-puits (Holt, 1995). Ceci laisse supposer qu'il puisse exister plusieurs populations sources au sein d'une même métapopulation source-puits. Sur le plan génétique, la différenciation entre la source et les puits est théoriquement faible dans les cas où (1) les phénomènes d'extinction sont fréquents et la plupart des recrues des population-puits proviennent de la population-source, et où (2) les phénomènes d'extinction sont rares et contrebalancés par de la migration entre population-puits. Néanmoins, le degré de différenciation génétique entre les puits peut être important si l'immigration se déroule de manière stochastique multipliant les effets de dérive génétique (Gaggiotti & Smouse, 1996). Le taux de déclin des populations doit également être considéré si l'on veut prédire l'effet conjoint de la migration et de la dérive sur la variabilité génétique (Dias, 1996). Si le taux de migration depuis la population source est constant dans le temps et l'espace et que le taux de déclin d'une population-puit est élevé, alors la diversité génétique de la population-puit approche celle de la population source, et il n'y a pas de différence génétique significative ni entre puits, ni avec la source. Au contraire, si le taux de migration varie de façon aléatoire dans l'espace et le temps, et que le taux de déclin d'une population-puit est faible, les effets de dérive génétique peuvent alors dominer et il s'en suit une augmentation du niveau de différenciation génétique entre les puits et avec la source (Dias, 1996).

II.3. Observation des structures à micro-échelle

Regarder le fonctionnement des populations à l'échelle locale (i.e. la parcelle) permet d'aborder les mécanismes micro-évolutifs associés au recrutement et à la survie des post-larves/ juvéniles jusqu'à la reproduction. Cette échelle d'observation est d'autant plus importante que de faibles différences biologiques au niveau d'un groupe de sous-populations (e.g. variance du succès reproducteur, asynchronie de la ponte, mécanismes associés à la

structure en métapopulation) peuvent avoir d'importantes conséquences sur le devenir des générations suivantes et orienter les mécanismes macro-évolutifs si les contraintes sont maintenues dans le temps (voir les modèles temporels d'isolement adaptatif de Hendry & Troy, 2005). L'observation à micro-échelle d'une population structurée localement (population composée de dèmes de différentes tailles) passe nécessairement par un suivi temporel car c'est la variation temporelle de la taille efficace (i.e. la part de géniteurs produisant une nouvelle génération d'adultes) qui conditionne la structure génétique à cette échelle. La taille efficace (N_e, l'effectif reproducteur permettant d'expliquer l'ensemble de la diversité génétique observée dans la population) est généralement beaucoup plus petite que la taille totale de la population (N), cette réduction pouvant être due aux fluctuations démographiques de la population et aux variations du succès reproducteur liées aux différences de fertilité entre les dèmes ou même à une asymétrie dans la distribution des sexes. Ce paramètre est d'importance en biologie de la conservation car il permet de définir le stock géniteur minimum, mais il est aussi très difficile à estimer car il faut un échantillonnage hiérarchique de la population locale. De plus, son estimation se fait sous l'hypothèse que la population est à l'équilibre mutation-dérive et ne reçoit pas de migrants en provenance de l'extérieur. La méthode de Waples (1989), basée sur la variation temporelle des fréquences alléliques à l'intérieur d'une population à générations non-chevauchantes permet d'estimer ce paramètre N_e en l'absence de sélection, de migration et de mutation. Cette méthode est la mieux adaptée chez les organismes possédant une forte fecondité et pour lesquels les juvéniles sont sujets à une forte mortalité (Waples, 1989).

L'hypothèse selon laquelle l'hétérogénéité temporelle des recrues est responsable de discontinuités génétiques spatiales à petite échelle a été proposée à partir d'études allozymiques à plus ou moins grande échelle géographique chez des espèces caractérisées par une longue phase larvaire (e.g. patelles, Johnson & Black, 1984; oursins, Watts *et al.*, 1990; crabes, Kordos & Burton, 1993; McMillen-Jackson *et al.*, 1994; bivalves, David *et al.*, 1997).

Pourtant, la plupart de ces études n'expliquent l'hétérogénéité locale qu'à partir des variations génétiques observées à plus grande échelle en mettant en avant l'effet possible de la sélection. A l'échelle d'une population locale subdivisée en dèmes et relativement fermée aux apports larvaires extérieurs, les changements génétiques spatio-temporels peuvent être expliqués par un différentiel significatif du succès reproducteur entre les dèmes (Hedgecock, 1994), ou par un fort degré d'asynchronie de la reproduction entre dèmes qui favoriserait le recrutement de cohortes génétiquement différenciées (David *et al.*, 1997). L'existence d'une structure génétique faible mais significative à micro-échelle spatiale et/ou temporelle a été définie sous le vocable de « structure chaotique » par Johnson & Black (1982). Un des mécanismes sous-jacents à ce type de structure pourrait également provenir de phénomènes répétés d'extinction et de recolonisation, c'est-à-dire une structure en métapopulation locale.

L'hétérogénéité de l'habitat à micro-échelle peut également modifier considérablement les fréquences alléliques dans une population à un locus donné en favorisant l'expansion des allèles/génotypes les plus performants eu égard à des contraintes sélectives locales particulières. Elle peut contrebalancer l'effet homogénéisant de la migration mais aussi l'effet diversifiant de la dérive génétique si ces contraintes sélectives agissent uniformément sur la totalité de la parcelle analysée. Les agrégats d'individus peuvent alors diverger en étant attirés vers des pics adaptatifs différents. Un mécanisme pouvant forcer les populations à passer d'un pic vers un autre peut alors être la modification des conditions environnementales. A cela viennent s'ajouter les processus stochastiques tels que des effets démographiques forts qui peuvent accentuer ces modifications de fréquences alléliques.

III. LA ZONE D'ETUDE : L'ATLANTIQUE NORD EST

Les particularités biogéographiques de l'Atlantique Nord Est sont le fruit d'une évolution des structures de peuplement en fonction de phénomènes historiques, géologiques et hydrodynamiques propres à la région arctico-boréale. Les recouvrements d'espèces marines

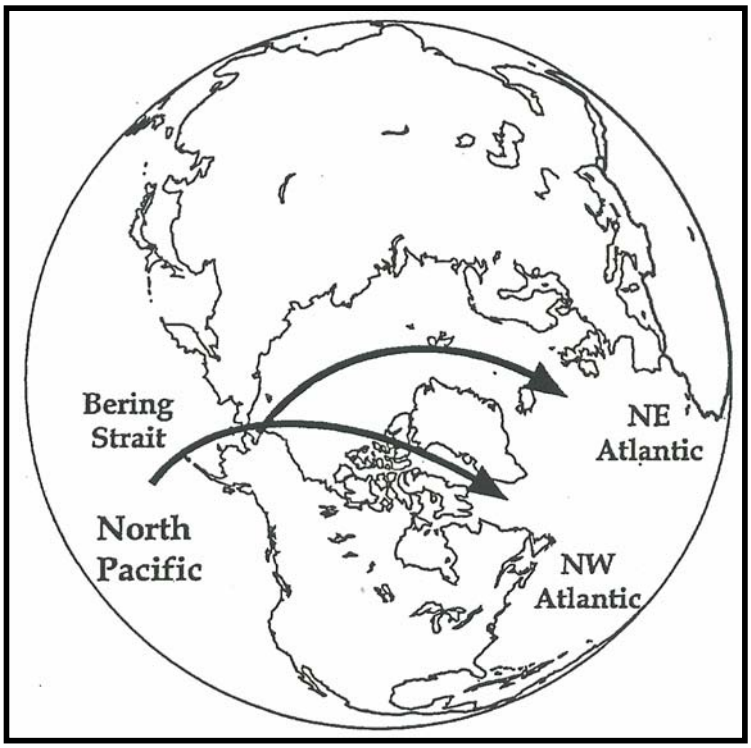


Figure 7. Echanges trans-arctiques ayant démarré vers 3.5 millions d'années selon les données paléogéographiques de fossiles de mollusques, après l'ouverture du détroit de Béring (située entre -7 et -4 millions d'années selon les estimations de Marincovitch & Gladenkov, 1999). Figure tirée de Cunningham & Collins (1998).

sont nombreux et s'effectuent plus particulièrement en limite d'aire de distribution des espèces, notamment au niveau des îles Britanniques et des approches occidentales de la Manche. Pour apprécier ce constat biogéographique, rappelons tout d'abord quelques faits concernant l'évolution historique de ces régions et de leurs peuplements.

III.1. Evolution historique des peuplements dans l'Atlantique Nord

La formation de l'Atlantique Nord est relativement récente (principalement durant la période du Crétacé supérieur) lorsqu'on la compare à celle du Pacifique. La réouverture du détroit de Béring entre 4 et 7 millions d'années (Marincovich & Gladenkov, 1999) a vraisemblablement permis l'échange d'un grand nombre d'espèces marines polaires et tempérées, avec une dominance d'envahisseurs provenant du Pacifique et colonisant l'Atlantique (Figure 7) notamment chez les mollusques, les cirripèdes, et les échinodermes (Vermeij, 1991 ; Cunningham & Collins, 1998). La fermeture complète de l'isthme de Panama (il y a 3 millions d'années ; Stehli & Webb, 1985) quant-à-elle a stoppé les échanges de faunes côtières entre Atlantique et Pacifique dans la ceinture tropicale (Knowlton *et al.*, 1993, Lessios & Weinberg, 1994, Knowlton & Weigt, 1998), amenant les courants marins à se modifier pour aller renforcer et réchauffer le Gulf Stream (Shackleton & Opdyke, 1977) et provoquer ainsi (1) l'extinction massive des mollusques tempérés froids dans l'Atlantique nord à la transition du Plio-Pleistocène (Stanley, 1986, Reeb & Avise, 1990) et (2) des échanges plus fréquents entre les populations marines du Golfe du Mexique et de l'Atlantique nord. Cependant, durant le dernier maximum glaciaire du Pleistocène (Last Glacial Maximum : LGM, il y a 18 000- 23 000 années), les régions Arctiques ont été de nouveau isolées complètement du Pacifique et partiellement de l'Atlantique en raison de la baisse du niveau de la mer d'environ 150 m (Poag, 1981). Les calottes glacières s'étendaient alors en Europe jusqu'au sud des îles Britanniques et aux Etats Unis jusqu'à Long Island Sound (Braatz & Aubrey, 1987), donnant lieu à un isolement des populations dans des zones dites

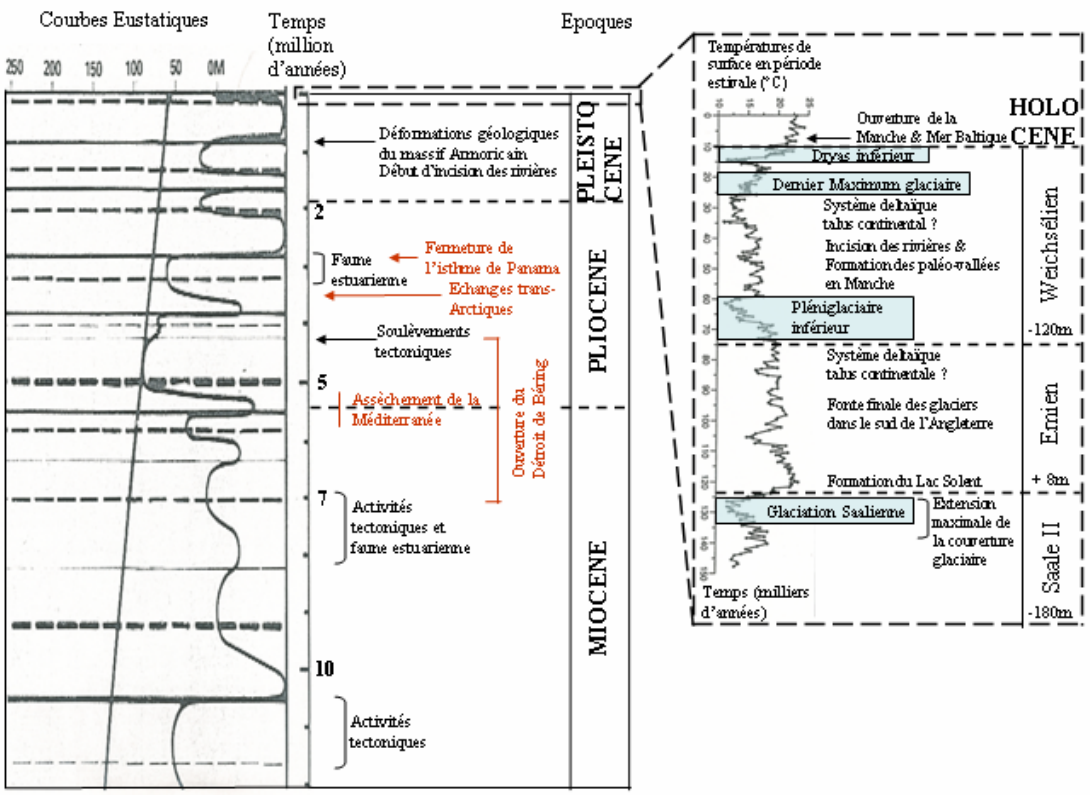


Figure 8. Evolution des courbes eustatiques de variation du niveau de la mer pendant les derniers 12 millions d'années ainsi que des températures de surface en été pendant les derniers 150 milliers d'années, en fonction des évènements géologiques et climatiques en Europe de l'Ouest (en noire). En rouge sont les évènements majeurs qui se sont déroulés ailleurs. (d'après les informations tirées de Kellaway *et al*., 1975 ; Smith, 1989 ; Haq *et al*., 1987 ; Conradsen, 1995 ; Marincovitch & Gladenkov, 1999 ; McKenzie, 1999 ; Reynaud *et al*., 1999 ; Chapman *et al*., 2000 ; Renssen & Vandenberghe, 2003 ; Brault *et al*., 2004).

« refuges », où les conditions environnementales sont restées propices à la survie des organismes. Au Nord ouest des Etats-Unis, les glaciers recouvraient la totalité des habitats intertidaux (Wares & Cunningham, 2001). Cette période est reconnue comme ayant été particulièrement difficile pour les espèces inféodées aux substrats rocheux de la zone intertidale (Riggs *et al.*, 1996). Pour certaines de ces espèces, ces extinctions furent suivies d'événements de recolonisation à partir de l'Europe où le substrat rocheux était plus abondant et les changements climatiques moins importants (Vermeij, 1991). Bien que les zones refuges se soient situées plus vraisemblablement dans le Golfe du Mexique, pour *Pagurus longicarpus,* l'existence d'un refuge glaciaire dans le Nova Scotia (nord-est des US) a aussi été inférée (Young *et al*., 2002). Très peu, voire aucune information n'est disponible pour ce qui concerne l'histoire évolutive des espèces inféodées aux sédiments meubles (mais voir *Streblospio sp*., Schulze *et al.*, 2000).

La chronologie des évènements tectoniques, géologiques et climatiques en Europe de l'Ouest est représentée par la Figure 8. Le bouclier armoricain connut plusieurs épisodes de transgression océanique. Il fut tout d'abord partiellement submergé au milieu du Miocène (il y a 8-9 millions d'années) notamment dans la zone s'étendant de Saint Malo à Nantes (Brault *et al*., 2004). Les fluctuations du niveau de la mer furent également importantes au Pliocène avec une première phase d'inondation il y a 4.5 millions d'années, qui a favorisé l'installation d'une faune marine, puis d'une nouvelle transgression, il y a 3.3 millions d'années favorisant l'installation d'une faune plus estuarienne (Brault *et al*., 2004). La période pré-LGM fut également caractérisée par des fluctuations de moindre amplitude du niveau de la mer (environ 10-15 m tous les 6000 ans : Haq *et al.*, 1987 ; Lambeck & Chappell, 2001). Le niveau de la mer est tombé à -100/ 150 m durant la dernière période glaciaire, le talus continental reliant alors le sud de l'Irlande aux pointes de la Cornouaille et de la Bretagne (Lambeck, 1997 ; Taberlet, 1998). Le réchauffement climatique et la déglaciation qui suivirent cette période (entre -18 000- 13 000 ans) furent stoppés spar un retour aux

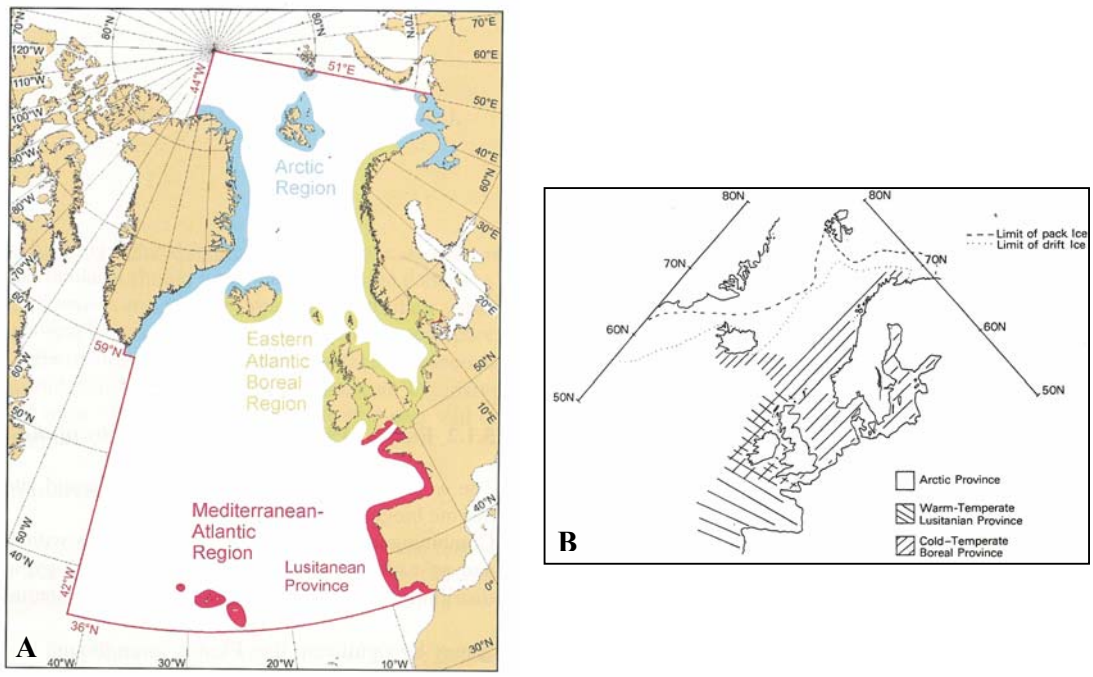
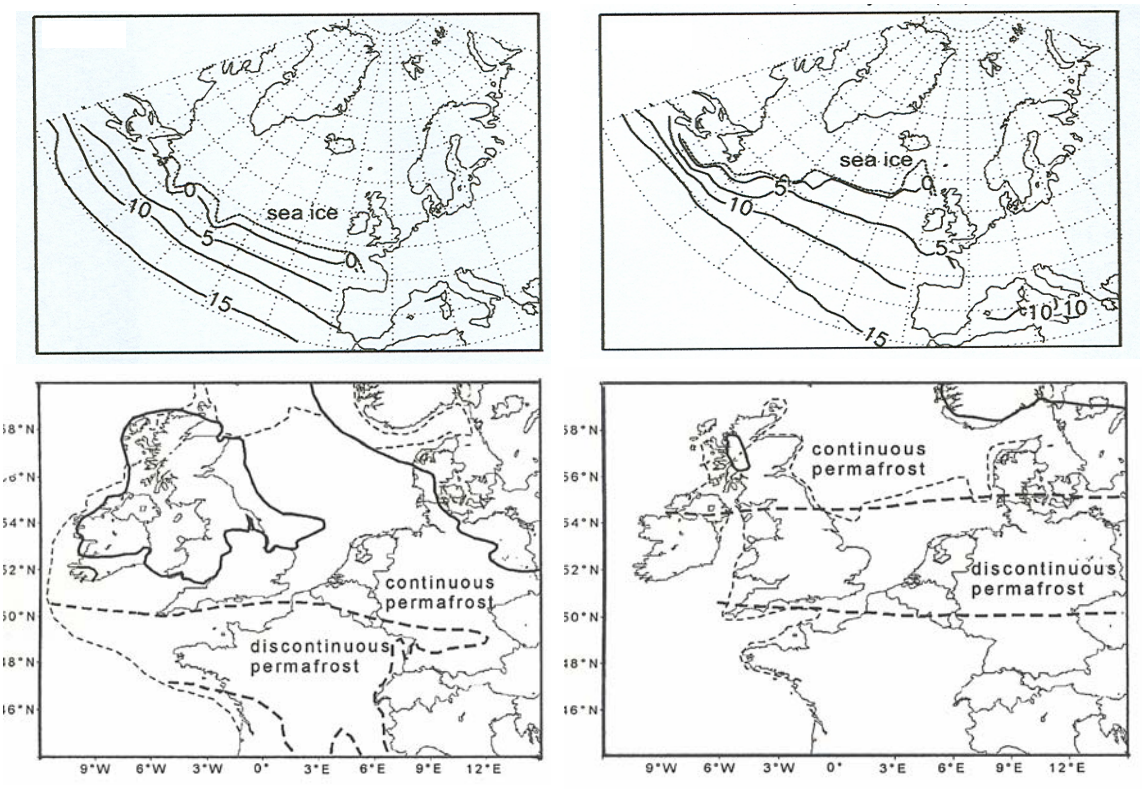


Figure 9. Régions biogéographiques actuelles dans l'Atlantique Nord Est selon Forbes (1856) et redessiné par Briggs (1995) (A) et selon Briggs (1974) dans Earll & Farnham (1983) (B).

conditions glaciaires durant la période des « Younger Dryas » entre -12 900 et 11 500 ans (Renssen & Vandenberghe, 2003). Les causes seraient dues à l'arrêt de la circulation thermohaline de l'Atlantique Nord en réponse à l'afflux soudain d'eau fraîche provenant de la fonte des glaciers. Ces évènements transgressifs se sont accompagnés d'une modification des isothermes au niveau des zones Lusitanienne et Boréale, qui ont conduit à un déplacement d'espèces Boréales et d'espèces tempérées-froides vers des régions situées plus au sud (Taberlet, 1998 ; Dinter, 2001) jusqu'en Méditerranée (Raffi, 1986). Durant la dernière période de réchauffement climatique qui suivit, le retrait des glaciers a de nouveau modifié les courants marins permettant une expansion de l'aire géographique vers le nord de ces espèces entre 15° et 20° de latitude.

III.2 Les frontières biogéographiques de l'Atlantique Nord Est

L'Atlantique Nord Est se divise en trois grandes provinces (Figure 9) : (1) la province Lusitanienne (tempérée chaude), (2) la province Boréale (tempérée froide) et (3) la province Arctique/subarctique (froide). Il est à noter que la faune côtière au sud-ouest des îles Britanniques n'appartient pas à la province Lusitanienne selon la classification de Briggs (1974). Les mers situées autour des îles Britanniques sont essentiellement de nature boréale mais la partie nord est plutôt caractérisée par des communautés boréo-arctiques, alors que la partie sud-ouest présente des caractéristiques lusitano-boréales. Plus particulièrement, Michaneck (1979) et Lüning (1990) donnent une frontière biogéographique basée sur la distribution des algues, entre espèces des zones tempérées froides et espèces des zones tempérées chaudes, à l'ouest de l'Irlande et aux approches occidentales de la Manche. La forte variabilité environnementale qui caractérise cette zone et les évènements historiques complexes qui s'y sont déroulés sont en partie la cause de la très grande biodiversité marine observée à l'heure actuelle sur les côtes Bretonnes.



(A) Le Dernier Maximum Glaciaire (B) Le Dryas inférieure

Figure 10. Carte représentant les limites du permafrost en Europe de l'Ouest, l'évolution du trait de côte et des températures de surface (période hivernale) entre (A) le dernier maximum glaciaire (entre -23 et -18 milliers d'années) et (B) le Dryas inférieur (entre -14 et -11 milliers d'années). La ligne noire correspond aux limites de la couverture glaciaire ; la ligne noire discontinue représente les limites du permafrost ; la ligne noire fine et discontinue représente le trait de côte. (tiré de Renssen & Vandenberghe, 2003).

III.3. Historique de la Manche

La formation de la Manche est un phénomène très récent, progressif depuis sa partie occidentale (Lambeck, 1997) et catastrophique dans sa partie orientale (Smith, 1989). Outre la formation du « Lac Solent » après la période de glaciation Saalienne il y a 128 000 ans (Kellaway *et al*., 1975, voir Figure 8), il y a 12 000 ans, la Manche et la Mer d'Irlande correspondent à des baies connectées à l'Atlantique (Figure 10), un permafrost s'étendant au-delà de 55°N (Lambeck, 1997 ; Renssen & Vandenberghe, 2003). Les échanges entre faunes Boréale et Lusitanienne ne se font alors qu'au niveau du talus continental à la pointe des îles Britanniques (Ecosse). Ce n'est qu'il y a moins de 9 000 à 10 000 ans, qu'un passage s'est établi entre la Manche et la Mer du Nord avec l'ouverture catastrophique du détroit du Pas-de-Calais (Smith, 1989). De gigantesques volumes d'eau se sont alors déversés sur les parties orientale et centrale encore émergées de la Manche. Les présents patrons de circulation océanique ne s'établissent qu'il y a 7000-8000 ans, après l'ouverture de la Manche et du détroit du Danemark. Vers Cherbourg, les rivages approchent leur position présente à ces mêmes dates et selon Lambeck (1997) le niveau de la mer atteint celui observé à l'heure actuelle, il y a 5 000 ans, même si des données plus récentes montrent que le niveau continue d'augmenter lentement aux approches occidentales de la Manche (Lambeck & Chappell, 2001).

III.2.2. La zone de transition biogéographique aux approches occidentales

La côte nord-ouest de la Bretagne (et la mer d'Iroise) est reconnue comme une zone de transition entre espèces Lusitaniennes et Boréales (Cabioch, 1968, mais voir Dinter, 2001). De plus, si l'on considère la position géographique des îles Britanniques, la Bretagne aurait été l'une des premières régions à avoir été recolonisée à partir des faunes situées plus au sud (notamment la région Ibérique) à la fin de la dernière période glaciaire. La phase de recolonisation aurait également été effectuée à partir de certains refuges glaciaires nordiques

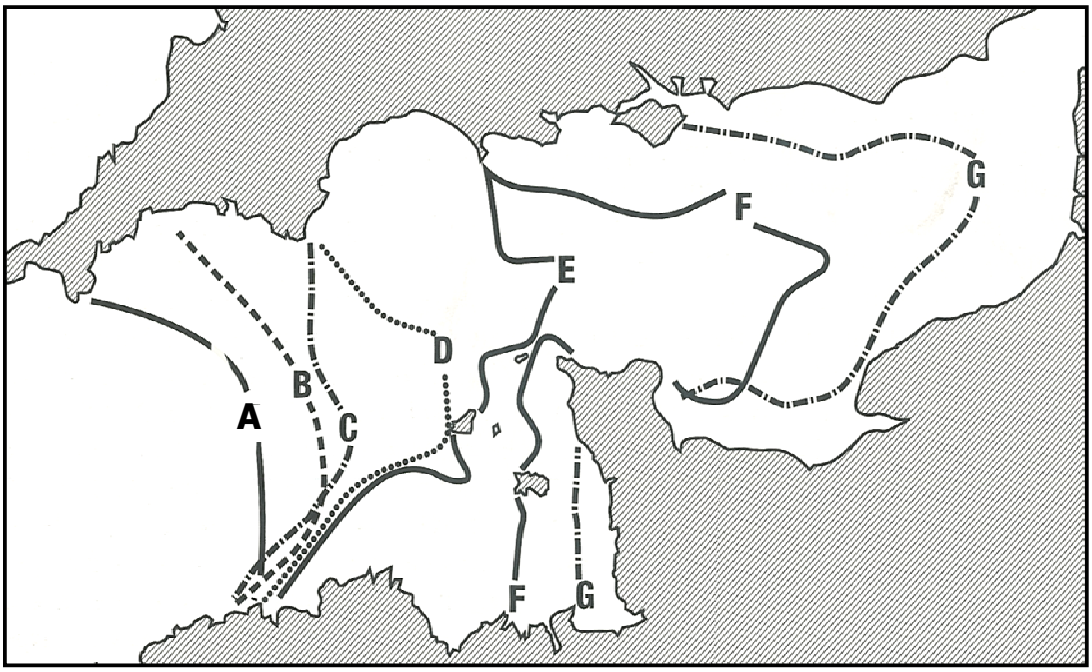


Figure 11. Courbes de pénétration des espèces Atlantiques en Manche. Limites orientales successives d'espèces de l'épifaune benthique, d'Ouest en Est : A : *Porella compressa* (Bryozoaire); B : *Diphasia pinaster* (Cnidaire); C : *Thuiaria articulata* (Cnidaire); D : *Lafoea dumosa* (Cnidaire); E : *Caryophyllia smithi* (Cnidaire); F : *Sertularella gayi* (Cnidaire); G : *Rhynchozoon bispinosum* (Bryozoaire). Tirée de Cabioch *et al*. (1977).

(Ecosse ; Norvège ; côte Ouest de l'Irlande, Stewart & Lister, 2001 ; Richter *et al*., 2001), ce qui aurait donné lieu au chevauchement actuel d'espèces en limite d'aire le long des côtes bretonnes.

Plusieurs études ont mis l'accent sur la position stratégique de la Baie de Morlaix dans la zone de transition en Manche orientale comme une région de limites successives d'aires pour de nombreuses espèces Boréales et Lusitaniennes du macrobenthos des substrats rocheux (Cabioch *et al*., 1977, Figure 11). Ce chevauchement d'aires permet d'expliquer la plus grande richesse spécifique en polychètes (Dauvin *et al*., 1994) et en amphipodes (Dauvin & Toulmont, 1988) dans la Manche. Cette zone frontalière est renforcée par la présence de structures hydrodynamiques relativement stables (fronts Celtique et de Ouessant et tourbillons Normano-Breton), révélées à partir de la modélisation de la circulation à long terme des courants marins (Salomon & Breton, 1993). Celles-ci interviennent potentiellement comme des barrières à la dispersion. Un afflux d'eau chaude provenant de l'Atlantique entre en Manche occidentale le long de la côte nord de la Bretagne à partir de la dérive de l'Atlantique Nord, facilitant ainsi l'exportation des larves de Bretagne sud vers le nord le long des côtes anglaises. Une partie de la masse d'eau retourne cependant en Mer Celtique le long de la côte sud de la Cornouaille pour générer le front Celtique (Beaumont, 1982). Un système de front océanique (le front d'Ouessant) se développe en été (à l'ouest de 5°W) à la confluence des eaux stratifiées (Atlantique) et non stratifiées (Manche), entre le nord ouest de la côte Bretonne et la pointe de la Cornouaille (Pingree *et al*., 1975). Bien que non montré, ce front océanique joue vraisemblablement un rôle important dans le maintien de la zone de transition en Mer d'Iroise, en tant que barrière physique à la dispersion larvaire pour certaines espèces à cycle bentho-pélagique se reproduisant au printemps.

IV. LE CHOIX DES MODELES BIOLOGIQUES

Dans le cadre de cette thèse, nous nous sommes intéressés à deux espèces marines d'annélides polychètes tubicoles inféodées aux substrats meubles sablo-vaseux, *Pectinaria koreni* (Malmgren) et *Owenia fusiformis* (Delle Chiaje). Ces deux modèles biologiques ont été sélectionnés à cause des connaissances acquises au cours des programmes nationaux (ie. PNDR- *Programme National sur le Déterminisme du Recrutement* ; PNEC- *Programme National d'Environnement Côtier*) sur ces animaux : (1) leur cycle de vie bentho-pélagique, (2) leur potentiel reproducteur considérable et leur répartition en agrégats à l'échelle locale d'une Baie, (3) la distribution fragmentée des populations adultes dans l'Atlantique Nord Est et la Manche, et (4) la modélisation du transport larvaire.

A l'échelle de l'Atlantique Nord Est, ces deux espèces présentent une distribution de type « insulaire » calquée sur la répartition des sédiments sablo-vaseux en tâches discontinues, notamment en Mer d'Irlande et le long des côtes françaises. A l'échelle de la Manche où les distributions sont relativement bien connues, les populations pourraient former soit une métapopulation, soit des groupes fonctionnels isolés à l'échelle de la Baie (Ellien *et al*, 2000 ; Barnay *et al.*, 2003). De plus, les larves de ces deux espèces ont une durée de vie supérieure à 15 jours et peuvent être transportées sur des distances considérables selon la circulation résiduelle des masses d'eau et l'intensité du forçage par le vent.

Pectinaria koreni (Malmgren, 1867)

L'annélide polychète tubicole *Pectinaria koreni* fait partie des Pectinariidae, famille phylogénétiquement proche des Alvinellidae, des Terebellidae et de Ampharetidae, qui appartient au groupe des Terebelliformes (Rouse & Pleijel, 2001). Holthe (1986) considère la famille comme comprenant 46 espèces dont 42 dans le genre *Pectinaria*, préalablement découpé en 3 genres : *Amphictene*, *Cistenides* et *Lagis*. Aucune analyse phylogénétique n'a été faite jusqu'à présent dans cette famille, mais il est intéressant de noter que le genre

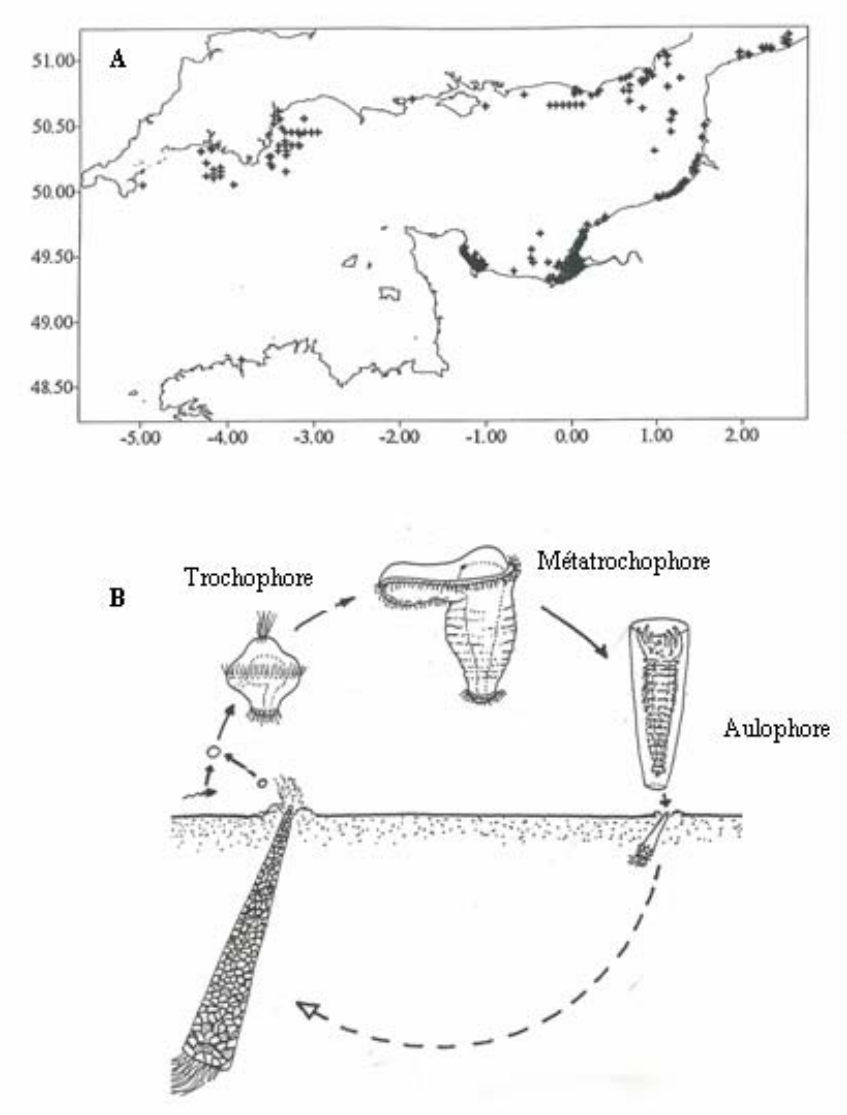


Figure 12. *Pectinaria koreni.* (A) Répartition des populations en Manche (tirée de Ellien, 2000) et (B) cycle de vie bentho-pélagique (dessin de F. Gentil).

Pectinaria est cosmopolite et qu'un fossile de tube provenant du Japon a été daté du Miocène (Katto, 1976).

Pectinaria koreni (syn. *Lagis koreni*) est une espèce monothélique univoltine avec une durée de vie de 15 à 18 mois (en Baie de Seine, Elkaim & Irlinger, 1987 ; Irlinger *et al.*, 1991). Sa répartition est calquée sur celle des sédiments sablo-vaseux dans les Baies et les estuaires (Figure 12). L'espèce est largement répartie le long des côtes de l'Atlantique Nord Est, des mers Boréales européennes (Mer de Barentz, Mer du Nord, Baltique, Manche) et des milieux lagunaires de Méditerranée et de la Mer Noire (Irlinger, 1985). Dans la Baie de Seine en Manche orientale, il existe deux principales périodes de reproduction, la première (Mars-Avril) étant plus importante que la seconde qui se déroule en Juin (Irlinger *et al*., 1991). La fécondité avoisine 20 000- 430 000 ovocytes par femelle (Ellien *et al*., 2000). Bien qu'à partir d'expériences en laboratoire, Cazaux (1981) ait estimé que la phase larvaire planctotrophique dure environ 58 jours, des observations *in situ* estiment celle-ci à environ 15 jours (Lagadeuc, 1990 ; Lagadeuc & Retière, 1993). Au cours de cette phase, la larve passe par 2 stades trochophores et 3 stades métatrochophores qui se maintiennent dans les eaux de surface (Figure 12). A la fin de cette phase larvaire, la métamorphose induit le passage au stade aulophore planctonique, stade qui se maintient à proximité du fond. Si le substrat n'est pas favorable lors de la première sédentarisation, les aulophores peuvent se remettre en suspension en produisant une voile de mucus pour se laisser entraîner par les courants (Lambert, 1991).

***Owenia fusiformis* (Delle Chiaje, 1841)**

Owenia fusiformis fait partie de la famille des Oweniidae, famille proche des Sabellaridae et incluse dans l'ordre des Sabellida (Rouse & Fauchald, 1997). Bien que cette famille soit composée de 37 (ou plus) espèces et au moins cinq genres, les relations phylogénétiques au sein de la famille n'ont jamais été étudiées.

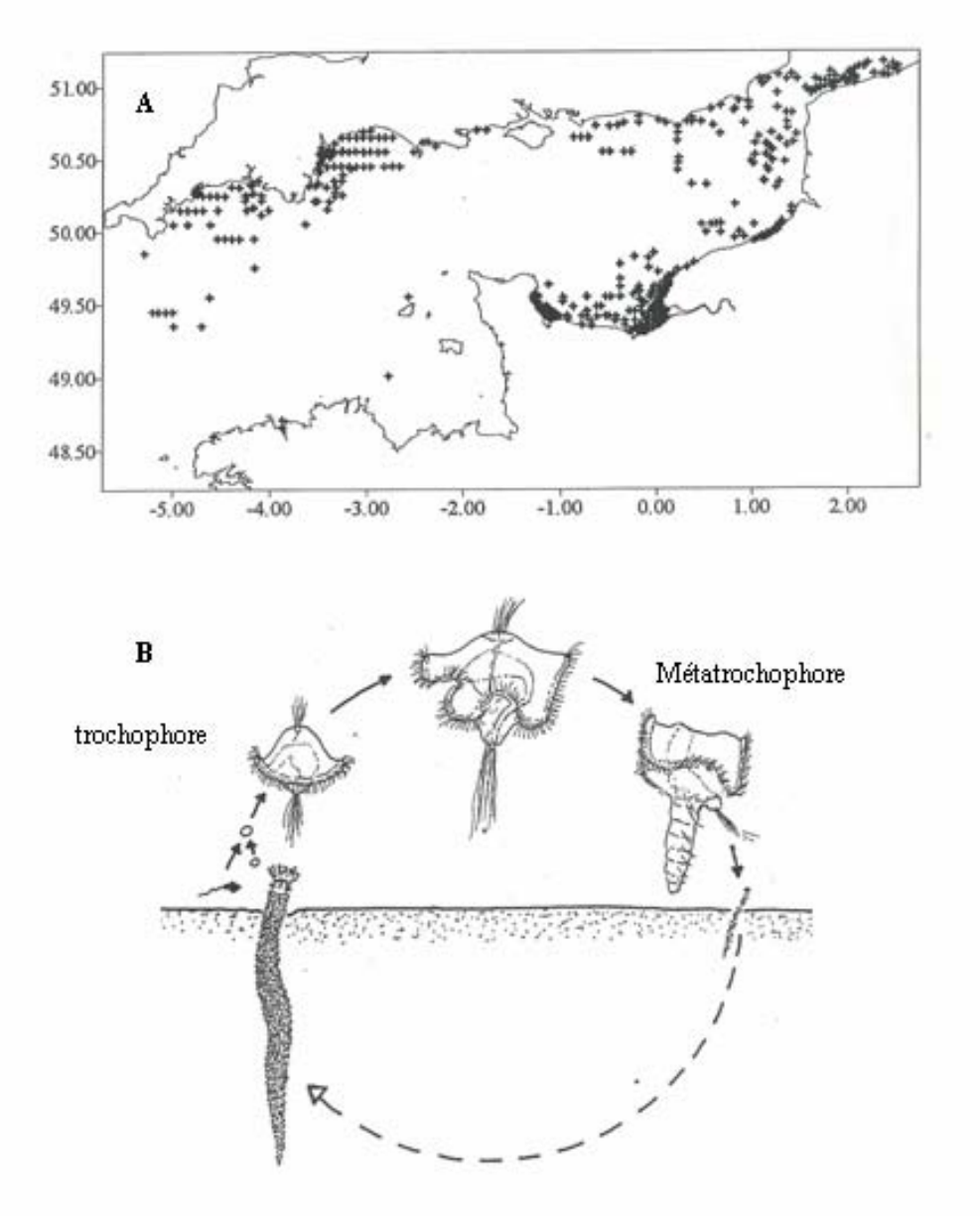


Figure 13. *Owenia fusiformis.* (A) Répartition des populations en Manche (tirée de Ellien, 2000) et (B) cycle de vie bentho-pélagique (dessin de F. Gentil).

L'espèce possède une durée de vie de 3 à 4 ans (Ménard *et al*., 1989), et est considérée comme ayant une distribution cosmopolite (Dauvin & Thiébaut, 1994), étant présente dans les océans Arctique, Atlantique, Pacifique et Indien. Blake (2000) a cependant rejeté l'hypothèse d'une espèce unique cosmopolite après avoir analysé un large éventail d'échantillons, sans pour autant décrire de nouvelles espèces. Koh & Bhaud (2001, 2003) ont conclu qu'*Owenia fusiformis* est un complexe d'espèces sur la base de nouveaux critères morphologiques dans la région antérieure (tentacules et thorax) et dans la région abdominale (crochets et soies) de l'animal. En Atlantique Nord Est, on retrouve la « dite » espèce avec *P. koreni* dans le peuplement à *Abra-alba*, bien qu'ayant une distribution plus étendue que cette dernière. Elle est très abondante dans les zones *Abra alba* sur les fonds sablo-vaseux de la Manche (Figure 13). L'espèce est caractérisée par une période de reproduction limitée au printemps de mai à juin et par une vie larvaire planctonique (la larve mitraria incluant les stades trocophore et métatrocophore) d'approximativement 28 jours (Wilson, 1932 ; Thiébaut *et al*., 1992). A partir de l'âge à la maturité sexuelle (2 ans), elle peut se reproduire annuellement durant toute sa vie (espèce itéropare), la femelle émettant en moyenne 70 000 ovocytes/an (Gentil *et al.,* 1990). En Baie de Seine, on observe pourtant une période de ponte principale centrée sur la période mai- juin.

V. OBJECTIFS DE LA THESE

Au regard de l'importance des échelles d'observation dans l'étude des populations marines naturelles, et, des grands bouleversements climatiques et tectoniques qui ont marqué l'évolution des espèces marines côtières dans les régions tempérées et boréales de l'Atlantique Nord, nous avons entrepris une analyse de la structure génétique des polychètes *Owenia fusiformis* et *Pectinaria koreni* à différentes échelles spatio-temporelles pour mesurer l'impact des phénomènes historiques dans l'Atlantique Nord Est, et les phénomènes

contemporains qui determinent la répartition actuelle de ces espèces en Manche et en Baie de Seine. Les objectifs de ce travail se divisent en trois grandes parties :

(I) La ségrégation des faunes de part et d'autre de la zone de transition Iroise-Manche et le niveau de pénétration des espèces de la province Lusitanienne dans la province Boréale posent de nombreuses questions quant aux processus qui initient et maintiennent les patrons faunistiques observés (effets climatiques, hydrodynamiques ou historiques). Ils posent également de nombreuses questions quant à l'intégrité génétique des espèces qui traversent cette zone frontalière. Ainsi, en comparaison de la côte Est des Etats Unis, on peut se demander si la zone de transition biogéographique aux approches occidentales de la Manche est aussi caractérisée par la présence de lignées évolutives distinctes au sein des espèces *Pectinaria koreni* et *Owenia fusiformis* ? Si oui, ces lignées ont-elles eu des histoires évolutives différentes ou congruentes corrélées à l'histoire de l'habitat des sédiments fins envasés qui ségrégent dans la zone de transition ? Pour étudier les principes et processus de colonisation à macro-échelle évolutive chez les genres *Owenia* et *Pectinaria*, eu égard à l'histoire géologique de l'Atlantique Nord Est, nous avons privilégié une approche phylogéographique par l'utilisation du polymorphisme enzymatique et nucléotidique de la sous-unité I du gene mitochondrial Cytochrome Oxidase (mtCOI).

(II) A ces patrons historiques de colonisation de la Manche se superposent les échanges actuels de gènes entre populations. Cette juxtaposition pose de nombreuses questions : quelle est la part des phénomènes contemporains sur la structure actuelle des populations de *P. koreni* autour des îles Britanniques (notamment entre la Mer d'Irlande et la Manche) ? Est-il possible de dissocier les flux géniques historique et contemporain à l'échelle de l'Atlantique Nord et de la Manche ? Si oui, à l'échelle de la Manche, existe-t-il des populations exportatrices de larves vers des population-puits ? De plus, les flux larvaires potentiels

obtenus par simulation hydrodynamique de la dispersion larvaire sont-ils en accord avec les échanges efficaces estimés par les méthodes de génétique des populations ? Pour analyser ces échanges, nous avons utilisé quatre locus microsatellites hypervariables développés par Weinmayr *et al*. (1999), et confronté les flux géniques contemporains obtenus entre les populations de *Pectinaria koreni* aux échanges larvaires estimés à partir de simulations de la dispersion en Manche.

(III) Mesurer les flux de gènes à grande échelle passe également par une meilleure connaissance du fonctionnement des populations et/ou de la métapopulation à micro-échelle, et nécessite l'estimation des paramètres démographiques de la population à partir des variations spatio-temporelles de la structure génétique à l'échelle locale d'une population subdivisée en dèmes. Les agrégats d'adultes sont-ils génétiquement différenciés à l'échelle d'une métapopulation ? Quel est le modèle de métapopulation qui convient le plus pour expliquer la structure génétique de *P. koreni* ? Existe t-il une possibilité pour qu'un recrutement discontinu des cohortes génère des entités génétiquement différenciés au sein d'une même baie ? Si oui, la structure génétique observée chez les juvéniles est-elle modifiée après la phase de recrutement ? Pour répondre à ces questions, nous avons donc entrepris l'étude des processus micro-évolutifs s'exerçant sur le fonctionnement spatio-temporel d'une métapopulation locale au niveau de la Baie de Seine, où les caractéristiques biologiques et la dynamique des populations de *Pectinaria koreni* sont bien connues. Cette structure a été appréhendée à partir de 4 échantillonnages temporels représentant 4 générations distincts au niveau des populations de géniteurs (les adultes), afin de mieux apprécier les effets génétiques inter/ intra-génération du renouvellement (« turnover ») de la métapopulation locale.

CHAPITRE I

ETUDE PHYLOGEOGRAPHIQUE DE PLUSIEURS LIGNEES EVOLUTIVES CHEZ LES POLYCHETES TUBICOLES INFEODEES AUX SEDIMENTS SABLO-VASEUX, DANS L'ATLANTIQUE NORD EST.

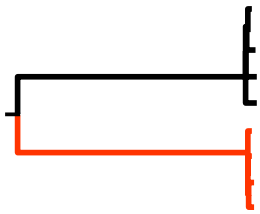
Introduction

Les grandes modifications géologiques et climatiques qui se sont déroulées en Europe du Nord au cours des dix derniers millions d'années ont joué un rôle d'activateur de la biodiversité en contribuant directement à l'isolement géographique de relicats faunistiques au niveau des zones « refuges » situées au sud, vers la zone Ibérique et après l'ère Messinienne (il y a 6 millions d'années) en Méditerranée, et au nord, dans des zones qui sont restés libres de glace durant la dernière grande glaciation du Weichsélien (e.g. au LGM), il y a environ 20 000 ans. Le repositionnement des isothermes durant les épisodes de réchauffement climatique qui suivirent ont donné lieu à une expansion géographique d'espèces à partir de ces zones refuges, qui se sont positionnées selon les frontières naturelles imposées par la biologie propre aux espèces.

La zone de transition biogéographique Iroise-Manche constitue une frontière progressive entre une faune associée aux régions tempérées chaudes (Lusitanienne) et une faune tempérée froide (Boréale), mais aucune étude n'a encore été entreprise pour savoir si les gradients faunistiques observés dans cette zone au niveau des peuplements s'accompagne également de ruptures phylogéographiques dans les populations naturelles d'espèces largement distribuées le long du littoral européen. Contrairement à la côte Est des Etats Unis, où les niveaux de divergence intraspécifique sont associés à une barrière géologique et hydrodynamique directement produite par la péninsule de Floride pendant le Pleistocène (il y a environ 2.5 millions d'années) et maintenus jusqu'à nos jours par l'hydrodynamisme local, l'ouverture de

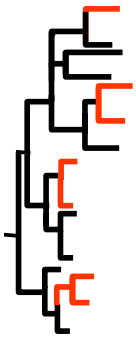
Encadré 2. Les signaux phylogéographiques

A)



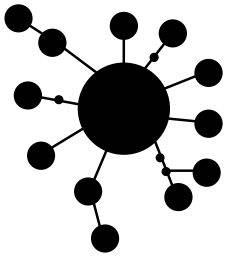
Monophylie réciproque
Histoire évolutive montrant l'évolution distincte entre 2 lignées/taxons (en rouge et en noire, divisés par un même évènement vicariant. Cette topologie peut rendre compte d'évènements de spéciation allopatrique, parapatrique et sympatrique.

B)



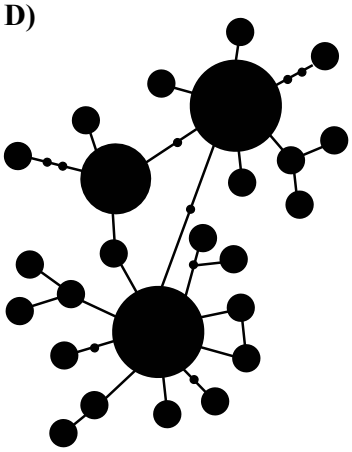
Paraphylie et polyphylie
Suite à un évènement vicariant, l'extinction locale d'une population située d'un seul côté de la barrière, est suivie par une recolonisation de l'aire géographique à partir d'une ou de plusieurs populations sources issues de l'autre côté de la barrière (en noire) qui ne se sont pas éteintes.

C)



Réseau d'haplotypes en forme étoilée
Si la recolonisation de l'aire géographique s'est faite à partir d'une seule population source et d'un petit nombre de migrants, celle ci peut être suivie d'une expansion démographique qui génère par mutation une diversification rapide des haplotypes mitochondriaux à partir d'un haplotype ancestral, qui se traduit aussi par des déviations par rapport à la neutralité.

D)



Réseau d'haplotypes en forme structurée
Si la recolonisation de l'aire géographique s'est faite à partir de plusieurs populations sources préalablement isolées par vicariance, celle ci peut être suivie de remises en contact secondaire. Les phases d'hybridation peuvent être suivies de phases d'expansion démographique et géographique avec une diversification des haplotypes mitochondriaux. Dépendant du niveau de divergence entre les populations qui se sont remises en contact, il y aura ou non des déviations par rapport à la neutralité. Si les haplotypes ancestraux (centraux) correspondent à une géographie particulière, on peut en déduire des zones refuges ou des patrons d'isolement par la distance avec effet fondateur.

la Manche sur l'Atlantique est beaucoup plus récente (à partir de -10 000 ans dans sa partie occidentale). Si des niveaux de divergence comparables à ceux de la côte Est des Etats Unis sont observés (entre 2 et 8 % de divergence), ce ne serait pas là le résultat d'une barrière historique produite par la pointe de Bretagne durant le Pleistocène, mais plutôt la conséquence d'effets historiques beaucoup plus anciens.

Afin de caractériser cette zone de transition biogéographique en terme de divergence intraspécifique qui pourrait être maintenue par des phénomènes contemporains (action hydrodynamique du front d'Ouessant et/ou mise en place d'un front d'hybridation), nous nous sommes intéressés aux populations naturelles de *Pectinaria koreni*. En effet, (1) son caractère univoltin peut conduire à d'importantes phases d'extinction locale et de recolonisations, (2) le succès dispersif de son stade larvaire de 15 jours dépend de l'action hydrodynamique locale, (3) la fragmentation de ses populations le long des côtes françaises et anglaises dans les baies et les estuaires supporte l'hypothèse de métapopulation, et (4) le fait que cette espèce soit beaucoup moins abondante dans la zone de transition par manque d'habitats appropriés, font que potentiellement des divergences génétiques peuvent exister entre les populations Atlantique, de la Manche et de la Mer d'Irlande. A l'inverse, *Owenia fusiformis* possède des générations chevauchantes et les populations sont distribuées de façon plus continue. On pourrait s'attendre à des niveaux de différenciation moindres, néanmoins, il y a de fortes chances que l'on puisse trouver des Unités Evolutives Significatives (UES) en Atlantique Nord Est, car les récents résultats d'études morphologiques (Koh & Bhaud, 2001, 2003) suggèrent fortement que l'espèce soit en fait, composée de plusieurs types morphologiques.

Nous avons donc utilisé le polymorphisme nucléotidique de la sous unité I du gène mitochondrial de la Cytochrome Oxidase (mtCOI), gène transmis maternellement et couramment utilisé dans les études de phylogéographie intraspécifique, car elle permet de retracer l'histoire évolutive d'une lignée (Encadré 2 : résumé des grands signaux phylogéographiques qui sont observés avec l'ADN mitochondrial). Les objectifs sont de (1)

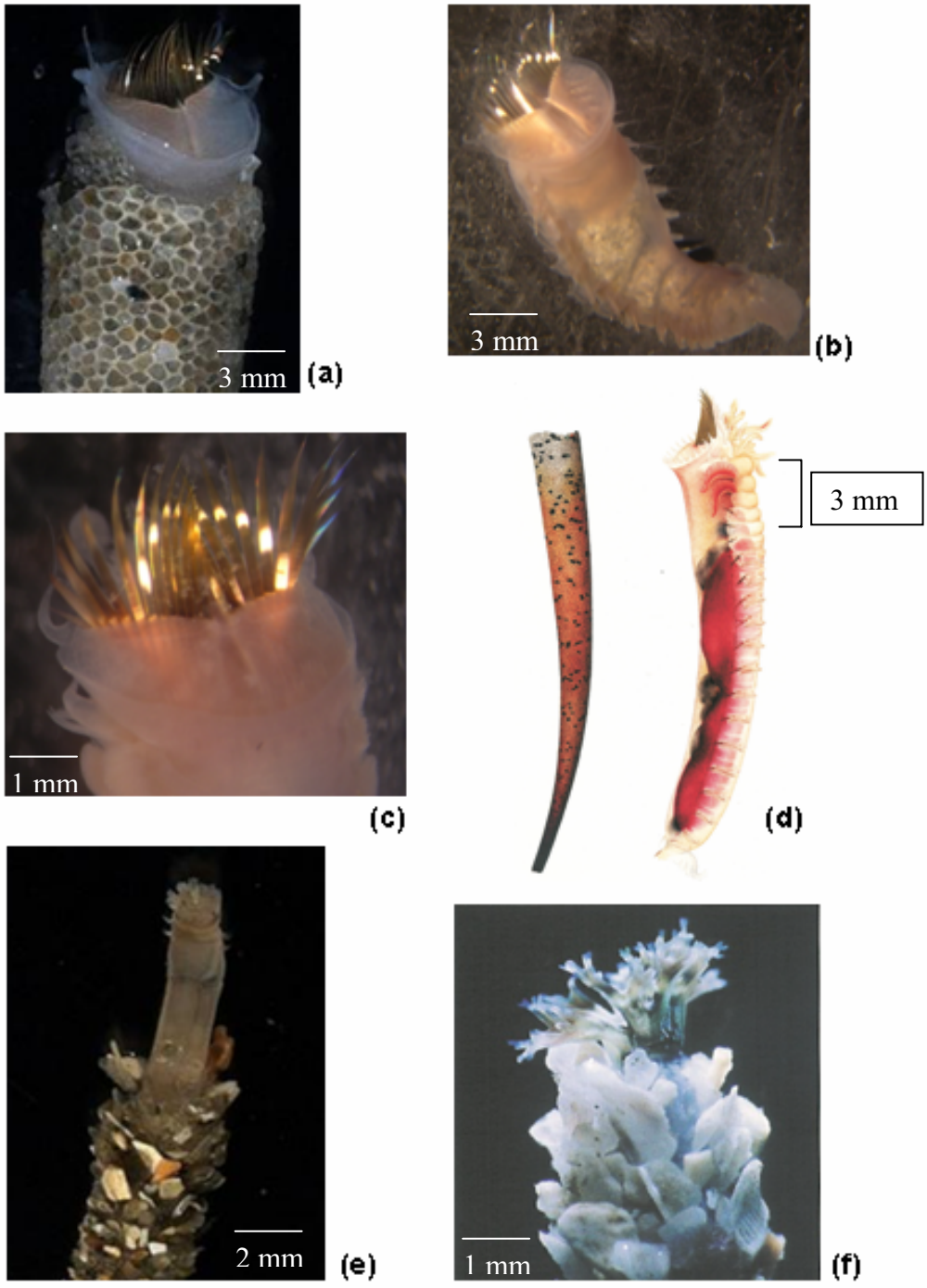


Planche I. (a) - (c) : *Pectinaria koreni* ; (d) *Pectinaria auricoma* ; (e) - (f) : *Owenia fusiformis*

comparer les patrons de colonisation et les histoires démographiques de *Pectinaria koreni*, *Pectinaria auricoma* et *Owenia fusiformis* dans l'Atlantique Nord Est, et (2) d'établir si la présence de lignées généalogiques hautement divergentes est due aux mêmes évènements de vicariance chez les espèces de polychètes inféodées aux substrats meubles. Les questions posées sont notamment : les histoires vicariantes et démographiques sont-elles congruentes ou différentes selon les espèces des sables fins-envasés, c'est-à-dire existe-t'il une aire géographique particulière où les histoires évolutives des espèces sont similaires et où les dynamiques ont pu co-évoluer ? Est-il possible d'identifier les zones ayant servi de refuge glaciaire pour les espèces inféodées aux sédiments fins envasés durant les grandes époques de glaciation (glaciations Saalienne et du Pleistocène) ? Quels sont les phénomènes historiques et les processus évolutifs à grande échelle qui ont influencé les patrons de colonisation de l'Atlantique Nord Est ? Enfin, si des lignées évolutives existent, ont-elles des aires de recouvrement au niveau de la zone de transition biogéographique Iroise-Manche ?

Ce chapitre se décompose en deux parties représentant 2 articles, avec un complément d'information et de discussion en fin de chapitre. Dans un premier temps, nous aborderons l'étude de la zone de transition biogéographique Iroise-Manche, ceci afin d'inférer la présence possible d'espèces cryptiques dans cette zone de recouvrement entre faunes Boréale et Lusitanienne. Dans un second temps, nous nous baserons sur la méthode dîte de phylogéographie comparée entre *Owenia fusiformis*, *Pectinaria koreni* et *Pectinaria auricoma* (Planche 1) pour étudier les principes et processus qui gouvernent la distribution des lignées évolutives dans l'Atlantique Nord Est et identifier les phénomènes historiques, autant au niveau génétique que démographique, qui ont conduit à la distribution et à la structure actuelle des populations.

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Sharp genetic break between Atlantic and English Channel populations of the polychaete *Pectinaria koreni*, along the North coast of France

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This study uses enzymatic and mitochondrial genes to infer the relative importance of historical processes and contemporary hydrodynamic features on the observed patterns of genetic structure in subdivided populations of *Pectinaria koreni* (Polychaeta: Pectinariidae) along the coasts of Brittany and the English Channel. Nucleotide sequence variation of a 603-bp fragment of the mtDNA cytochrome oxidase subunit I gene revealed a surprisingly deep phylogeographic break of about 16% divergence separating the Brittany and Channel populations, which coincides with a biogeographic boundary along the western coast of Brittany. Deep sequence divergence with fixed haplotype differences and the inversion of allele frequencies at two enzyme loci suggests the occurrence of potential cryptic or sibling species of *P. koreni*. The two clades showed opposite features. Channel populations exhibited bimodal match–mismatch curves due to two highly divergent haplotypes occurring at high frequencies and no overall heterozygote deficiencies at enzyme loci, suggesting respectively, a historic secondary contact between two differentiated populations followed by contemporary panmixia. On the contrary, Brittany populations displayed unimodal curves with low nucleotide diversity and highly significant heterozygote deficiencies, probably reminiscent of a recent population expansion and recolonisation of Brittany with contemporary admixture of divergent populations.
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Introduction

Marine biogeographic boundaries are transition zones of significant ecological complexity (ie because of overlaps in faunal assemblages; see Holt and Keitt, 2000 for biological complexities arising from species range limits) and important in terms of evolutionary processes (ie mating isolation, maintenance of hybrid zones, speciation). Such boundaries may give rise to biogeographic barriers, which are filters for many species depending on their ability to disperse, reproduce and survive various environmental conditions and the level of habitat fragmentation, potentially leading to differences in the genetic structure of populations on either side. Studies of allozymes and/or mitochondrial DNA divergence in invertebrate species living across such barriers have revealed major phylogeographic breaks concordant with biogeographic boundaries, plate movements and contemporary oceanographic conditions, which may act to maintain or not such breaks (*Crassostrea virginica*, Reeb and Avise, 1990; *Tigriopus californicus*, Burton, 1998; *Linkia laevigata*, Williams and Benzie, 1998; *Streblospio* spp, Schulze *et al*, 2000; *Perna canaliculus*, Apte and Gardner, 2002).

The Iroise Sea and the north western part of the Brittany coast (France), is a biogeographic transition zone between temperate and cold-temperate/boreal marine faunal assemblages (Cox and Moore, 2000; Dinter, 2001). Unlike the Cap Canaveral (East coast of Florida) and Point Conception (California) boundaries, the North west coast of Brittany has been less studied in terms of phylogeographic structure, despite some interesting dynamic features revealed by the modelling of long-term currents in the English Channel (Salomon and Breton, 1993). An inflow of warmer/higher salinity water derived from the North-Atlantic drift enters the western English Channel along the north coast of Brittany, thereby facilitating larval export from the south to the north coast. However, in the case of the shallow-water gastropod *Hydrobia* sp, the ecological conditions specific to each biogeographical region seem to have prevented dispersal across most of the north coast of Brittany, thus leading to strong spatial isolation between *Hydrobia glyca* and *H. acuta neglecta* (Wilke and Pfenninger, 2002). This water then circles northwards to return to the Celtic Sea along the south coast of Cornwall. Some of this water also passes north-eastwards on the English side of the Channel (see Beaumont, 1982 and references within). A frontal system known as the Ushant front develops between north Brittany and the tip of Cornwall in

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summer at the confluence of mixed and stratified waters, west of 5°W (Pingree *et al*, 1975). Structures such as eddies (ie circular currents) may promote the isolation of populations and are constrained locally along both coasts of the Channel (Salomon and Breton, 1993). Finally, a strongly oriented west–east current separates (1) the eastern from the western province of the English Channel, at the Wight-Cotentin section and (2) the English and French coasts in the eastern province, between Cherbourg and the straights of Dover.

Genetic studies of Atlantic and English Channel populations of invertebrates have mostly concerned commercially exploited or invasive species (for example, *Mytilus* sp, Bierne *et al*, 2003; *Aequipecten* (*Chlamys*) *opercularis*, Beaumont *et al*, 1982; *O. edulis*, Saavedra *et al*, 1995; *Pecten maximus*, Wilding *et al*, 1997; *Crepidula fornicata*, Dupont *et al*, 2003). However, the impact of human activities interferes with the study of natural evolutionary processes by reinforcing secondary contacts between previously isolated populations. To infer historical processes that have led to present patterns of genetic structure in Atlantic and English Channel populations of marine invertebrates, it is necessary to study native species with a large distribution and presenting a number of populations throughout their range. The tubeworm *Pectinaria koreni* (Malmgren) is a polychaete species with nonoverlapping generations that presents all these characteristics. It belongs to the Atlantic boreal fauna and is widely distributed along the European coastlines from Scandinavia to the Mediterranean Sea (Holthe, 1978). In addition, the species possesses a bentho-pelagic lifecycle (ie a benthic reproducing adult stage and a 15-day dispersive larval stage) representative of most of the commercially exploited invertebrates, but is not itself exploited. *P. koreni* is a gonochoric species (sex ratio 1:1) and has a main breeding period occurring between March and July, with the release of 20 000–430 000 oocytes per female (Irlinger *et al*, 1991). As populations are confined to muddy-fine sediments (necessary to construct their tube) in shallow waters of bays and estuaries, the species thereby exhibits a highly fragmented distribution along its range.

This study uses polymorphic enzymatic and mitochondrial genes in 12 populations of *P. koreni* sampled along both coasts of the English Channel and southern Brittany, to infer the relative importance of historical processes and contemporary hydrodynamic features on the observed patterns of genetic structure. Owing to the very high levels of sequence polymorphism in *P. koreni* (Weinmayr, 1999), microsatellites were found mainly to reflect contemporary gene flow and thus were not appropriate to infer possible phylogeographic breaks between populations. On the contrary, allozyme markers (six loci screened across 306 individuals sampled within the seven most representative populations) and a partial fragment of the mitochondrial cytochrome oxidase submit I (COI) gene (206 individuals sampled in 12 populations) were found polymorphic and divergent enough to test the hypothesis of a genetic break across the Iroise transition zone between populations of *P. koreni*.

Material and methods

Sampling sites

Samples of *P. koreni* were obtained by sampling the top 10 cm of the sediment with a 0.25 m^{-2} Hamon grab or, in some cases where individuals were scarce, by dredging. Populations from bays and estuaries along both coasts of the English Channel were sampled during the 2000 PECTGENE cruise on board the 'NO Côte de la Manche'. Additional populations of the Baie du Mont S^T Michel and Morgat were sampled by foot during the lowest tides in April 2001. Samples from Morlaix Bay were gathered over a 2-year period as only one to two individuals were collected each time. It therefore seems that this site is not representative of a truly established population. Finally, the Lorient and Concarneau populations on the south coast of Brittany were sampled in April 2002 on board the 'NO Côte d'Aquitaine'. After sampling, the individuals were kept in liquid nitrogen until DNA and protein extractions. Table 1 shows the location of the populations sampled along the coast of Brittany and the English Channel.

Allozyme electrophoresis

Horizontal enzyme electrophoresis was conducted on 12% starch gels and electrophoretic procedures were performed according to the methods of Pasteur *et al* (1987). Depending on the size of the animals, frozen samples of a whole or of a piece of body (cephalic disk and gills) were each macerated with 60–100 µl of grinding buffer (0.05 M Tris/HCl, pH 8.0), prior to centrifugation at 15 000 rpm for 5 min. The supernatant was then absorbed onto 4 mm × 12 mm filter-paper wicks

Table 1 Names (abbreviations) and locations of the *P. koreni* populations sampled along the coasts of Brittany and the English Channel

Population names	*Latitude*	*Longitude*	$N_{ALLOZYMES}$	N_{COI}
Great Western Bay (GWB)	50°18.50′N	3°25.00′W	—	14
Beachy Head (BH)	50°46.00′N	0°01.60′E	—	12
Rye Bay (RB)	50°53.73′N	0°53.75′E	50	25
Gravelines (GR)	51°01.40′N	2°04.70′E	50	21
Pays de Caux (PC)	50°24.20′N	1°31.60′E	—	13
Baie de Seine (BS)	49°27.20′N	0°01.50′E	47	25
Baie des Veys (BV)	49°32.00′N	1°15.50′W	—	22
Mont S^T Michel (MSM)	48°03.82′N	1°33.00′W	27	15
Morlaix (MRL)	48°42.83′N	3°52.62′W	—	7
Morgat (MO)	48°15.00′N	4°30.00′W	50	26
Concarneau (CC)	49°51.50′N	3°57.50′W	32	8
Lorient (LO)	47°43.28′N	3°21.94′W	50	18

N is the number of individuals analysed using allozymes and a 603-bp mtDNA COI fragment.

(Whatman No. 1) and electrophoresed using the following buffers: (1) Tris-borate-EDTA (TBE), pH 8 for mannose phosphate isomerase (*Mpi*, E.C. 5.3.1.8), phosphoglucomutase (*Pgm*, E.C. 5.4.2.2) and phosphoglucose isomerase (*Pgi*, E.C. 5.3.1.9); (2) Tris-citric-boric-lithium hydroxide (LiOH), electrodes pH 8.29, gel pH 8.26, for L-amino peptidase (*Lap*, E.C. 3.4.11.1), malic enzyme (*Me*, E.C. 1.1.1.40); (3) Tris-citrate (TC), electrodes pH 6.3, gel pH 6.7, for malate dehydrogenase (*Mdh*, E.C. 1.1.1.37). Buffer systems (1) and (2) were run at 150 V, 80 mA for 6 h and buffer system (3) at 100 V, 60 mA for 6 h; all at 4°C. Amperage was maintained constant and all six enzyme systems (six loci) were visualised using enzyme-specific stains according to Pasteur *et al* (1987).

Mitochondrial COI sequencing of PCR products

Total genomic DNA was extracted according to the extraction method given in Jolly *et al* (2003). Partial sequences (710 bp) of the mitochondrial COI gene were amplified with the universally applicable primers described by Folmer *et al* (1994), following the author's PCR conditions, but with an annealing temperature of 50°C. However, PCR reactions were performed into a 26 µl reaction volume consisting of 1 × PCR buffer (supplied with polymerase enzyme), 2 mM $MgCl_2$, 0.12 mM dNTP, 0.2 µM of forward and reverse primers, 0.5 U of Thermoprime Plus *Taq* polymerase (ABgene), 25–50 ng CTAB-extracted genomic DNA. The PCR products were then purified and sequenced on ABI 3100 using BigDye© (Perkin-Elmer) terminator chemistry, following the manufacturer's protocol. Forward and reverse sequences were proofread in Chromas 2.23 and aligned manually in BioEdit Sequence Alignment Editor (Hall, 1999).

Statistical analyses

Allozymes: For each population, allele frequencies, mean number of alleles per population (N_{ALL}), observed (H_0) and expected heterozygosity (H_{NB}; Nei, 1987) were estimated using GENEPOP 3.3. software (Raymond and Rousset, 1995) and Fstat 2.9 (Goudet, 1995) was used to estimate allelic richness (R_S). The null hypothesis of independence between loci was tested from genotypic disequilibrium analysis and isolation-by-distance was tested with a Mantel test. Deviations from Hardy–Weinberg equilibrium were examined for each population, at each locus, by calculating Wright's fixation index F_{IS} as estimated by Weir and Cockerham (1984) f, which was then tested using exact tests. Overall levels of genetic differentiation was analysed by calculating the estimator $\hat{\theta}$ of Wright's F_{ST} statistic for each locus, and tested using exact tests for the null hypothesis of identity of allelic distribution across populations. Multilocus $\hat{\theta}$ values were also calculated between pairwise combinations of populations. All the above analyses were performed using GENEPOP 3.3 (Raymond and Rousset, 1995). Group comparisons were used to test for regional structure between populations of the Brittany coast and the English Channel using Fstat 2.9 (Goudet, 1995) and a analysis of molecular variance (AMOVA) analysis between groups was performed using ARLEQUIN *vs* 2 (Schneider *et al*, 2000). The relationships between Atlantic and English Channel populations were illustrated by a tree based on UPGMA clustering of Reynolds *et al* (1983) distance using the POPULATION 1.2.28 software (Langella, 2002).

Mitochondrial DNA: For each population, haplotype diversity ($h_{e\text{-}HAP}$) and nucleotide diversity (π) were examined with DnaSP *vs* 3 (Rozas & Rozas, 1999) and match–mismatch curves for all populations were created using the same program. In addition, Tajima's D statistic (Tajima, 1989) was investigated as an index of departure from population equilibrium. To analyse the relationships between Atlantic and English Channel populations, a neighbour-joining tree was constructed (using all informative sites) based on Kimura-2 parameter (Kimura, 1980), with MEGA *vs* 2.1 (Kumar *et al*, 2001), with 1000 resampling of the data set (bootstraps) and with *P. auricoma* as outgroup. The same software was used to test for the relative rate constancy of a molecular clock. Estimates of the time of divergence (T) between Atlantic and English Channel population clusters was carried out by computing the average distance (D_{AVG}) and standard error between clusters according to the Kimura-2 parameter model (Kimura, 1980) with MEGA *vs* 2.1 (Kumar *et al*, 2001) and estimating T according to specific mutation rate of the mtCOI (see Chevaldonné *et al*, 2002). An AMOVA analysis of variance between groups and populations was performed using ARLEQUIN *vs* 2 (Schneider *et al*, 2000). Minimum-spanning networks were created using TCS *vs* 1.13 (Clement *et al*, 2000) to infer the most parsimonious branch connections between the sampled haplotypes among (1) the English Channel populations and (2) the Brittany populations.

Results

Allozymes

Six enzyme loci were found polymorphic and average genetic diversity (H_{NB}) for polymorphic loci was 44. 8%. No genotypic disequilibrium was detected, indicating that loci give independent information. Despite no isolation-by-distance at the scale of our study (Mantel test P-value = 0.162), the distribution of allelic frequencies across populations (Table 2) suggests the existence of a pronounced cline at the *Pgi* locus for both *Pgi*-97 and *Pgi*-100, with an inversion of allele frequencies in Lorient. Clinal variation was also observed at the *Mdh* locus (*Mdh* 93 and *Mdh* 100) together with a slight inversion of *Mpi*-100 for *Mpi*-95 in all three populations of Brittany. Rare alleles were found not only in the highly polymorphic *Mpi* enzyme (*Mpi* 77 and 106) but also at the *Lap* locus (*Lap* 104) and at the *Mdh* locus (*Mdh* 114). The latter locus was found monomorphic only in the Mont Saint Michel population. This can be explained by a lower sample size at this site ($N = 27$) as this population was also characterised by a lower allelic richness ($R_S = 2.675$) (Table 3). There were also significant differences ($P < 0.04$) in both allelic richness (R_S) and gene diversity (H_S) between the 'Brittany' ($R_S = 3.174$; $H_S = 0.503$) and the 'English Channel' groups ($R_S = 2.907$; $H_S = 0.398$). Except for Gravelines, no significant deviations from Hardy–Weinberg expectations were recorded within the English Channel populations or in the Mont Saint Michel (Table 4). On the contrary, all three Brittany populations showed highly significant F_{IS} values at least three loci

Table 2 *P koreni*. Allele frequencies ([a] denotes a rare allele) and sample size (*N*) at each site

N *Locus* *Allele*	*Rye Bay* *50*	*Gravelines* *50*	*Baie de Seine* *47*	*Mont S[T] Michel* *27*	*Morgat* *50*	*Concarneau* *32*	*Lorient* *50*	*All* —
Lap								
94	0.080	0.150	0.096	0.200	0.228	0.167	0.076	0.142
100	0.890	0.840	0.851	0.760	0.739	0.817	0.902	0.828
104[a]	0.030	0.010	0.053	0.040	0.033	0.017	0.022	0.029
Mdh								
93	0.050	0.136	0.044	0.000	0.061	0.300	0.205	0.114
100	0.910	0.830	0.933	1.000	0.829	0.520	0.682	0.815
107	0.030	0.034	0.011	0.000	0.098	0.060	0.091	0.046
114[a]	0.010	0.000	0.011	0.000	0.012	0.120	0.023	0.025
Me								
90	0.160	0.122	0.083	0.039	0.133	0.205	0.222	0.138
100	0.740	0.743	0.798	0.846	0.733	0.659	0.681	0.743
105	0.100	0.135	0.119	0.115	0.133	0.136	0.097	0.119
Mpi								
77[a]	0.032	0.000	0.023	0.019	0.000	0.048	0.000	0.017
88	0.053	0.030	0.023	0.056	0.020	0.081	0.082	0.049
93	0.117	0.070	0.102	0.093	0.080	0.145	0.184	0.113
95	0.202	0.330	0.273	0.315	0.400	0.242	0.316	0.297
100	0.383	0.400	0.455	0.333	0.370	0.242	0.276	0.351
104	0.202	0.150	0.125	0.148	0.110	0.226	0.112	0.153
106[a]	0.011	0.020	0.000	0.037	0.020	0.016	0.031	0.019
Pgi								
97	0.071	0.011	0.172	0.125	0.190	0.323	0.670	0.223
100	0.714	0.913	0.641	0.833	0.730	0.613	0.320	0.681
106	0.214	0.076	0.188	0.042	0.080	0.065	0.010	0.193
Pgm								
91	0.044	0.063	0.090	0.022	0.023	0.241	0.095	0.083
94	0.211	0.125	0.256	0.413	0.216	0.407	0.250	0.268
100	0.700	0.688	0.577	0.565	0.636	0.315	0.631	0.587
106	0.044	0.125	0.077	0.000	0.125	0.037	0.024	0.062

(*Me*, *Mpi* and *Pgi*). Large heterozygote deficiencies were reflected in another two loci (*Mdh* and *Pgm*) in Concarneau, and in the *Pgm* loci in Lorient. This result is possibly associated either with the co-occurrence of highly differentiated groups of individuals (Walhund effect) or with a high level of inbreeding. The AMOVA analysis of variance (Table 5) chosen to test for regional structure between populations of the English Channel and Brittany revealed a significant level of genetic heterogeneity among the two groups ($F_{CT} = 0.029$; $P = 0.032$), but which only accounted for 0.18% of the total variance. As a comparison, variation among populations within groups was 0.95% ($F_{SC} = 0.043$; $P < 10^{-4}$). Pairwise values of genetic differentiation (Table 6) indicated the isolation of the three populations present along the coast of Brittany (Concarneau, Lorient and to a lesser extent Morgat), while within the English Channel, Gravelines was the only population that appeared genetically isolated from all others except with Rye Bay. These pairwise values of differentiation are illustrated in the UPGMA tree (Figure 1) which showed a major genetic split between the Atlantic (Concarneau, Lorient) and the English Channel populations, with Morgat closely related to the latter group but with low bootstrap value. Removal of the above population slightly improves bootstrap values.

mtCOI

The individuals were screened for variation from a 603-bp partial mitochondrial COI sequence, and a total of 81 haplotypes were detected that revealed 122 polymorphic sites. Of these variable sites, 80% were parsimoniously informative and 20% were singletons. Transition/transversion ratio was 2.76 and total haplotype ($h_{e\text{-}HAP}$) and nucleotide diversity (π) were 0.926 ± 0.011 and 0.0619 ± 0.008, respectively. Genetic variation for all populations is indicated in Table 3. The individuals from Morlaix (MRL) exhibited the lowest haplotype diversity and the highest nucleotide diversity, but which was due to the presence of only one individual exhibiting a highly divergent haplotype characteristic of the English Channel group. While there was no significant difference in haplotype diversity between the English Channel and Brittany populations ($0.85 \pm 0.04 \leqslant h_{e\text{-}HAP} \leqslant 0.88 \pm 0.02$), nucleotide diversity was two-fold lower within the latter group ($\pi = 0.0031$; *t*-test = 1.227***, $P \leqslant 0.001$) after exclusion of the Morlaix individual presenting a 'Channel' haplotype. The AMOVA analysis of molecular variance (Table 5) reveals a significant level of genetic heterogeneity between the two groups ($F_{CT} = 0.123$; $P = 0.001$). Among population variation in both groups only accounted for 0.04% (English Channel) and 1.03% (Brittany) of the total variance (in terms of F_{ST}), thus

Table 3 *P. koreni*. Allozyme and mtCOI genetic variation at each site

	English Channel							*Brittany*				
	GWB	*BH*	*RB*	*GR*	*PC*	*BS*	*BV*	*MSM*	*MRL*	*MO*	*CC*	*LO*
Allozymes												
N_{ALL}	—	—	4	3.7	—	3.8	—	3.3	—	3.8	4	3.8
H_0	—	—	0.339	0.368	—	0.363	—	0.314	—	0.347	0.297	0.316
H_{NB}	—	—	0.414	0.398	—	0.419	—	0.375	—	0.466	0.583	0.487
			(±0.214)	(±0.198)		(±0.217)		(±0.258)		(±0.133)	(±0.174)	
R_S	—	—	3.025	2.934	—	2.994	—	2.675	—	3.028	3.427	3.066
MtCOI												
N_{HAP}	9	7	16	10	6	12	10	11	4	13	6	11
H_{eHAP}	0.835	0.894	0.917	0.871	0.833	0.893	0.857	0.905	0.714	0.849	0.893	0.889
	(±0.101)	(±0.063)	(±0.045)	(±0.048)	(±0.071)	(±0.038)	(±0.054)	(±0.072)	(±0.181)	(±0.053)	(±0.111)	(±0.064)
Π	0.0053	0.0068	0.0068	0.0069	0.0061	0.0074	0.0059	0.0072	0.0430	0.0030	0.0025	0.0035
$P_{HAP}(\%)$	5.4	3.4	7.5	3.4	2	6	4.5	5.4	3.4	17	5.1	13.6

Allozymes: observed (H_0) and expected heterozygosity (H_{NB}; Nei, 1987) (SD), allelic richness (R_S) based on a sample of eight diploid individuals; mtCOI: number of haplotypes (N_{HAP}), unbiased haplotype diversity (H_{eHAP}) and nucleotide diversity (π). Private haplotypes (P_{HAP}) in each population are represented as a percentage of the total number of individuals in each clade.

possibly reflecting genetic homogeneity within each group. This is further reflected in the neighbour-joining tree, which reveals two distinct clades (Figure 2) separating the populations of the English Channel (clade 1) from those present along the coasts of Brittany (clade 2) (also see map of the distribution of haplotype frequencies, Figure 3). Relative rate constancy of a molecular clock was not rejected at 5% significance level, thereby allowing estimates of the time of divergence between the two clades.

Limited divergence was found within each clade ($D_{AVG} = 0.7$ and 0.3% respectively). Only Gravelines and Great Western Bay were significantly isolated from each other ($F_{ST} = 0.07$; $P = 0.018$). The only clade 1 haplotype present in the Morlaix population was excluded from further analysis as it was one of the most common haplotype present in the Channel (HAP4; see Figure 3) and divergence between the two clades was high ($D_{AVG} =$ 16.4%). As a comparison, the average distance between *P. koreni* and the congeneric species *P. auricoma* is about 27% based on Kimura-2 parameter. As divergence was very deep, network picturing of haplotype relationships were calculated and drawn separately for each clades. The haplotype networks (Figure 4) were based on the 55 and 26 haplotypes present in clades 1 and 2, respectively (ie none was shared between the clades). Within clade 1, two groups of haplotypes were revealed in the network and which were shared across the sampled populations of the Channel. This was further confirmed with mismatch distribution of pairwise nucleotide differences where a bimodal topology was observed for all populations (Figure 5). Tajima's D values were not significant and ranged from -1.49 to $+1.12$. On the contrary, the curves generated for clade 2 populations of the Brittany coast were unimodal and coincided with a clustering of haplotypes into one group (Figure 4). Conversely, Tajima's D was negative ($-2.01 < D < -1.64$) and deviated significantly from neutral expectations (P-value < 0.01–0.02) in three Brittany populations (Morgat, Lorient and Concarneau).

Discussion

Our results indicate a major phylogeographic break with 16% divergence between the English Channel populations of *P. koreni* and those present along the coast of Brittany, and which appears to be associated with a marine biogeographic boundary along the north-western Brittany coast. The break in the spatial distribution of both mitochondrial haplotypes and enzymatic allele frequencies was not attributable to the presence of *P. auricoma* in the Brittany populations as individuals of this species were singled -out on the basis of (1) tube morphology and (2) diagnostic alleles at two enzyme loci (*Pgi* and *Mdh*). In addition, when *P. auricoma* was used as an outgroup, the level of divergence ($D_{AVG} = 27\%$) was far beyond that observed between the two *P. koreni* clades. Although Brittany and English Channel populations of *P. koreni* were thought to belong to one species, the observed deep sequence divergence with fixed haplotype differences and the inversion of allele frequencies at two enzyme loci suggests potential cryptic or sibling species. Other arguments in favour of crypticism include the fact that microsatellite markers developed from Baie de Seine individuals (Weinmayr,

Table 4 *P. koreni*. Correlation of homologous alleles between individuals within populations (F_{IS}) for all variable loci

	Rye Bay	*Gravelines*	*Baie de Seine*	*Mont S^T Michel*	*Morgat*	*Concarneau*	*Lorient*
Lap	−0.087	−0.168	0.204	0.180	0.034	0.251	−0.077
Mdh	−0.059	0.000	−0.042	—	−0.130	0.501***	−0.256
Me	0.346	0.166	−0.030	−0.124	0.693***	0.914***	0.944***
Mpi	0.248	0.382***	0.092	0.086	0.249*	0.291**	0.347*
Pgi	0.222	−0.073	0.300	0.153	0.490**	0.635***	0.473**
Pgm	0.096	−0.207	0.227	0.421*	0.115	0.413*	0.426*
Multilocus average	0.159	0.139**	0.145	0.156	0.264***	0.337***	0.477***

Tests of significance were performed with Genepop 3.3 (Raymond and Rousset, 1995) (significance levels after sequential Bonferroni correction). *$P<0.05$; **$P<0.01$; ***$P<0.001$.

Table 5 F_{ST}− based hierarchical AMOVA between the 'English Channel' and 'Brittany coast' groups of *P. koreni* for both allozymes and mtCOI.

Source of variation	*Percent variation*	*Fixation indices*	P-*value*
Allozymes			
Among groups	0.18	$F_{CT}=0.029$	0.032
Among populations within groups	0.95	$F_{SC}=0.043$	0.000
Within populations	98.87	$F_{ST}=0.072$	0.000
MtCOI			
Among groups	12.30	$F_{CT}=0.123$	0.001
Among populations within groups	0.13	$F_{SC}=0.001$	0.404
Within populations	87.60	$F_{ST}=0.124$	0.000

'English Channel': RB, GR, BS, MSM; 'Brittany coast': MRL, MO, CC, LO.

Table 6 *P. koreni*. Pairwise F_{ST} values based on data for six enzymatic loci for all samples

Populations	*Rye Bay*	*Gravelines*	*Baie de Seine*	*Mont S^T Michel*	*Morgat*	*Concarneau*
Gravelines	0.007					
Baie de Seine	−0.003	0.019**				
Mont S^T Michel	0.020	0.015**	0.010			
Morgat	0.015*	0.009*	0.007	0.010		
Concarneau	0.077***	0.071***	0.062***	0.075***	0.053***	
Lorient	0.065***	0.157***	0.077***	0.124***	0.082***	0.050***

*$P<0.05$; **$P<0.01$; ***$P<0.001$. Tests of significance were performed with Genepop 3.3 (Raymond and Rousset, 1995) (significance levels after sequential Bonferroni correction).

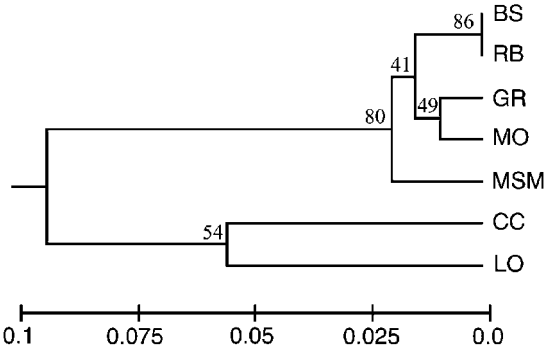

Figure 1 UPGMA tree showing the relationship between populations of *P. koreni* based on allozyme data using Reynolds *et al*'s (1983) distance. Bootstrap values were estimated from 1000 replications.

1999) fail to amplify those from the Brittany clade (MT Jolly, personal observation), and that the data support the concept of allopatric relationships within cryptic radiations (see Wilke and Pfenninger, 2002 and references within). Only partial sympatry occurs at Morlaix and which needs to be investigated further to test for reproductive isolation between clades. However, this one sympatric population might just be the result of random unidirectional dispersal events (from MSM towards MRL) rather than long-term stability within the population.

Although estimates of the evolutionary rate of the mtCOI gene vary between taxa (Chevaldonné *et al*, 2002, even with the highest mutation rate $r\leqslant 2.2\%$ Myr (Knowlton *et al*, 1993), estimates of the time of divergence ($T\geqslant 3.7$ Mya) predate the Pleistocene period and the formation of the English Channel. Assuming that the time of divergence between the two clades predates the Pleistocene era would indicate that divergence was not the direct result of a geographic barrier produced by Brittany after the formation of the English Channel during the last interglacial some 9000 ya (Smith, 1989; Lambeck, 1997). The two entities would have emerged at a time when sea levels were low and where the present distribution of the species was very different. Yet, if we accept the concept of allopatric relationships within cryptic radiations, one species would have taken a more northern distribution than the other. One plausible scenario, which is in accordance with the presence of two groups of haplotypes and the significantly higher

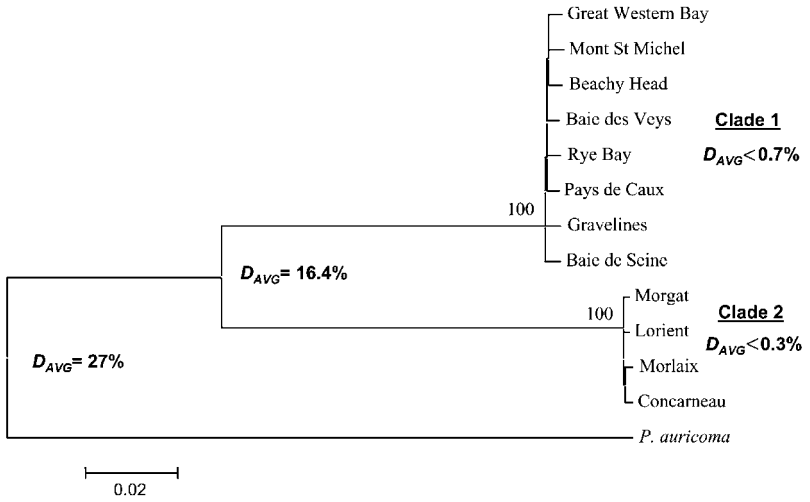

Figure 2 Neighbour-joining tree showing the relationship among COI haplotypes of *P. koreni* using Kimura-2 parameter distance method (Kimura, 1980). Bootstrap values were estimated from 1000 replications. D_{AVG} is the average distance between two clades using the same distance method.

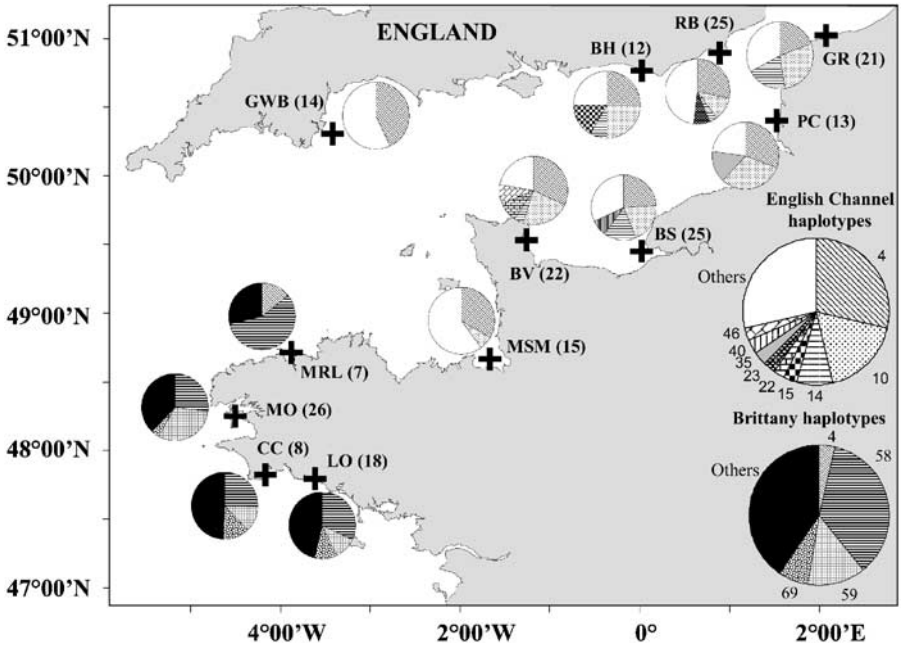

Figure 3 Map showing the distribution of COI haplotype frequencies across 12 sampled populations of *P. koreni* along the coasts of Brittany and the English Channel. Site abbreviations are as in Table 1. Numbers of samples per population (*N*) are within parentheses. 'Others' represent haplotypes with less than 6% frequency in each population.

nucleotide diversity in the Channel group (see discussion below), may be that the more northernly distributed species had a wider range and colonised the English Channel from more than one area. First, via the western approaches of the English Channel between the end of the 'Last Glacial Maximum' (LGM) (circa 19 kya) and the 'Younger Dryas' cold phase (12.9–11.5 kya) during which the English Channel was partly open to the sea in the west (Lambeck, 1997) and where continuous permafrost was located above 55°N (Renssen and Vandenberghe, 2003). Second, via the North Sea, after the catastrophic opening of the Dover Straights in the late Pleistocene (some 10 kya) when a great volume of water spilled out of the North Sea across the emerged eastern and central portions of the Channel (Smith, 1989). Subsequently, the two differentiated populations could have made secondary contact after the formation of the Channel and which would have also been facilitated by the rise in water levels following the retreat of the ice sheets and the setting up of holocene marine currents. As the palaeoshorelines were approaching their present locations in Brittany and the Cherbourg coast by 8000 ya (Lambeck, 1997), the range of the more southernly distributed species was restricted by the pattern of holocene marine currents still maintained today in the western province of the English Channel. This is in accordance with hydrodynamic modelling of the potential larval dispersal of *P. koreni* (Ellien, 2001) using the model of Salomon and

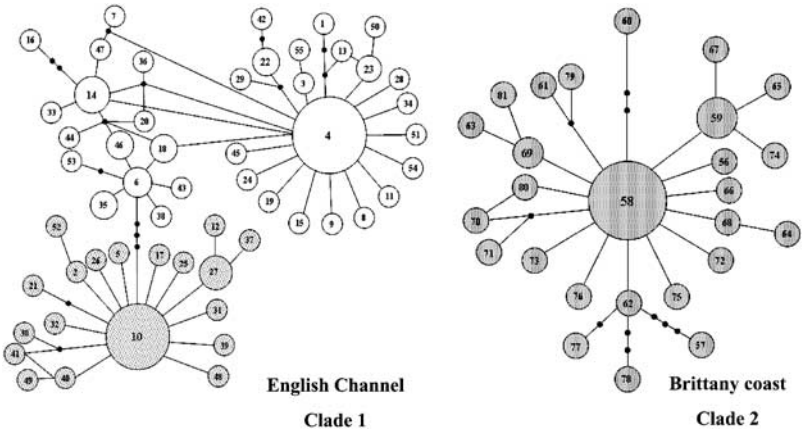

Figure 4 Haplotypic networks showing the evolutionary relationships between mitochondrial haplotypes of the English Channel and Brittany group of populations (clades 1 and 2, respectively). Circles represent haplotypes, with the area of each circle representative of the frequency with which it occurred in the total sample. Black dots represent hypothetical haplotypes not detected in the survey.

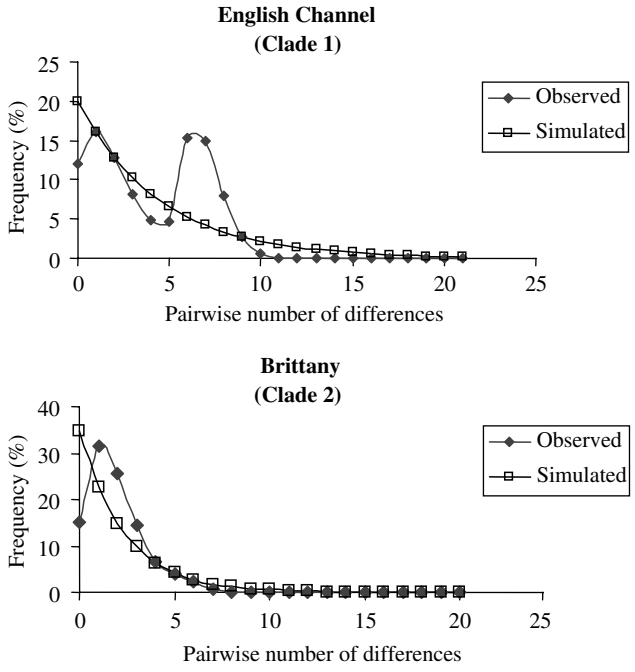

Figure 5 Observed and simulated match–mismatch curves under constant population size, generated for both the English Channel and Brittany populations of *P. koreni*.

Breton (1993), in which wind-driven currents may only favour larval export from the south to the north coast of Brittany.

In addition to the deep genetic break, there are some finer-scale patterns of differentiation within both Channel and Brittany populations, which may correspond to both Pleistocene habitat fragmentation or to the effects of contemporary gene flow and natural selection.

Within the English Channel group, the haplotype network reveals at least two groups of haplotype shared across all populations (except in GWB but probably due to sampling drift), no significant F_{IS} (except for GR but only at the *Mpi* locus), no significant pairwise F_{ST} values for sequence data (except between GR and GWB) and bimodal mismatch curves with nonsignificant values of Tajima's *D*. This suggests that populations of the English Channel are in a relatively stable state of panmixia. The fact that all populations of the Channel share the two most common clade 1 haplotypes is likely to have resulted from a secondary contact between differentiated populations (historic admixture) after each most common haplotype evolved separately in two distinct areas. Such areas could have served as temporary refugia during the LGM (23–19 kya) where local diversification would have allowed for the separate evolution of the two most common haplotypes and from which animals have subsequently dispersed and intermingled. Potentially, small northern ice-free refugia could have existed in the northern North Sea area and in small localities along the coast of Scotland and Norway (Luttikhuizen *et al*, 2003). Additional sampling from these areas should confirm this. Within the Channel, some contemporary restriction on gene flow is revealed. For instance, the occurrence of unique haplotypes in the populations of Beachy Head and of the Pays de Caux most likely suggests that dispersal on either side of the populations is hampered probably because local hydrodynamism favours retention of larvae. Our results also support the hypothesis of an east–west separation of populations on either side of the Pointe du Cotentin, but only when the Morgat population is excluded.

We observed a close genetic similarity between Morgat and the English Channel populations for enzymatic data, whereas all COI sequences in this population were members of the Brittany clade. Clinal variations from north to south appear at three enzymes, which may either reflect the effects of natural selection, migratory events between populations belonging to the two clades (but without reproduction) or hybridisation between clades.

The populations of Brittany display completely opposite phylogeographic and demographic patterns than those within the English Channel. The fact that all populations show a substantial degree of genetic differentiation at allozyme loci but none at mtCOI sequences may correspond to the effects of recent habitat fragmentation around south Brittany. In addition, although nucleotide diversity was significantly lower

than in the Channel, mean haplotype diversity remained high and similar, suggesting no recent bottlenecks in these populations. Rather, high haplotype diversity, low nucleotide diversity, unimodal mismatch distributions and significantly negative values of Tajima's *D* (Tajima, 1989) could reflect population expansion along the Brittany coast during or following the Pleistocene. Diversification, as reflected by the relatively high percentage of private haplotypes within each population, would have followed due to habitat fragmentation. Interestingly, the highly significant F_{IS} values observed at most allozyme loci in all Brittany populations seem to reflect recent contacts between genetically differentiated populations (ie Walhund effect).

Coastal currents may export larvae along the Brittany coasts. However, depending on the seasonal and inter-annual variability of current fields (see Lazure and Jegou, 1998 for hydrodynamic modelling), the average pattern may be modified yearly. During the reproductive period of *P. koreni* (April–June), there is an inversion of the circulation patterns that may favour connectivity between coastal populations. Alternatively, differential mortality of allochtonous larvae or reproductive asynchrony between bays could also be contributing to this genetic sorting.

Reproductive compatibility trials between the two *P. koreni* clades and detailed investigation at the morphological level (to confirm potential morphological crypticism) need to be performed experimentally to test the extent to which molecular phylogeny is reflecting species phylogeny. However, the fact that within the Brittany group highly significant F_{IS} values are associated with the presence of only one group of haplotype could also indicate a change in the reproductive characteristics of *P. koreni*. This may be worth investigating in future studies, especially if reproductive isolation between the clades is revealed. Further sampling is needed in populations present along the Atlantic coast, Irish Sea and North Sea to investigate more closely the phylogeographic history of the two potential cryptic species of *P. koreni* and to delimit accurately putative refugia. In addition, the spatial patterns of the two taxa need to be investigated if the animals occur in areas of stable sympatry to test for any locally sharp separation of the two entities. This will enable us to address phylogeographic issues ranging from the causes of population genetic patterns (ancient or recent processes) to the objective identification of a cryptic/sibling species complex in *P. koreni*.

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Comparative phylogeography of two coastal polychaete tubeworms in the North East Atlantic supports shared history and vicariant events.

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Abstract

The historic processes which have led to the present-day patterns of genetic structure in the marine coastal fauna of the North East Atlantic are still poorly understood. While tectonic uplifts and changes in sea level may have caused large scale vicariance, warmer conditions during glacial maxima may have allowed pockets of diversity to persist to a much wider extent than in the North Western Atlantic. The large scale geographic distribution of deeply divergent lineages of the coastal polychaete tubeworms *Pectinaria koreni* (2 clades) and *Owenia fusiformis* (3 clades) were compared using a fragment of the mitochondrial Cytochrome Oxidase I gene (mtCOI). All lineages were present along the biogeographic transition zone on the north coast of Brittany (France) and we found evidence pointing towards congruence in the timing of cladogenic events between *Pectinaria sp.* (*P. auricoma/P. belgica* and *P. koreni*) and *Owenia sp.*, suggesting a shared history of vicariant events. More conserved 16SrRNA sequences obtained from 4 species of Pectinariidae together with mtCOI sequences of *P. koreni* seem consistent with an initial establishment of pectinariids in the north, and a southward colonisation of the NE Atlantic. Phylogeographic patterns in *O. fusiformis* were also consistent with a north/ south pattern of lineage splitting and congruent levels of divergence were detected between lineages of both species. We observed signatures of both persistence in small northern glacial refugia, and of northwards range expansion from regions situated closer to the Mediterranean. However, whether the recolonisation of the NE Atlantic by both species actually reflects separate interglacial periods is unclear with regards to the lack of molecular clock calibration in coastal polychaete species.

Keywords: mtCOI, biogeography, congruent vicariance, colonisation pathways

Introduction

The molecular information used to elucidate marine biogeographic patterns and demographic histories has highlighted the complexity of the historical processes which have shaped the evolution of species or species complexes in relation to their spatial distribution. Alongside numerous cases of crypticism within widely distributed taxonomic units (see Knowlton, 2000), congruent genetic breaks between reciprocally monophyletic populations of co-distributed marine species at biogeographic boundaries have emphasised the importance of historical vicariance and the role of contemporary hydrodynamic features in maintaining such biogeographic patterns (Reeb & Avise, 1990; Burton, 1998). To generalise about patterns of colonisation and the evolution of species' range limits, however, is difficult since different species have different life history traits and different ancestral colonisation pathways. Comparative phylogeographic approaches are therefore essential for inferring the historical causes leading to dissimilar or congruent patterns of colonisation and population processes (bottleneck or range expansion), and in terms of biodiversity, in identifying areas of endemism as a basis for the protection of marine biodiversity and ecosystems (Moritz & Faith, 1998).

In the North Eastern Atlantic (NEA), biogeographic patterns of division all point towards the area around the British Isles as being a transition area (see Dinter, 2001 for a review), with Boreal-Arctic characteristics in the north and Boreal-Lusitanian ones at the south western approaches. Such a range of environmental features, together with the complex historical events which have shaped the NEA during geological times, have led to the establishment of a great variety of marine assemblages in this area. While the transition of the Mediterranean sea to full marine conditions 5.3 million years ago (mya; McKenzie, 1999) opened up new territories to exploit for Atlantic marine organisms which were then capable of migrating through the Gibraltar Strait, the re-opening of the Bering Strait between 7.4 and 4.8 mya (Marincovich & Gladenkov, 1999) enabled a large number of Pacific invaders to move to the

Atlantic some 3.5 mya (Vermeij, 1991; Cunningham & Collins, 1998). Present day communities of the boreal-temperate region around the British Isles represent a combination of species that survived in northern glacial refugia during Pleistocene glacial maxima and species that returned from more temperate ones following the reorganisation of isotherms and patterns of marine currents during interglacial periods. The Pleistocene's Last Glacial Maximum (LGM, 18-23 kya) especially seems to have affected differently the biota living on either side of the North Atlantic. The phylogeographic patterns of most north western Atlantic rocky intertidal invertebrates are consistent with an extinction event followed by recolonisation from Europe at the end of the last glacial period (Wares & Cunningham, 2001). In addition, most species of marine invertebrates found on the west coast of the United States exhibit a genetic break between Atlantic and Gulf of Mexico populations, as a result of historical isolation, contemporary hydrodynamism and non-equilibrium processes (Wares, 2002). In Europe, no such congruent patterns have yet been confirmed, which suggests either more complex responses to Quaternary ice ages and multiple postglacial colonisation routes, or simply a lack of studies using appropriate sampling methods. While southern marine refugia would have existed closer to the Iberic peninsula and the Mediterranean, small glacial ones may have also occurred further north, around the British Isles (south west Ireland, north west Scotland) and the coast of Norway (Richter *et al*., 2001; Stewart & Lister, 2001; Luttikhuizen *et al*., 2003). The occurrence of such glacial refugia may be inferred from the levels of divergence and population subdivision whereby a substantial amount of population subdivision occurred earlier and survived the LGM in western Europe.

An integrative survey of intraspecific phylogeographic patterns among marine taxa in north-western Europe is lacking, especially for soft sediment species for which a fossil record is non-existent. Comparing such patterns between sympatric, soft sediment and rocky intertidal species and recognising the phylogeographic differences or similarities among taxa is essential to tracing back historical responses of marine biota to climate change and habitat

fragmentation, and thus to predict the evolution of populations when habitats are altered. Recently, Jolly *et al* (2005) revealed a parapatric boundary between two clades of the polychaete *Pectinaria koreni*, coinciding with the Boreal-Lusitanian biogeographic transition zone along the coast of Brittany. Based on a 2.2% mutation rate for the mtCOI in polychaetes (Chevaldonné *et al*., 2002), these clades may have separated more than 3.7 mya. To find congruent historical relationships among other co-distributed taxa, we extended the geographical sampling area of *P. koreni* and compared its phylogeographic structure with another polychaete tubeworm *Owenia fusiformis*, samples of which we obtained over the same geographic range from the Mediterranean to the Irish and Baltic Sea. While *O. fusiformis* has been regarded as a worldwide distributed cosmopolitan species (Dauvin & Thiébaut, 1994), it may in fact be composed of several taxonomic units (Koh & Bhaud, 2001). Both *Owenia fusiformis* and *Pectinaria koreni* share the same muddy fine sediment habitat and their distribution is highly fragmented along the coastlines of Western Europe. Both are gonochoric species with equal sex ratios but differ in their respective life history traits. *O. fusiformis* is a polythelic species which can live up to four years and with a long-lived larval stage (around 28 days) while *P. koreni* is a monothelic and univoltine species which possesses a relatively shorter dispersive larval stage (around 15 days).

We describe and compare the ranges of newly discovered lineages of *O. fusiformis* and *P. koreni* over the whole NEA, based on sequences of the mitochondrial Cytochrome Oxidase I gene (mtCOI), focussing our attention on the area around the British Isles. New COI data gathered for *P. koreni* along its geographic range (Irish Sea, North Sea, Baltic Sea) was extended to its congener *P. auricoma*, a species which is absent from the central and eastern English Channel. To fully compare the spatial evolution of Pectinariid species (four distinct species) with *O. fusiformis* over the NEA, we used (1) the 16S ribosomal RNA gene to investigate the phylogenetic positioning of European Pectinariids (ie. *P. koreni*, *P. auricoma* and *P. belgica*) in relation to *P. gouldii* from the North Western Atlantic and compared both

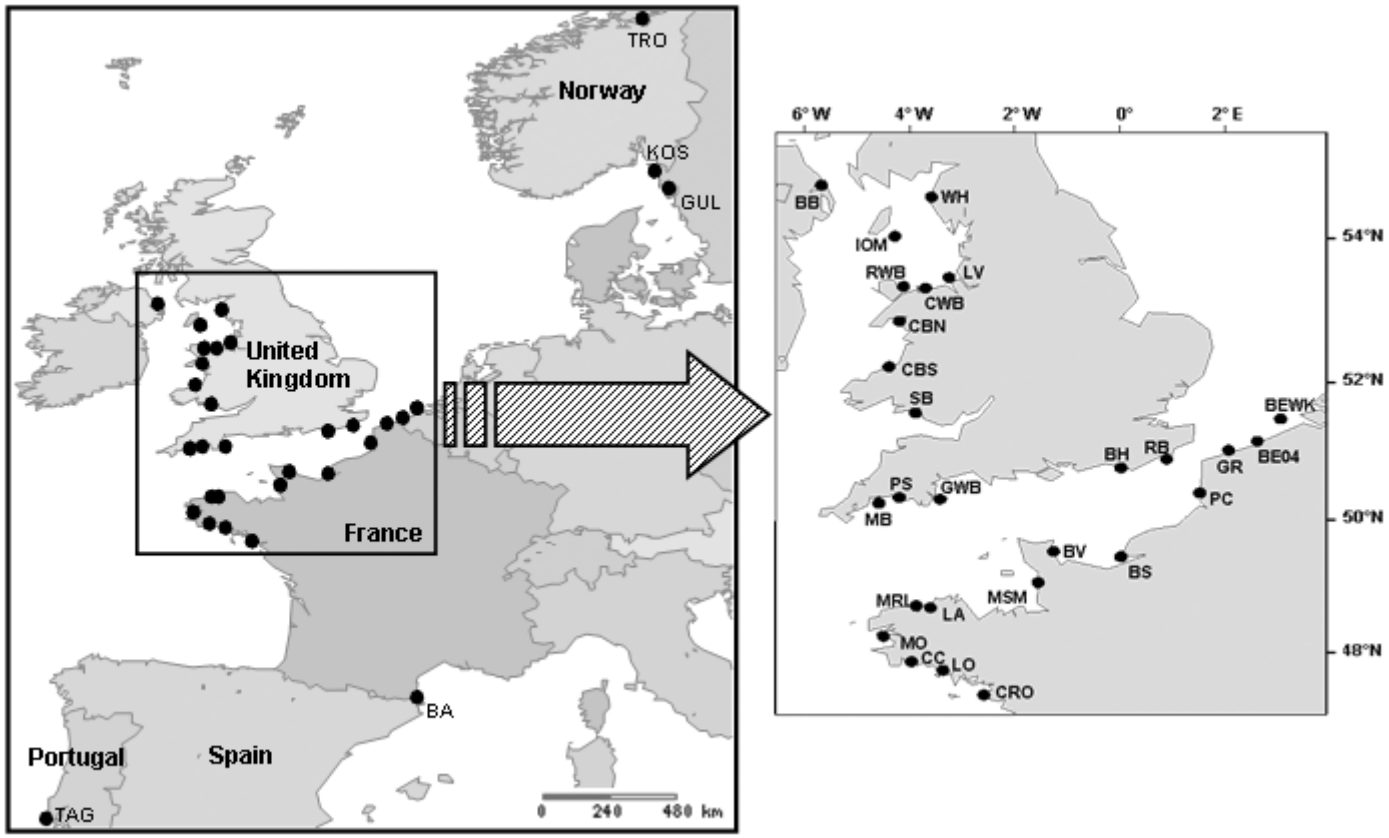

Figure 1. Location of the sampling sites (abbreviations) within and around the North East Atlantic. See Table 1 for population names and number of samples for each species.

16sRNA and COI data and (2) compared the COI trees between *Pectinaria sp.* and *Owenia fusiformis* throughout the NEA, to draw conclusions regarding the colonisation pathways of those polychaete species and the possibility for a shared history of vicariance. Furthermore, we aimed at understanding how differences in the life cycle of *P. koreni* and *O. fusiformis* may have influenced the observed patterns of genetic structure.

Materials and methods

Specimen collection

Individuals of *P. koreni*, *P. auricoma* and *O. fusiformis* were obtained by sampling muddy fine sediments in bays and estuaries, and stored in liquid nitrogen until DNA extraction. Populations of *P. koreni* were previously sampled along both coasts of the English Channel during the 2000 PECTGENE cruise while those on the south coast of Brittany were sampled in April 2002 along with *P. auricoma* (see Jolly *et al*., 2005). Additional populations of *P. koreni* were sampled from the Belgium coast in July 2003 and from the Irish Sea during the PECTIRL cruise in June 2004, together with *P. auricoma*. The southernmost samples of *P. koreni* and *P. auricoma* were obtained from the Tagus estuary in Portugal, and the northernmost from the Trondheimfjiord (Norway) in the Boreal-Arctic province and from the Kosterfjiord and Gullmarsfjiord (Sweden) in the Skagerrak region of the Baltic. A total of 25 localities were sampled which included 347 *P. koreni* and 26 *P. auricoma* (table 1). A total of 305 O*wenia fusiformis* were sampled from nearly all these localities, with a few additional individuals obtained from the Mediterranean (Banyuls, France). For phylogenetic analysis of the 16SrRNA gene, 3 individuals of *P. belgica* were obtained from the Gullmarsfjiord area of Sweden and individuals of *P. gouldii* were provided from Woods Hole on the North East coast of the USA. The geographic locations of the sampling sites are represented in figure 1 and details concerning the numbers of individuals (N) and species sampled are provided in table 1.

Table 1. Geographic details, name of the populations and species sampled. N is the number of individuals included in genetic analysis. *Pectinaria auricoma* (Pa), *P. belgica* (Pb), *P. koreni* (Pk), *Owenia fusiformis* (Of).

Biogeographic location	Name of the populations sampled (abbreviations)	Species sampled (N)
Arctic		
	Trondheimfjiord (TRO)	Pk (1)
Baltic		
	Kosterfjiord (KOS)	Pa (4), Of (2)
	Gullmarsfjiord (GUL)	Pa (5), Pb (3), Pk (4)
North East Atlantic		
North Sea		
	Belgium coast (BEO4; BEWK)	Pk (35), Of (18)
	Gravelines (GR)	Pk (20)
English Channel		
	Rye Bay (RB)	Pk (25)
	Beachy Head (BH)	Pk (12), Of (5)
	Pays de Caux (PC)	Pk (13), Of (18)
	Baie de Seine (BS)	Pk (25), Of (19)
	Baie des Veys (BV)	Pk (22), Of (21)
	Mont Saint Michel (MSM)	Pk (15)
	Baie de Lannion (LA)	Pk (10), Of (38)
	Baie de Morlaix (MRL)	Pk (16), Of (5)
	Great western Bay (GWB)	Pk (11), Of (22)
	Plymouth Sound (PS)	Of (18)
	Mevagissey Bay (MB)	Of (18)
Irish Sea		
	Belfast Bay (BB)	Of (18)
	Whitehaven (WH)	Pk (19)
	Isle of Man (IOM)	Of (6)
	Liverpool Bay (LV)	Pk (21)
	Colwyn Bay (CWB)	Pk (23)
	Red Wharf Bay (RWB)	Pk (6), Of (19)
	Cardigan Bay (CBN; CBS)	Pa (6), Pk (1), Of (23)
	Swansea Bay (SB)	Pk (1), Of (2)
Atlantic Ocean		
	Morgat (MO)	Pk (26)
	Concarneau (CC)	Pa (8), Pk (10), Of (21)
	Lorient (LO)	Pk (18), Of (19)
	Le Croisic (CRO)	Pk (7)
	Tagus estuary (TAG- Portugal)	Pa (3), Pk (6), Of (9)
Mediterranean		
	Banyuls Bay (BA)	Of (4)

DNA extraction and direct sequencing of PCR products

Total genomic DNA was extracted using a CTAB (CetylTrimethylAmmoniumBromide) extraction procedure. Gills were removed from frozen or ethanol-preserved animals, homogenised in 600µl of a 60°C preheated 2% CTAB buffer solution (containing 1.4M NaCl, 0.2% 2-mercaptoethanol, 20mM EDTA, 100mM Tris-HCL pH 8, 0.1 $mg.ml^{-1}$ proteinase K) and digested for two hours at 60°C. Proteins, lipids and carbohydrates were removed using a single Chloroform-Isoamyl alcohol (24:1) precipitation, DNA was precipitated with 370µl cold 100% isopropanol and was stored at –20°C for one to two hours. The tubes were finally centrifuged at 15 000 g for 5 min. DNA pellets were washed with 70-75% ethanol and re-suspended in 20-60µl of 1xTE buffer (10 mM Tris-HCL; 20 mM EDTA pH 8).

Partial sequences (around 600bp for *Pectinaria* and 500bp for *Owenia*) of the mitochondrial Cytochrome Oxidase subunit I gene (mtCOI) were amplified with the universal primers LCO1490 and HCO2198 (Folmer *et al.*, 1994). DNA amplifications were performed with the following conditions: (1) an initial denaturation step at 94°C for 2 min, (2) 5 cycles of denaturation at 94°C for 35 s, annealing at 45°C for 35 s and elongation at 72°C for 70 s, followed by 35 cycles of annealing at 50°C and (3) a final elongation at 72°C for 7 min. As saturation of mtCOI sequences may have been reached between the distinct Pectinariid species, partial sequences (584bp) of the 16S ribosomal RNA gene were obtained from one to three individuals of each species for phylogenetic reconstruction. 16SrRNA sequences were amplified using the universal primers 16Sar-L and 16Sbr-L described by Palumbi *et al*. (1991). DNA amplifications of this gene consisted of (1) an initial denaturation step at 94°C for 2 min, (2) 35 cycles of denaturation at 94°C for 35 s, annealing at 50°C for 35 s and elongation at 72°C for 70 s, and (3) a final elongation at 72°C for 7 min. All PCR reactions were performed into a 26 µl reaction volume consisting of 1xPCR buffer; 2mM $MgCl_2$; 0.12mM dNTP; 0.2µM of forward and reverse primers; 0.5 U of Thermoprime Plus *Taq*

polymerase (ABgene); 25ng genomic DNA. The PCR products were then purified and sequenced on ABI 3100 using BigDye© (Perkin Elmer) terminator chemistry following the manufacturer's protocol. Forward and reverse sequences were proofread in Chromas 2.23 and aligned manually in BioEdit Sequence Alignment Editor (Hall, 1999).

Phylogenetic analysis

Phylogeny of Pectinariid species using the 16SrRNA gene

Since phylogenetic relationships between Pectinariid species could neither be resolved using the mitochondrial COI sequences (high level of saturation) nor using the translated protein sequences (ie. only one codon distinguishes *P. auricoma* from the other species), Maximum Likelihood (ML) analyses of 16SrRNA sequence data were performed using PAUP 4.0b10 (Swofford, 2002), with midpoint rooting, stepwise addition and TBR branch swapping. The optimal substitution model of sequence evolution was selected by using the Akaike Information Criterion (AIC) in Modeltest 3.06 (Posada & Crandall, 1998). The General Time Reversable model (GTR+1) with invariable sites (I= 0.7795), unequal base frequencies (A= 0.3012; C= 0.2010; G= 0.2237; T= 0.2741), unequal substitution rates and equal among site rate variation was selected (AIC= 2701.2744) and the model parameters were implemented in heuristic searches in PAUP. Inferred sequence gaps were treated as missing data and nodal support was estimated with 1000 re-sampling of the dataset (bootstraps), using maximum likelihood and parsimony.

Phylogenetic analyses of lineages of Pectinaria and Owenia using the mtCOI gene

To best represent the phylogenetic relationships within the Pectinariidae, a Minimum Evolution (ME) tree was constructed (using all informative sites) based on Kimura-2 parameter (K2-P, Kimura, 1980), with MEGA v. 2.1 (Kumar, 2001). The same tree construction method was used to represent the phylogenetic relationships between lineages of

Owenia fusiformis. The program PHYLTEST 2.0 (Kumar, 1996) was used to test for the relative rate constancy of a molecular clock across lineages and divergence estimates between lineages were calculated by computing the average distance (D_{AVG}) according to the K2-P model (Kimura, 1980) with MEGA v. 2.1 (Kumar *et al.*, 2001). Minimum-spanning networks based on the mtCOI gene were constructed for each species/ lineage using TCS v.1.13 (Clement *et al*., 2000) to infer the most parsimonious branch connections at the 95% confidence level between haplotype pairs. To test for congruent histories of vicariance among taxa, we used PAML version 3.14 (Yang, 1997) to compare the phylogenetic trees of *Pectinaria* (*P. auricoma, P. koreni*) and *Owenia* lineages under alternate sequence data sets and constrained the topologies (branch length included) according to the KH test (Kishino & Hasegawa, 1989).

Haplotype diversity and coalescent estimation of population parameters

For each population of *P. koreni* and *O. fusiformis*, haplotype diversity ($h_{e\text{-}HAP}$) and nucleotide diversity (π) were examined with DnaSP 3.51 (Rozas & Rozas, 2000) and mismatch analyses of COI sequences were performed using ARLEQUIN v. 2 (Schneider *et al.*, 2000). As all substitutions appeared to be synonymous, Fu & Li's *D* statistic (Fu & Li, 1993) was investigated as an index of departure from population equilibrium. The frequency distribution of pairwise K2-P distances among sequences was compared to an expected distribution under a model of sudden expansion by estimating the τ statistic (Rogers and Harpending, 1992). The time since the most recent expansion (t) was calculated from mismatch distribution analysis of parameter τ using the equation $\tau = 2\mu t$ (Rogers and Harpending, 1992) where μ is the per-nucleotide mutation rate 2.2 x 10^{-8} (Chevaldonné *et al*., 2002) multiplied by the sequence length (600bp in *P. koreni*; 578bp in *P. auricoma*; 506bp in *O. fusiformis*).

Ancestral population parameters, Θ (Θ = 2 N_eμ for mtDNA, where N_e is the effective population size and μ is the mutation rate) and g (the exponential population growth parameter) were jointly calculated using FLUCTUATE (Khuner *et al*., 1998), for species/lineages exhibiting no spatial differentiation among populations (*P. koreni* clade 2, *P. auricoma* and *O. fusiformis* clade 3). The joint estimates of Θ and g (using all nucleotide positions) were based on an average of ten convergent replicate analyses. For both *P. auricoma* and *O. fusiformis* clade 3, we used 20 short chains of 200 steps each and two long chains of 20 000 steps, with sampling increments of 20. For *P. koreni* clade 2, we used 10 short chains of 2 000 steps each and 5 long chains of 20 000 steps with sampling increments of 20. The transition: transversion ratios estimated with PAUP* 4.0 were used as input for each analysis and UPGMA trees were used as initial starting topologies. The estimate of Θ during the Last Glacial Maximum (20 kya) is based on g and a mutation rate of μ = 2.2 10^{-8} substitution per site per generation (2.2% per my, Chevaldonné *et al*., 2002). A graph of change in relative effective population size was generated from the equation $\Theta_t = \Theta e^{-(g\mu)t}$ (Khuner *et al*., 1998), where Θ_t is the effective population size at a given time *t* in the past, and Θ the present effective population size calculated jointly with g, the growth parameter.

Genetic structure, isolation by distance and past gene flow

Whithin each lineage, an analysis of variance between groups of populations (AMOVA) was performed to test for regional structure in the partitioning of haplotypes using ARLEQUIN v. 2 (Schneider *et al.*, 2000). Isolation by distance was then tested with a Mantel test based on pairwise genetic/geographical distances (Reynolds *et al*., 1983) for species/lineages exhibiting a significant level of regional partitioning, using GENEPOP 3.3 (Raymond & Rousset, 1995). For the Mantel test, sampling locations with less than 5 individuals were removed to avoid biased genetic distances. The “Great Circle Distance

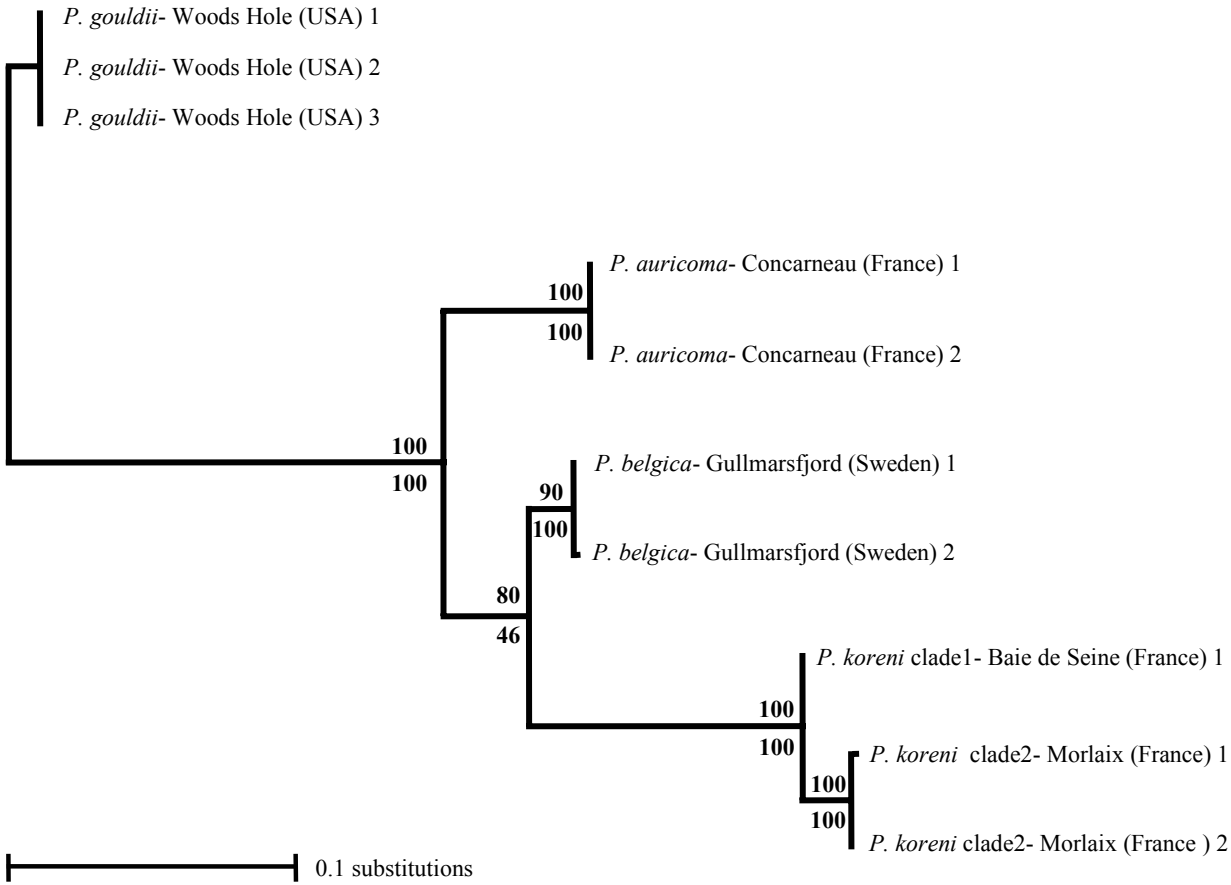

Figure 2. Optimal ML tree of 10 mtDNA haplotypes (16SrRNA) with bootstrap percentages from 1000 replicates. Above nodes are the values from the ML analysis and below nodes are those from the MP analysis.

Calculator" (available online at http://www.gb3pi.org.uk/great.html) was used to calculate the shortest distance between two geographic locations, following the coastlines.

To estimate past gene flow and migration patterns among populations of lineages showing some genetic structuring (*P. koreni* clade 1; *O. fusiformis* clade 1 and 2), we used the software package MIGRATE 1.761 (Beerli & Felsenstein, 1999) which takes into account historical and unsymmetrical gene flow. To obtain past gene flow for a full migration model, we used 10 short chains with 500 steps and 10 000 sampled genealogies, and 3 long chains with 5 000 steps and 100 000 sampled phylogenies. Populations with less than 4 individuals were discarded from the analyses.

Results

Phylogenetic investigation of Pectinariidae using the 16SrRNA gene

Phylogenetic analysis of 16SrRNA sequences (GenBank Accession Nos. DQ319856-DQ319865) revealed four strongly supported taxonomic units with 100% bootstrap values whether estimated by ML or MP methods (figure 2). The level of divergence obtained between the North East American species *P. gouldii* and the other European pectinariids in both ML and MP trees suggested an ancestral separation between the two continents during the colonisation of the NEA. Amongst European tubeworms, *P. belgica* clustered with *P. koreni* although bootstrap support was weaker (46- 80%) than for the other nodes. This was due to a different position of *P. belgica* relative to *P. auricoma* and *P. koreni* in the MP tree (not shown), whereby *P. belgica* and *P. auricoma* represented sister species. In both trees however, *P. gouldii* was positionned as ancestral to its congeneers.

Comparison of the mtCOI topologies between Pectinaria and Owenia lineages

MtCOI haplotypes are deposited in GenBank (*P. koreni*, Accession Nos. DQ319510-DQ319855); *P. auricoma*, Accession Nos. DQ319484- DQ319509; *O. fusiformis*, Accession

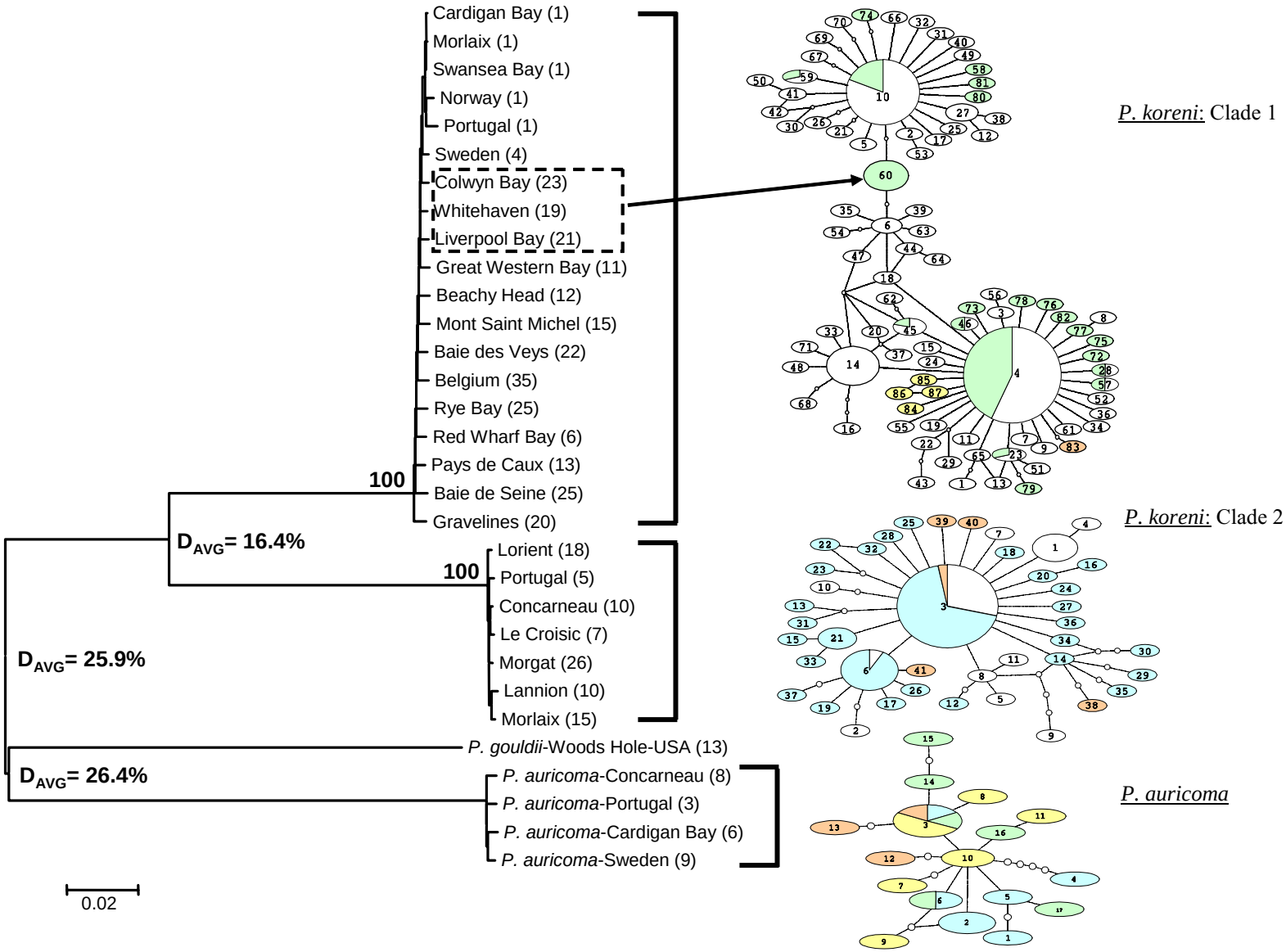

Figure 3. Minimum Evolution (ME) tree using Kimura 2-parameter distance between populations of Pectinariids and associated parsimony networks of COI haplotypes for each mtDNA clade. Small circles represent "missing" haplotypes. Colours represent the distribution of haplotypes in relation to their geographic locality; white: English Channel; green: Irish Sea; Blue: Atlantic, yellow: Baltic/ Arctic; orange: Portugal.

Nos. DQ319193- DQ319483). To test whether *Pectinaria sp.* (i.e. *P. koreni* and *P. auricoma*) and *Owenia sp.* phylogenies were congruent, we estimated the likelihood of obtaining the *Pectinaria sp.* tree topology given *Owenia sp.* sequences and *vice versa* using the relative rate tests of Kishino & Hasegawa (1989). We constrained the branch lengths of *Pectinaria* on *Owenia* sequences and estimated a likelihood value (LnLi= -1379.297) that was compared with the ML tree (LnLi= -1102.456) for *Owenia sp.* alone. The difference between the two likelihood values was not significant (P_{KH}= 0.094), thus the *Pectinaria sp.* topology was not significantly worse than the *Owenia sp.* topology for explaining evolutionary relationships among *Owenia sp.* mtDNA sequences. The reciprocal test (*Owenia-Pectinaria* topologies versus *Pectinaria sp.* sequences with constraints on branch lengths) however, was significant but only marginally so (LnLi= -1750.92; LnLi= -1408.381; P_{KH}= 0.049). Nonetheless, looking at the positioning of *P. belgica* in the 16sRNA ML/ MP trees (figure 2, MP tree not shown), we cannot discard the fact that *P. belgica* would have been more appropriate to test for co-evolutionnary processes between the two genera. Thus, providing that *P. belgica* or *P. auricoma* correspond to *Owenia sp.* clade 3, the timing of cladogenic events seems to be quite similar in both *Pectinaria sp.* and *Owenia sp*., surely as a consequence of shared histories during the colonisation of the NEA.

Pectinaria koreni

Jolly *et al.* (2005) previously observed a parapatric boundary between two strongly supported and highly divergent clades (clade 1 and 2; D_{AVG}= 16.4%). Herein, clade 1 (i.e. North Sea, English Channel, Irish Sea, Baltic Sea and Boreal-Arctic province) represented 87 haplotypes (N= 256) and clade 2 (the southern populations from the Atlantic) represented 41 haplotypes (N= 91) (figure 3). Interestingly, one individual from the Tagus estuary (Portugal) belonged to the "northern" clade 1. Elsewhere, note that all four sequences from the Skagerrak area of the Baltic and the one from Norway (Hap 84) represented unique

Table 2. Haplotype (H_e) and nucleotide diversity (π), estimates of Fu & Li's D-statistic and AMOVA tests with associated levels of significance calculated for the mitochondrial COI clades of *Pectinaria koreni*, *P. auricoma* and *Owenia fusiformis*.

COI haplotype groups	Haplotype diversity (H_e)	Nucleotide diversity (π)	Fu & Li's *D*	Among population variation (%)	Within population variation (%)
P. koreni					
Clade 1	0.871± 0.017	0.0064± 0.0002	-7.459*	1.85 *	98.15
Clade 2	0.862± 0.032	0.0033± 0.0003	-5.805*	0	100
P. auricoma	0.938± 0.034	0.0051± 0.0007	-2.292	0	100
O. fusiformis					
Clade 1	0.924± 0.018	0.0077± 0.0004	-6.530*	1.03	98.97
Clade 2	0.948± 0.013	0.0072± 0.0006	-4.093*	1.16	98.84
Clade 3	0.823± 0.06	0.0029± 0.0004	-1.759	0	100

*P< 0.05

haplotypes all derived from the ancestral Hap 4, more frequently distributed in the Irish Sea. The haplotype network of clade 1 revealed a pattern reminiscent of admixture by secondary contact between two groups of widely distributed and ancestral haplotypes (Hap 4 and Hap 10) connected together by a central and private haplotype found only in the Irish Sea (Hap 60; figure 3). Note that Hap 10 is also found in much lower frequency in the Irish Sea than in the English Channel.

Nucleotide diversity was twofold smaller in clade 2 although haplotype diversity was high and similar in both clades (table 2). While the AMOVA did not reveal any geographic structure within clade 2, variation among clade 1 populations represented 1.85% (P= 0.015) of the total variance. Further analysis conducted between groups of the Irish Sea and the English Channel (grouped here with the North Sea) revealed a significant level of genetic structuring representing 4.4% of the total genetic variation (F_{CT}= 0.044; P< 0.001) and which was associated with isolation-by-distance (Mantel test P= 0.001). In clade 1, nucleotide diversity (π) was also significantly lower in the Irish Sea (π = 0.0048± 0.00044; *U*-test= 0, P= 0.012) compared to the English Channel (π = 0.0068± 0.00047). Although at first glance there was no significant difference in haplotype diversity (H_e) between the Irish Sea ($H_{e\text{-}HAP}$= 0.7748± 0.09735) and the English Channel ($H_{e\text{-}HAP}$= 0.9022± 0.05468), it was significantly lower in the Irish Sea when the Red Wharf Bay (RWB) population (N= 6) which lacked Hap 60 was excluded ($H_{e\text{-}HAP}$= 0.7219± 0.08923; *U*-test= 2, P= 0.048).

Estimates of Fu & Li's *D* (D_F) statistic were used to test deviation from neutral expectations and mismatch analyses were used to test for conformity to a sudden expansion model of demographic history. The D_F statistic for *P. koreni* clade 1 takes on a significantly negative value (D_F= -7.459*; table 2) and the haplotype composition fitted the expectation of an exponential population growth (τ = 7.133; P= 0.860) although the mismatch curve for this clade was clearly bimodal (curve not shown). Clade 2 also deviated from neutral expectations (D_F= -5.802*; table 2), but was associated with a star-like haplotype network (figure 3), the

Table 3. Estimates of Θ (the parameter representing the effective population size and substitution rate) and g (the exponential growth parameter) using Fluctuate (Khuner *et al*., 1998) for species/lineages exhibiting no among population variation. The estimates, based on all codon positions (the average of ten replicate analysis) indicate the maximum-likelihood estimate of the joint probability Θ and g, presented with their standard deviation. The estimates of Θ during the Last Glacial Maximum (20 kya) are based on g and a mutation rate of $\mu = 2.2\ 10^{-8}$ substitution per site per generation.

species/clades	Θ (no growth)	Θ (joint estimation)	g	Relative Θ_t (20kya)
P. koreni clade 2	0.0352	0.18443 ± 0.01697	1978.6 ± 81.7	0.0722 ± 0.0164
P. auricoma	0.0196	0.28025 ±0.06417	1737.9 ± 142.7	0.1305 ± 0.0603
O. fusiformis clade3	0.0074	0.0448 ± 0.01091	2803.4 ± 338.4	0.01305 ± 0.0094

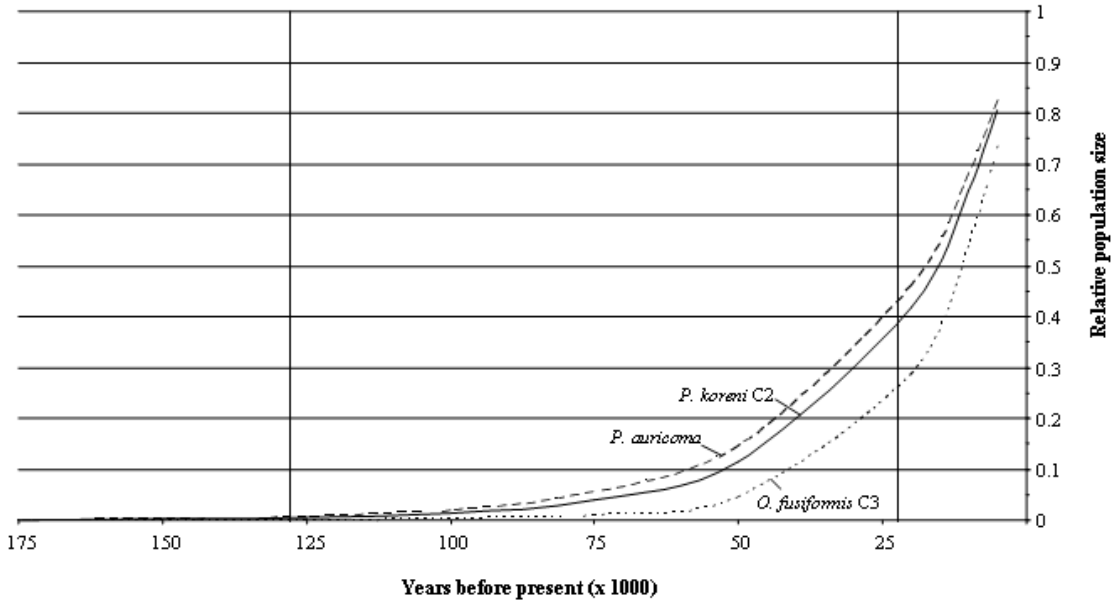

Figure 4. Graph of change in relative effective population size over time based on Metropolis-Hastings Monte Carlo coalescent analysis. Vertical bars correspond to glacial maxima.

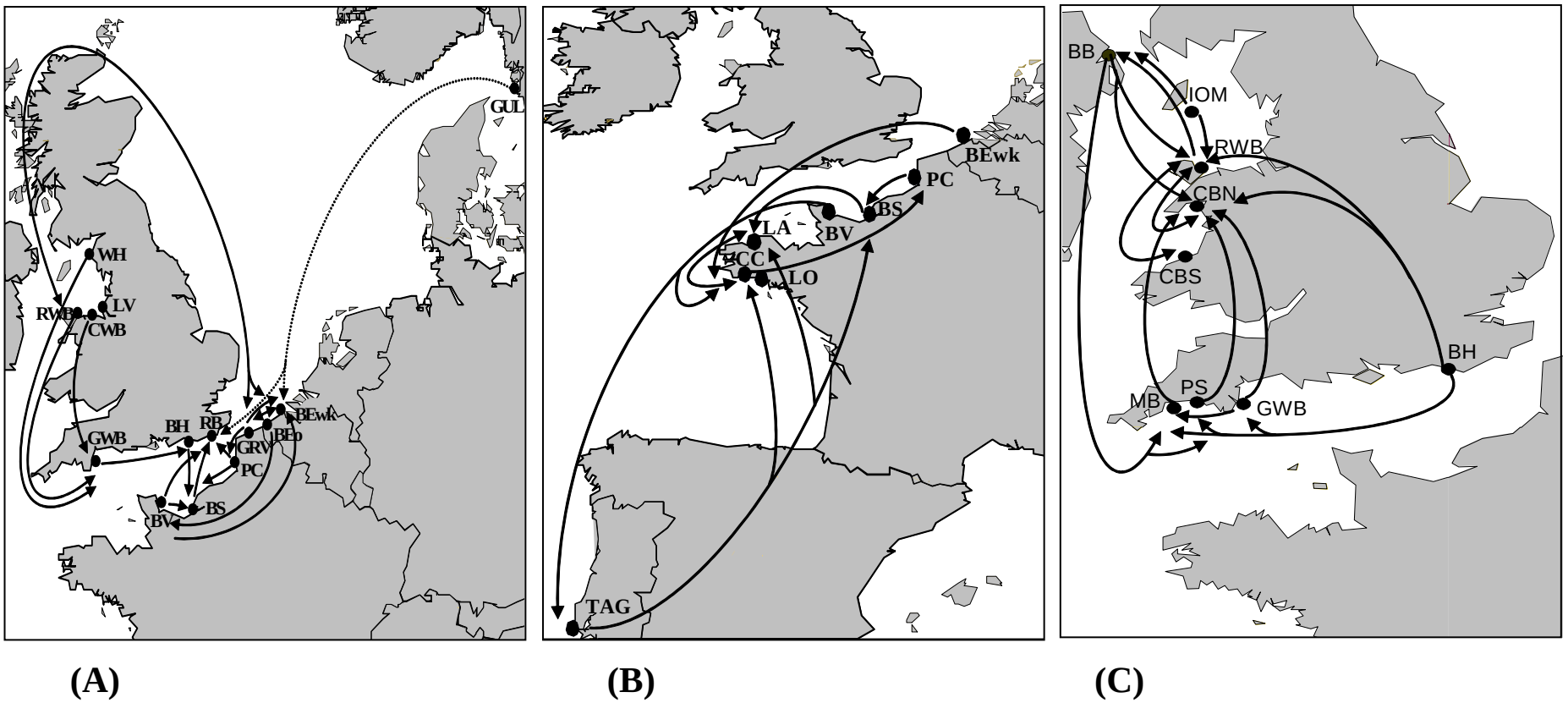

Figure 5. Geographic distribution of clades and gene-flow patterns in: (A) *Pectinaria koreni* clade 1, (B) *Owenia fusiformis* clade 1 and (C) *O. fusiformis* clade 2. For clarity, only gene flow estimates of $Nm \geq 10$ are represented.

composition of which fitted an exponential population growth (τ = 1.912; P= 0.449) with values of g and Θ (table 3) suggesting that populations of *P. koreni* clade 2 were circa 1% of their present population size 77,692 years ago (figure 4). This estimate roughly corresponds to that of the time since expansion (t = 72,424 generations ago) based on our mismatch analyses (with τ = 1.912 and μ = 1.32 x 10-5), knowing that the species has a one year generation time (Irlinger *et al.,* 1991).

Coalescent gene flow analyses carried out on *P. koreni* clade 1 (figure 5a) suggests considerable long distance dispersal ($4.9 < Nm < 20.8$) in a N-S direction between populations of the Irish Sea (RWB, CWB, WH) and the population of GWB, situated on the south west English coast. Relatively little gene flow ($0.9 < Nm < 4.9$) was observed within the Irish Sea, while the strongest (Nm= 280.7) was observed in a W-E direction between GWB and BH, on the south English coast of the English Channel. In addition, RB (eastern English Channel) and BEo (Belgium coast) populations seem to have received migrants from both Scandinavian Fjords (GUL, but N= 4) and the Irish Sea. These results indicate two distinct colonisation pathways for *P. koreni* clade 1, around the British Isles (via the western English Channel and the North Sea), which might explain the secondary admixture of two ancestral haplotypes.

Pectinaria auricoma

A total of 17 haplotypes (N= 26) was found for this species. Haplotypes from the Baltic Sea were centrally distributed within the haplotype network (figure 3) with an "ancestral" haplotype (Hap 3) distributed throughout the geographic range of this species (from the Baltic Sea to Portugal). This haplotype occurs at higher frequency in the Baltic Sea, possibly indicating an N-S pattern of colonisation of the NEA for this species. Haplotype diversity was high ($H_{e\text{-}HAP}$= 0.938± 0.034), the level of which was slightly higher when compared to that of populations of *P. koreni* (clade 1 and 2), but with an intermediate level of nucleotide diversity (π = 0.0051± 0.0007) (table 2).

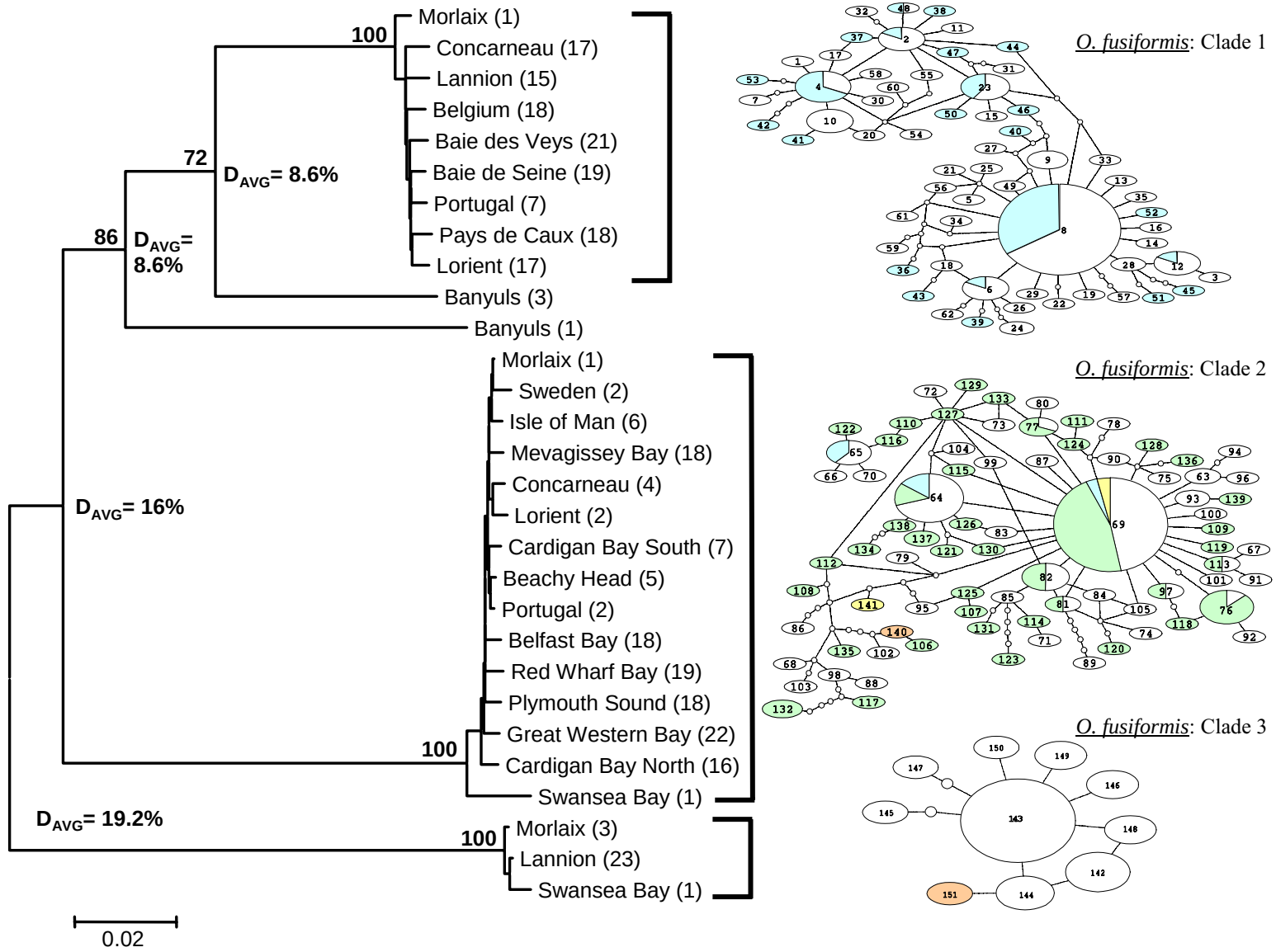

Figure 6. Minimum Evolution (ME) tree using Kimura 2-parameter distance between populations of *Owenia fusiformis* and associated parsimony networks of COI haplotypes for each mtDNA clade. Small circles represent "missing" haplotypes. Colours represent the distribution of haplotypes in relation to their geographic locality; white: English Channel; green: Irish Sea; Blue: Atlantic; yellow: Baltic; orange: Swansea Bay.

Populations of *P. auricoma* did not deviate from neutral expectations (D_F= -2.292; P>0.05) although mismatch analysis fitted the sudden growth model (τ = 2.9; P= 0.529). In addition, the estimated effective size of the population at the present time is only twice that of the population 20 kya, which in turn is 6 times larger than when the effective size was estimated with no growth (table 3). This suggests that most of the population growth in *P. auricoma* pre-dated the LGM. The coalescent analysis of change in effective population size over time (table 3) suggests that *P. auricoma* populations were circa 1% of their present population size 148,765 years ago (figure 4), which roughly corresponds to the time since expansion (t = 114,173 generations) based on our mismatch analyses (with τ = 2.9 and μ = 1.27 x 10-5).

Owenia fusiformis

Analyses of the mtCOI gene revealed three strongly supported clusters (86-100% bootstrap support; figure 6), with average divergence estimates (D_{AVG}) based on K2-P ranging from 16% to 19.2%. Clade 1 represented 62 haplotypes (N= 133) and was abundant on the French coast of the Atlantic and the English Channel, and on the Belgium coast of the North Sea. Clade 2 which represented 79 haplotypes (N= 141) was present in the Skagerrak area of the Baltic Sea and was distributed around the British isles, from the Irish Sea to the southern English coast. Clade 2 was also found in sympatry with clade 1 along the north coast of Brittany (France) and also in the Tagus estuary (Portugal). Divergence estimates between those two clades was 16% based on Kimura 2-P distance model. A third and seemingly more divergent clade of *O. fusiformis* (clade 3 grouping the Morlaix, Lannion and Swansea Bays; D_{AVG}= 19.2%) accounted for 10 haplotypes (N= 27), with the individual sampled in Swansea Bay representing a unique haplotype (Hap 151; figure 6). In addition, two haplotypes differing by 8.6% were found to occur in the Mediterranean. The basal positioning of these

two divergent haplotypes which clustered with clade 1 suggests that this clade may have emerged in the southern parts of the NEA.

AMOVA analyses indicated no significant genetic structure within either of the clades (table 2), although variation among populations of clade 2 was marginally non-significant and represented 1.16% of the total genetic variation (P= 0.068). Within clade 2, no pattern of isolation-by-distance was detected between Irish Sea and English Channel groups (Mantel test: P= 0.111). Whithin both clade 1 and 2, haplotype networks displayed similar and complex topologies with one ancestral haplotype (clade 1= Hap 8; clade 2= Hap 69), together with less frequent ones (Hap 4 and 23 in clade 1; Hap 64, 76 and 82 in clade 2), suggesting similar histories. The most divergent haplotypes in the network (figure 6) were actually associated with populations sampled from Cornwall and Devon (MB, PS and GWB) and from the Irish Sea, possibly reflecting a longer history of isolation among those populations. Haplotype diversity was high for all clades ($0.823 < H_{e\text{-}HAP} < 0.948$) and no significant differences were detected across populations of both clade 1 and 2. In clade 3, however, both haplotype diversity ($H_{e\text{-}HAP}$= 0.823± 0.06) and nucleotide diversity (π = 0.0029± 0.0004) appeared to be significantly lower than clade 2 (*t*-test $H_{e\text{-}HAP}$ = 5.285, P< 0.00; *t*-test π = 4.599, P= 0.001) (table 2).

Of the three clades of *O. fusiformis*, only clade 2 in which D_F= -4.093* (P<0.02) exhibited a mismatch pattern inconsistent with a rapid population expansion (τ = 4.149**; P= 0.010). By contrast, clade 1 and clade 3 fit expectations of exponential population growth (τ = 7.068, P= 0.750 and τ = 1.623, P= 0.349 respectively), although the match-mismatch curve was bimodal in clade 1 (curve not presented). No deviation from neutral expectations (D_F = -1.759; P> 0.1) was found in clade 3, but values of Θ reflected a 3.4 increase in the effective population size during the last 20 ky, whereas the effective size 20 kya was only 1.7 times larger than when Θ was calculated with no growth (table 3). The coalescent analysis of change in relative population size over time (figure 4) suggests that *O. fusiformis* clade 3 was

circa 1% of its present population size 76,548 years ago. While *O. fusiformis* may live up to four years (Gentil *et al*., 1990), one major recruitment event is observed yearly in the English Channel, so there is mostly one generation per year. Accordingly, this is reflected by the time since expansion of clade 3 based on our mismatch analyses (t = 73,108, with τ = 1.623 and μ = 1.11 x 10-5).

The results of the coalescent gene flow analyses that were carried out on both *O. fusiformis* clade 1 and clade 2 are shown in figure 5b-c. In clade 1, both N-S ($41.5 < Nm < 2.8\ 10^{15}$) and S-N ($Nm = 6.6\ 10^{13}$) patterns of gene flow were detected, oriented towards the CC population on the south coast of Brittany (France). The S-N pattern, however, only relied on the small number of individuals sampled in Portugal and thus should be regarded with caution. In clade 2, there was considerable gene flow within the English Channel, in an E-W direction (opposite to *P. koreni* clade 1) from BH towards populations situated in the western province of the Channel ($11.8 < Nm < 1.9\ 10^{16}$). While both N-S and S-N patterns of long distance dispersal were observed between populations of the Irish Sea and those of the English Channel, the latter pattern was considerably stronger.

Discussion

Vicariance events and colonisation pathways of the NE Atlantic

From the analyses of 16sRNA sequences, it appears that a Pectinariid ancestor gave rise to a north-western Atlantic species (*P. gouldii*) and a European species ancestral to *P. auricoma P. koreni* and *P. belgica*. While it is thought that the colonisation of the North Atlantic mostly involved the movement of Pacific invaders through the Bering straits some 3.5 Mya. (Cunningham & Collins, 1998), whether *Owenia sp.* and *Pectinaria sp.* were present in the North Atlantic before or after the trans-Arctic interchange took place is not clear since no fossil records can attest for this interchange in polychaetes. A molecular clock was therefore applied but not calibrated. Assuming a 2.2% mutation rate of the mtCOI gene for polychaetes

(Chevaldonné *et al*., 2002) and the observation of congruent divergence levels (around 16%) between clade 1 and 2 of both *O. fusiformis* and *P. koreni*, divergence time estimates (3.64-3.68 Mya) would fall close to the trans-arctic interchange (around 3.5 mya). A similar mutation rate would bring, however, the separation of the more divergent *O. fusiformis* clade 3, or the separation of *P. koreni* from the other European Pectinariids, to around 4.5 Mya, closer to the Mio-Pliocene transition (circa 5 mya), a period of major climatic and tectonic activity with important topographic consequences in western Europe (Brault *et al*., 2004). Similarly, the separation of European pectinariids from *P. gouldii* would be even older which suggests that the applied mutation rate could actually be a lower bound value.

Although samples of *Pectinaria sp.* from the Mediterranean are needed together with COI data from *P. belgica*, according to the KH test (Kishino & Hasegawa, 1989) the timing of cladogenic events in *Pectinaria* looks very similar to that of *O. fusiformis*, thus both genera may well have co-evolved in the NEA, especially since they share the same type of soft sediment habitat. This might further be explained by a common history of vicariance/ speciation events at the origin of the separation of *O. fusiformis* clade 3 and of *P. belgica/ P. auricoma* species on one side and *P. koreni* on the other. This ancestral lineage splitting also seems concomitant with the separation of two cryptic species of *Amphiura brachiata*, an echinoderm inhabiting the same soft sediment habitat than our polychaete tubeworms (Muths *et al*., submitted). Such events may have occurred during a southward range expansion of the ancestor species as suggested by the central position of *P. auricoma*'s Baltic Sea haplotypes and the peripheral position of the other haplotypes, mostly from the Irish Sea and the Atlantic.

The congruence in the timing of the lineage splitting event observed in the mtCOI gene from both *P. koreni* and *O. fusiformis* (D_{AVG}= 16-16.4%) between reciprocally monophyletic "northern" (*P. koreni* clade 1; *O. fusiformis* clade 2) and "southern" groups (*P. koreni* clade 2; *O. fusiformis* clade 1 + Banyuls) can also only be explained by a strong vicariant effect involving physical restriction on larval dispersal. This probably happened at a time of lowered

sea level, probably of Miocene-Pliocene age, to fully allow for large scale allopatric isolation in both genera. The additional breaks between haplotypes of *O. fusiformis* belonging to the "southern" group might be indicative of late Pleistocene differentiation/speciation across the Gibraltar Straits or within the Mediterranean. The stronger degree of genetic structuring and the lack of clear signature of recent range expansion among the "northern" clades might indicate persistence in small northern glacial refugia during the LGM. This hypothesis is reinforced by the haplotype network of *P. koreni* clade 1 which shows an admixture of two divergent haplotypes which have evolved separately before secondary contact maybe in the vicinity of the Irish Sea. This is suggested by the occurrence of a private Irish Sea haplotype linking the two ancestral ones, which might have evolved in small ice-free areas either along the South Western coast of Ireland or the North Western coast of Scotland (Stewart & Lister, 2001; Richter *et al*., 2001) before the colonisation of the Irish Sea by this species. Isolation in glacial refugia may also be inferred for *O. fusiformis* clade 2, since the most divergent haplotypes were always found associated with populations of the south-west English coast. While the pattern of divergence among *O. fusiformis* haplotypes is similar to that of *P. koreni*, the haplotype networks are somewhat different in that there is no such clear-cut separation between ancestral haplotypes. Since a similar history of vicariance between the two species would have given similar results, the discrepancies found probably means that secondary contact between divergent haplotypes was faster in *O. fusiformis* due to the species' higher dispersal potential and overlapping generations.

According to gene flow patterns estimated from coalescent analyses (figure 5), the colonisation of the English Channel seems to have taken two different paths in both genera: via the western approaches of the English Channel and via the North Sea. However, although both N-S and S-N patterns of long distance dispersal were observed, within the English Channel, *P. koreni* clade 1 shows a predominant W-E pattern of gene flow while in *O. fusiformis* clade 2, dispersal tended to be inversed in an E-W direction. This maybe indicates

the occurrence of separate refuges either along the extreme SW English coast, SE Ireland or NW Scotland (see references above). In contrast, *P. koreni* clade 2 shows clear evidence of recent northward range expansion but which was hampered at the western entrance of the English Channel (see Jolly *et al*., 2005). As opposed to the previous clades, the colonisation pathways of *O. fusiformis* clade 1 are unclear since both a northward and/ or a southward range expansion from a northern refuge may be suggested from the data. Indeed, both a strong S-N (from TAG to CC) and N-S (from BEwk to CC and from BV to TAG) pattern of gene flow was observed, which maybe suggests multiple range contractions and expansions. Taking into consideration the shared history of vicariance between *Pectinaria sp.* and *Owenia sp.*, we may however suggest that both *O. fusiformis* clade 1 and *P. koreni* clade 2 expanded their range northwards from a probable southern refuge, but with a faster rate of colonisation by the former due to its higher dispersal capabilities. By subsequently colonising the higher latitudes of the NEA by rapid post-glacial dispersal, the geographic ranges of these "southern" clades (*O. fusiformis* clade 1; *P. koreni* clade 2) seemingly overlapped with those of their north European counterparts. This may be suggested by the presence of one individual from *P. koreni* clade 1 in the Tagus estuary (Portugal), and the occurrence of both *O. fusiformis* clade 1 and 2 at four sites along the Atlantic (coasts of Portugal and France). While such a pattern of recolonisation is consistent with that found among cryptic species of Hydrobia (see Wilke & Pfenninger, 2002), it may also be the result of human mediated dispersal via increases in human activity (eg. ferry) between the north and the south.

Genetic diversity and demographic histories

Both *O. fusiformis* clade 3 and *P. auricoma* do not deviate from neutral expectations, although in both species the observed raggedness values were not significantly different from the mismatch distribution expected under a sudden growth model. While both species might have co-evolved in the NEA, they may not have shared a common demographic history.

While *P. auricoma* shows a much larger increase in population size before the LGM than after, the former species has comparatively much lower haplotype and nucleotide diversities, with a mismatch distribution (not shown) consistent with a more recent growth of the population. While the time since expansion of *P. auricoma* pre-dates that of *O. fusiformis* clade 3, estimates fall around the time of the Saalian glacial maximum (around 128 kya) rather than closer to the LGM. This is interesting because the Saalian glaciation is thought to have been more widespread than the LGM around the British Isles and was responsible for the damming-up of the fluvio-glacial Lake Solent (Kellaway *et al*., 1975), thus maybe partly explaining the absence of *P. auricoma* from the central and eastern part of the English Channel. In the case of *O. fusiformis* clade 3, growth and diversification seem to have happened within the population after the LGM but coalescent simulations indicate low population sizes despite a relatively higher growth parameter. This suggests that this lineage might be composed of remnant populations which may have persisted over a more restricted area and at lower population sizes after the LGM.

Although *P. koreni* clade 1 and *O. fusiformis* clade 2 shared similar histories and present an admixture of 2 ancestral haplotypes, paired match-mismatch curves (not shown) do not overlap and the haplotype composition of *P. koreni* clade 1 fits a model of exponential growth whereas that of *O. fusiformis* clade 2 does not. Thus, both species/lineages probably did not have shared a similar range expansion although they might have shared similar colonisation pathways from a unique refuge near to the Irish Sea. Within *P. koreni* clade 1, the deviation from neutral expectations of D_F may reflect population expansion after the LGM, and the isolation-by-distance pattern with lower genetic diversity in the Irish Sea could indicate founder events in this area following range expansion during the "Younger Dryas" (circa 12 kya), when the Irish Sea and the English Channel were opened to the Atlantic (Renssen & Vandenberghe, 2003). The unique haplotypes found in the Baltic Sea samples derive directly from a large common haplotype and from each other, indicating that they have evolved after

expansion but in relative isolation. This may have either happened just after the LGM since a connection already existed with the Norwegian Sea (Renssen & Vandenberghe, 2003) or after 9.6 kya when the arctic-subarctic environment of the Skagerrak-Kattegat area was succeeded by boreal conditions (Conradsen, 1995). *O. fusiformis* clade 2 shares similar levels of haplotype and nucleotide diversity with *P. koreni* clade 1 and the lineage has retained near-to-significant levels of genetic structuring (P= 0.068) which could indicate that *O. fusiformis* clade 2 persisted in at least one glacial refugia. In addition, the haplotype network shows a complicated and interconnected star-like distribution, with a high level of haplotype paraphyly and no broad scale geographic differences in haplotype distribution. The more "southern" clades seem to have shared a history of expansion from refugia situated further towards the Mediterranean. The molecular signature of *P. koreni* clade 2 is clearly consistent with recent population expansion and diversification (Jolly *et al*., 2005) with a dramatic increase in effective population size during the past 20 ky. *O. fusiformis* clade 1, however, has twice as much nucleotide diversity as the former genus, a bimodal haplotype network, an older estimate of the time since expansion (τ = 7.068) and a larger population size. This indicates that this clade may have expanded and stabilised more rapidly, or that its distribution was originally more fragmented over the continental slope at the time of reduced sea level.

We have described the geographic distribution and the demographic histories of distinct lineages of the polychaete tubeworms *Owenia fusiformis* and *Pectinaria koreni* within the NEA shelf seas. The data sets point towards an initial colonisation of the NEA from the north and a common history of vicariance which has affected both genera in a similar way, splitting the ancestral range into northerly and southerly distributed populations. While there seems to be enough genetic structure in *P. koreni* clade 1 and *O. fusiformis* clade 2 to suggest persistence in small northern glacial refugia during the LGM, further sampling of the North Sea and the western coast of Ireland is needed to reveal putative refugia and to test for the

hypothesis of two distinct colonisation pathways around the British Isles. Range expansion does not seem to have occurred at the same time in both species, which perhaps reflects the effects of, at least, two separate glacial maxima on range contraction and expansion, the Saalian (*c.* 128 kya) and the Weichselian (*c.* 20 kya). In the case of *P. koreni*, samples are needed from the Mediterranean to confirm the pattern seen in *O. fusiformis*, which may be cases of speciation within the Mediterranean or across the Gibraltar straits during low sea levels. The northward shift in the polar front during the last deglaciation ultimately allowed the re-colonisation of the NE Atlantic coastline. Coastal polychaetes have been shown to exhibit deeply divergent lineages (Schulze *et al*., 2000), and the mutation rate estimate of 2.2% per million years used in this study is probably a lower-bound value. Although the most likely explanation for the observed deep phylogenetic breaks in both *P. koreni* and *O. fusiformis* is allopatric speciation due to strong historical geological events, this must have been followed by niche expansion, diversification and some degree of ecological specialisation to increasingly available niches following the rise and fall in sea level after the Mio-Pliocene transition. Indeed, a striking feature in both taxa comes from the distinct distribution of their respective lineages. *O. fusiformis* clade 3 is distributed intertidally with respect to its other lineages whereas lineages of *P. koreni* are distributed on either side of a biogeographic transition zone. Finally, the fact that both taxa may have shared similar vicariant events and that all lineages are present along the biogeographic transition zone on the north coast of Brittany, warrants more research into the possibility of vicariant co-evolution between species living in the same muddy-fine sediment habitat.

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Supplementary material

Gene flow (4Nm) estimates for *Pectinaria koreni* clade 1 (A), *Owenia fusiformis* clade 1 (B) and *Owenia fusiformis* clade 2 (C). For locality codes see Table 1. Receiving populations are in columns, donating populations in rows. For clarity, gene flow estimates of 4Nm > 10 are in bold.

A) *Pectinaria koreni* clade 1

	GWB	BH	RB	PC	BS	BV	GRV	BEo	BEwk	WH	LV	CWB	RWB	GUL
GWB	-	**280.7243**	0.0000	0.2374	4.8852	0.0000	0.0000	1.8530	3.9844	0.0000	**34.2552**	0.4256	0.0000	0.0000
BH	1.9794	-	0.0000	0.0000	**30.9425**	1.4033	0.6148	0.0000	1.9913	0.0000	7.4288	0.4260	0.0000	1.8170
RB	2.9663	0.0000	-	0.0000	4.9015	0.0000	2.9528	0.0000	0.0000	0.0000	**12.3813**	0.8520	0.3062	0.0000
PC	8.7142	5.2959	**36.3102**	-	**16.2779**	1.7916	6.0920	4.6325	0.0000	2.6525	0.0000	0.0000	0.0000	0.4192
BS	0.9888	0.0000	**18.3781**	0.1187	-	1.4361	0.0000	0.0000	0.0000	0.6015	0.0000	0.0000	0.0000	0.8385
BV	0.9888	0.0000	**25.2175**	5.4013	0.0000	-	1.9005	0.0000	**19.0159**	0.5739	**17.2835**	0.0000	0.0000	0.0000
GRV	9.0854	0.0000	4.5935	**16.2900**	0.0000	3.2311	-	0.0000	**12.9470**	0.9159	**17.3337**	0.0000	0.0000	0.1395
BEo	2.9762	5.2959	0.0000	0.0000	0.0000	**14.3164**	2.4604	-	0.0000	0.0000	**12.3766**	0.0000	0.0000	0.2795
BEwk	0.0000	0.0000	0.0000	0.1187	0.0000	0.0000	6.1492	**33.2540**	-	1.2166	4.9525	0.0000	0.0000	0.0000
WH	**11.8652**	0.0000	0.0000	1.7803	0.0000	0.0000	3.0740	4.6325	0.0000	-	0.0000	0.2130	0.0000	0.1397
LV	4.9438	5.2959	0.0000	0.1187	0.0000	2.4460	6.7618	0.0000	0.0000	0.0000	-	0.2130	0.0000	0.1397
CWB	**20.7641**	0.0000	0.0000	0.5003	0.0000	1.1091	3.8048	1.8530	5.9536	0.0000	4.9525	-	0.0000	0.4192
RWB	**17.6269**	0.0000	0.0000	0.3561	0.0000	**10.3366**	5.5905	**16.6646**	2.9869	0.0000	0.0000	0.0000	-	2.9441
GUL	0.0000	0.0000	**13.7847**	0.4747	0.0000	0.0000	0.0000	1.8513	**17.0148**	0.0000	0.0000	3.6165	0.9812	-

B) *Owenia fusiformis* clade 1

	BEwk	PC	BS	BV	LA	CC	LO	TAG
BEwk	-	0.0000	0.0000	4.8310	0.0000	**2.85E+15**	0.0000	4.0129
PC	0.5229	-	**12.6398**	0.0000	0.0000	0.292906	1.2831	4.6799
BS	0.0000	0.0000	-	0.0000	**12.9312**	**3.33E+14**	0.0000	2.0044
BV	7.1927	9.8278	0.0000	-	0.0000	**9.76E+14**	0.0000	**22.2418**
LA	0.0000	0.0000	6.5381	0.346796	-	**41.4520**	**35.4053**	0.0000
CC	0.348627	**52.6516**	0.0000	0.0000	**59.1471**	-	0.0000	0.0000
LO	0.0000	0.0000	1.3687	0.5481	**42.2765**	0.277934	-	0.0000
TAG	0.5191	0.0000	**13.3151**	0.173398	**46.2149**	**6.61E+13**	0.0000	-

C) *Owenia fusiformis* clade 2

	BH	GWB	PS	MB	CBs	CBn	RWB	IOM	BB
BH	-	**11.8192**	**45.9904**	**1.94E+16**	0.0000	**5.09E+15**	**2.58E+15**	0.0000	0.0000
GWB	0.0498	-	0.0000	**2.07E+16**	5.7313	**8.10E+15**	**1.29E+15**	0.0000	**14.1457**
PS	0.0498	0.0000	-	**7.59E+15**	**11.4279**	**3.04E+16**	**1.55E+16**	0.0000	1.5718
MB	0.4486	**28.8935**	**27.1753**	-	7.7718	**5.08E+15**	**1.29E+15**	0.4087	3.1186
CBs	0.3738	**21.0123**	0.0000	0.3068	-	0.8613	**1.35E+16**	1.4271	**20.4094**
CBn	0.1246	6.5659	**31.2473**	**8.43E+14**	6.5349	-	**2.12E+16**	0.0000	**26.7258**
RWB	0.0499	**13.1363**	**152.2071**	0.5549	2.4348	**2.54E+15**	-	0.7120	**15.6310**
IOM	0.0498	**45.9344**	**29.1225**	0.3484	4.5032	0.6829	**3.72E+16**	-	**39.2941**
BB	0.5720	6.5453	**10.4523**	**4.22E+14**	6.9807	**4.03E+15**	**1.29E+15**	0.0000	-

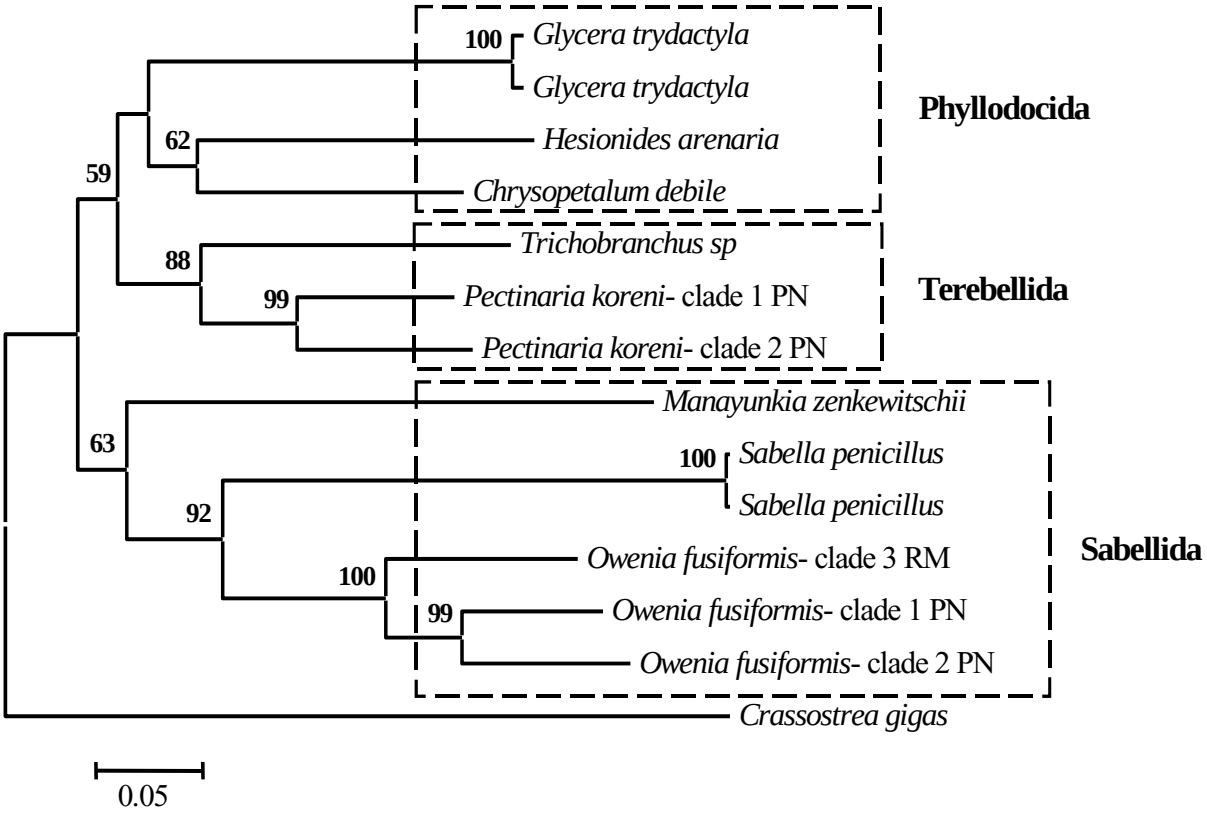


Figure 14. Arbre phylogénétique en Neighbour-Joining, construit à partir des séquences traduites du gène mtCOI. *Hesionides arenaria* (AF221569); *Chrysopetalum debile* (AF221567); *Trichobranchus sp.* (AF342674); *Manayunkia zenkewitschii* (AJ428405); *Crassostrea gigas* (NC001276) ; *Glycera trydactyla* (séquence non déposée) ; *Sabella penicillus* (séquence non déposée).

COMPLEMENT D'INFORMATION SUR LES 2 ARTICLES

Nos résultats montrent la présence d'espèces cryptiques au sein d'une espèce morphologique (*P. koreni* = 2 espèces cryptiques ; *O. fusiformis* = 3 espèces cryptiques), ainsi qu'une zone de chevauchement entre clades dans les baies de Morlaix et de Lannion. Pour compléter ce chapitre, nous nous sommes intéressé (1) au replacement de ces clades dans leur contexte phylogénétique, en utilisant certaines séquences du gène mtCOI déposés dans GenBank, puis (2) à la distribution particulière de ces clades dans les baies de Morlaix et de Lannion.

La position des clades de *P. koreni* et d'*O. fusiformis* dans l'arbre phylogénétique obtenu à partir des séquences traduites du gène mtCOI (Figure 14), correspond aux limites reconnues respectivement par Rouse & Pleijel (2001), pour les ordres des Terebellida et des Sabellida. Non seulement l'arbre montre clairement la présence de plusieurs espèces cryptiques au sein de nos modèles biologiques, mais également le positionnement ancestral du clade 3 d'*O. fusiformis* par rapport aux deux autres.

L'existence d'espèces cryptiques, ou de lignées évolutives distinctes, nous permet de souligner un point intéressant : celui de la différenciation génétique associée à une modification des caractéristiques reproductrices, propres à chaque lignée. Concernant cette hypothèse, il est à noter que dans le cas du polychète *Eupolymnia nebulosa*, la reconquête post-glaciaire de l'Atlantique à partir de zones refuges situées en Méditerranée, s'est accompagnée de la mise en place d'un nouveau mode reproductif, lequel n'a jamais pu être observé en Méditerranée (Lenaers & Bhaud, 1992). La modification du système de reproduction d'une espèce est un moyen très efficace de spéciation rapide qui pourrait expliquer certains patrons de différenciation chez nos espèces. Dans ce contexte, il est intéressant de noter que Dehorne (1911) a rapporté des caractères hermaphrodites chez

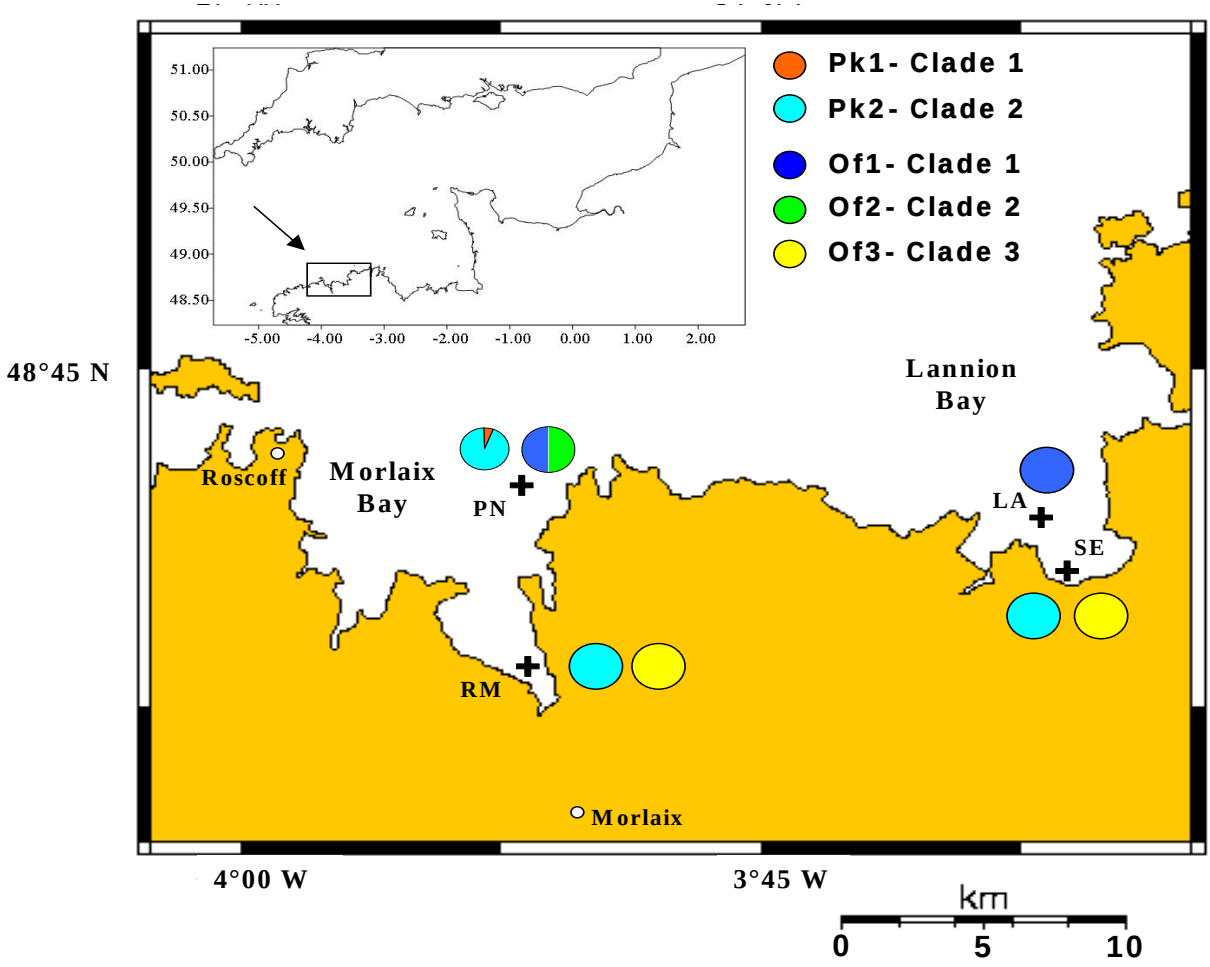


Figure 15. Distribution géographique des lignées évolutives (clades) de *Pectinaria koreni* (Pk) et d'*Owenia fusiformis* (Of) dans une zone de recouvrement : les baies de Morlaix et de Lannion.

Pectinaria koreni, alors même que l'espèce apparaît clairement gonochorique en Baie de Seine avec une sexe ratio équilibrée (Irlinger *et al*., 1991). L'observation de Dehorne (1911) n'a cependant pas été confirmée et il serait intéressant d'étudier plus précisément les populations et les caractères individuels de *P. koreni* dans l'Atlantique. En ce qui concerne *Owenia fusiformis*, espèce jusqu'à présent considérée comme cosmopolite, le fait que l'on retrouve deux lignées cantonnées l'une vers l'intertidal (clade 3 ; Figure 15), l'autre vers le subtidal (clade 1), peut également suggérer un changement des caractéristiques de développement ou de comportement larvaire ou reproducteur. De plus, le fait que le clade 3 d'*O. fusiformis* présente une divergence plus profonde que celle observée aux autres lignées pourrait suggérer une spécialisation écologique ancestrale.

La très nette frontière parapatrique observée chez *P. koreni* au niveau du gène mtCOI et le cline très progressif de fréquences constaté pour certains allèles des locus enzymatiques (*Pgi*, *Pgm*, *Mdh*), laissent penser à une action croissante de la sélection contre la migration de part et d'autre d'une barrière. En effet, la largeur d'un cline de fréquences alléliques diminue lorsque la migration diminue et que la sélection augmente. Ceci peut s'expliquer par un front d'hybridation ou par de la sélection diversifiante le long d'un gradient environnemental (i.e. la température). Cette constatation est renforcée par le fait que les individus échantillonnés à Morgat se regroupent avec le clade 1 (Manche) au niveau enzymatique, mais appartiennent au clade 2 (Atlantique) sur la base des séquences mitochondriales. L'explication la plus probable est qu'il y ait eu introgression de gènes nucléaires suite à une remise en contact des deux clades le long des côtes bretonnes. Ceci suggère qu'il y a toujours de la migration à travers la zone de transition et peut être de l'hybridation, mais que la sélection pourrait maintenir des allèles favorables de part et d'autre de la barrière ou contre-sélectionner les hybrides dans la zone de transition. Certes, la rareté de l'espèce à la pointe de Bretagne et le niveau de divergence entre les deux clades Atlantique et Manche, rendent sans doute les phénomènes

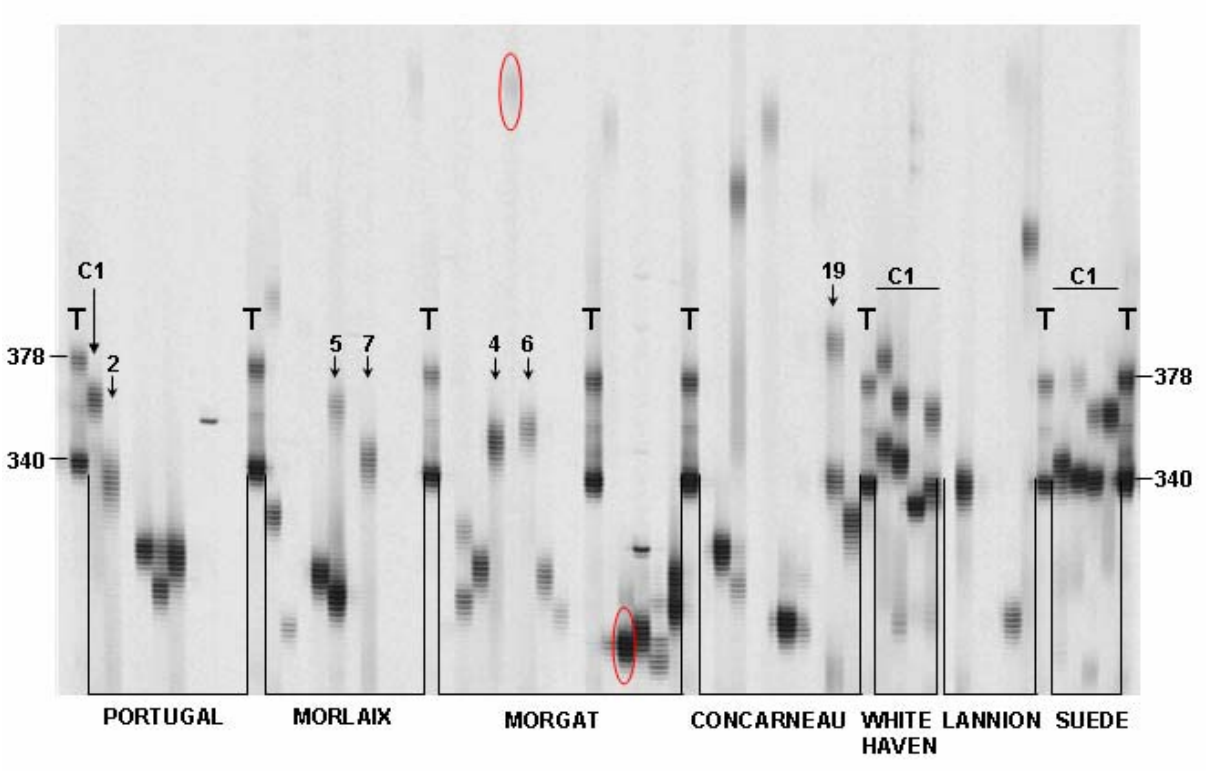


Figure 16. Amplification du locus microsatellite PKATGT1 chez des individus de *Pectinaria koreni* appartenant aux deux lignées évolutives: C1 indique les individus dont les haplotypes mitochondriaux appartiennent au clade 1; tous les autres individus ont une signature mitochondrial du clade 2. « T » est le témoin de taille (clade 1) ; les allèles entourés en rouge sont retrouvés en très faible fréquence (2 individus sur 200) dans la population de la Baie de Seine, alors qu'ils sont diagnostiques du clade 2.

d'hybridation peu fréquents, voire extrêmement rares. Cependant, l'échange de gènes entre lignées fait partie intégrante des processus évolutifs conduisant à la mise en place de barrières génétiques lorsque la spéciation n'est pas complète. Face à ces questions, il est intéréssant de noter que seul le locus microsatellite PKAT/GT1 (Weinmayr, 1999) a pu être amplifié avec succès sur les individus provenant des deux clades, pour les mêmes conditions d'amplification (Figure 16). Ce locus présente des tailles d'allèles très différentes entre clade 1 et clade 2, bien que quelques allèles soient retrouvés en situation d'hétérozygotie entre les deux clades. La présence d'individus hétérozygotes porteurs d'un allèle diagnostique du clade 1 et d'un allèle diagnostique du clade 2 peut s'expliquer soit par de l'hybridation orientée mâle clade1/ femelle clade 2 (cf. individu 1 et 5 de Pierre Noire dans la Baie de Morlaix), et/ou par de l'introgression de certains génotypes du clade 1 vers le clade 2. A titre d'exemple, les individus 7 (Pierre Noire), 4 et 6 (Morgat) sont homozygotes pour des allèles appartenant au clade 1 et l'individu 19 (Concarneau) est hétérozygote pour deux allèles appartenant au clade1, alors que ces quatre individus présentent une signature mitochondriale appartenant au clade 2. A l'inverse, aucun individu ayant une signature mitochondriale du clade 1 ne présente d'allèles PKATGT1 du clade 2. Cette présence atypique d'allèles clade 1 dans clade 2 peut également s'expliquer par une certaine rétention du polymorphisme ancestral au niveau du clade 2, car l'on retrouve certains des allèles appartenant au clade 2 (les allèles de plus petite ou de plus grande taille entourés en rouge) dans la population de la Baie de Seine, en faibles fréquences (voir le chapitre suivant utilisant le polymorphisme de taille du locus microsatellite PKAT/GT1 dans le clade 1 de *P. koreni*). Il n'y a pas assez de données pour valider l'hypothèse d'une zone d'hybridation en Mer d'Iroise, et il serait judicieux d'entreprendre des croisements expérimentaux entre mâles et femelles des deux clades, afin d'observer la transmission des allèles parentaux au marqueur microsatellite PKAT/GT1 par rapport à celle des haplotypes mitochondriaux.

CONCLUSION

La découverte d'un niveau de divergence intraspécifique identique chez *Pectinaria koreni* et *Owenia fusiformis* suggère que l'isolement géographique des populations est dû à un fort effet vicariant prédatant le dernier maximum glaciaire. Les lignées auraient divergé en allopatrie, pour ensuite se rencontrer au niveau de la zone de transition biogéographique Iroise-Manche. L'analyse phylogénétique et les signatures démographiques des lignées évolutives indiquent une histoire démographique différente entre lignées et espèces, ce qui pourrait être le reflet de multiple évènements de contraction/ expansion de l'aire géographique durant les derniers épisodes glaciaires du Pléistocène (Saale, il y a environ 128 000 ans ; Weichsel ou dernier maximum glaciaire, il y a environ 23 000 ans). Après la dernière période glaciaire, la re-colonisation de l'Atlantique Nord Est semble s'être produite d'une part, à partir de zones « refuges » situées en Méditerranée ou dans la région ibérique (*P. koreni* clade 2 ; *O. fusiformis* clade 1) et, d'autre part, à partir de refuges glaciaires situées beaucoup plus au nord (*P. koreni* clade 1 ; *O. fusiformis* clade 2). Bien que ces zones refuges n'aient pu être identifier avec précision, celles-ci auraient pu se situer au Sud Ouest de l'Angleterre et de l'Irlande, ainsi qu'au Nord Est de l'Ecosse. Dans le cas de *P. koreni* clade 1 on peut noter deux voies de colonisation à partir de la Mer d'Irlande vers la Manche, la première passe par les approches occidentales de la Manche selon un axe Ouest-Est, la seconde passe par les approches orientales de la Manche, depuis la Mer du Nord. Ces signaux phylogéographiques sont cependant moins évidents chez *O. fusiformis*.

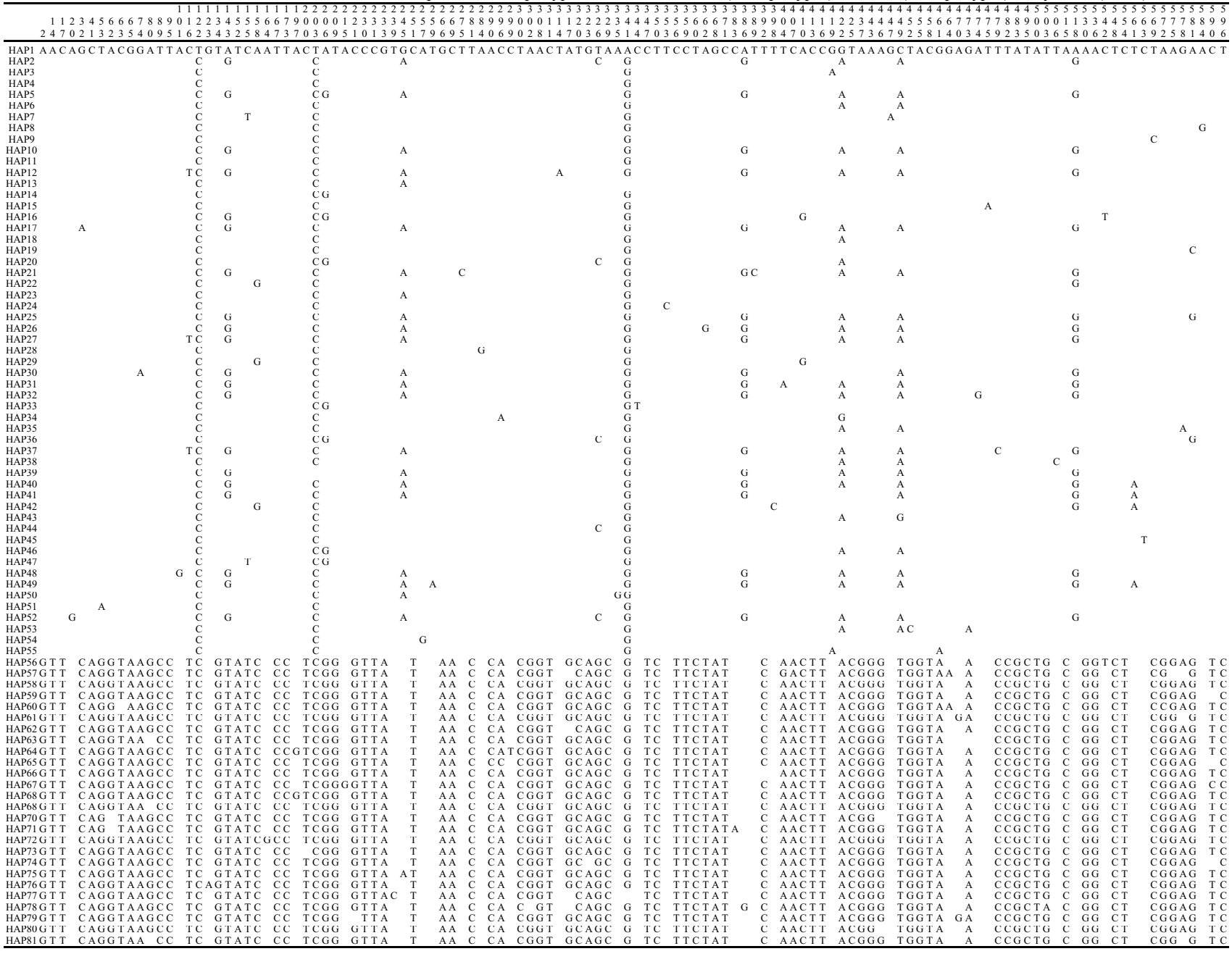
Outre de forts signaux phylogénétiques convergents, le fait que l'on ne retrouve pas exactement les mêmes empreintes génétiques et démographiques chez les deux espèces (cf. réseaux d'hapotypes, article 2) est probablement dû à des différences dans le cycle de vie et notamment la phase larvaire. Ainsi, la remise en contact des populations ayant évolué dans différents refuges pourrait avoir été beaucoup plus rapide chez *O. fusiformis* que chez *P.*

koreni, grâce aux capacités de dispersion larvaire de cette espèce. Une autre différence entre les deux taxons est le degré de chevauchement géographique des clades, avec chez *Owenia sp.* un plus fort chevauchement. Bien que ceci puisse aussi expliquer le fait qu'il y ait une plus grande zone de sympatrie chez les lignées d'*O. fusiformis* sur les côtes françaises de l'Atlantique, la probabilité de transports artificiels de larves entre le Sud et le Nord par les eaux de ballast est une hypothèse alternative à ne pas négliger. Bien que toutes nos espèces européennes (*P. koreni*, *P. belgica*, *P. auricoma* et *O. fusiformis*) soient présentes également en Méditerranée, le manque d'échantillons au sud de l'aire de distribution nous empêche d'approfondir cette question de la rencontre des clades. Pourtant, la phase de recolonisation de l'Atlantique du Nord Est aurait pu mettre en place une zone de chevauchement de ces lignées évolutives (ou clades) dans laquelle certaines lignées auraient pu être avantagées.

Pour mieux comprendre les patrons évolutifs observés, il est nécessaire de regarder les échanges de gènes à plus petite échelle. De telles études permettent en effet de mieux comprendre la dynamique et le degré de connectivité des populations actuelles. Pour cela, nous avons entrepris une étude ciblée sur les populations naturelles de *P. koreni* clade 1. Ce clade présente l'intérêt d'être centré sur le pourtours des îles Brittaniques et a fait l'objet d'un très gros effort d'échantillonnage autant à l'échelle de la Manche et de la Mer d'Irlande qu'à l'échelle de la Baie de Seine (les campagnes PECTGENE en 2000, PECTIRL en 2004 et PECTOW 1-2-3 en 2003).

ANNEXES
CHAPITRE I

Annexe I.1 : *Pectinaria koreni* . Position des sites variables pour les 81 haplotypes du mtCOI. Clade 1 (55 haplotypes); Clade 2 (26 haplotypes). Jolly *et al.* (2005).



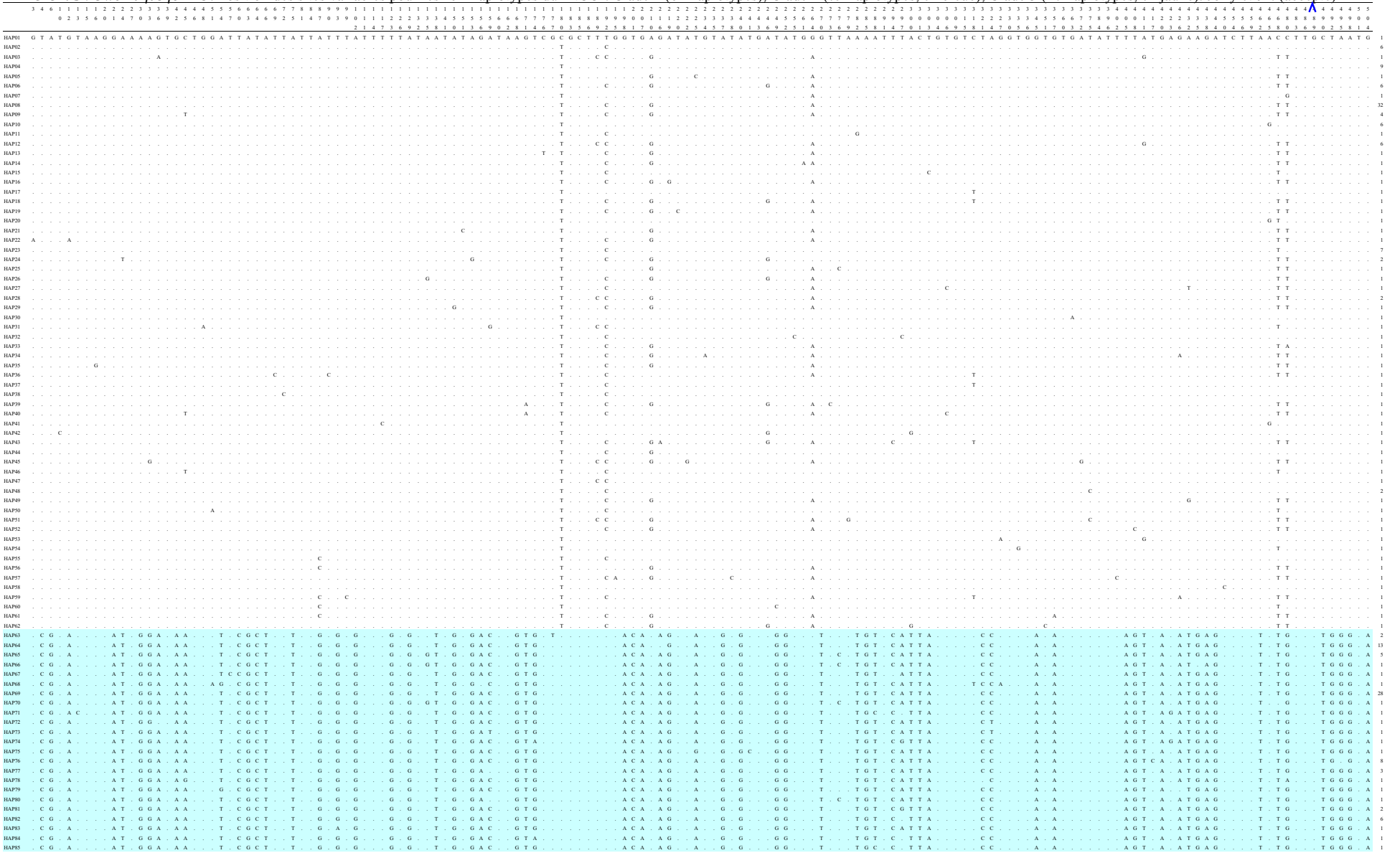
Annexe I. 2 : Distribution des haplotypes (colonnes) entre populations (lignes) of *P. koreni*. Voir Tableau 1 de l'article de Jolly *et al.* (2005) pour les codes des baies échantillonnées.

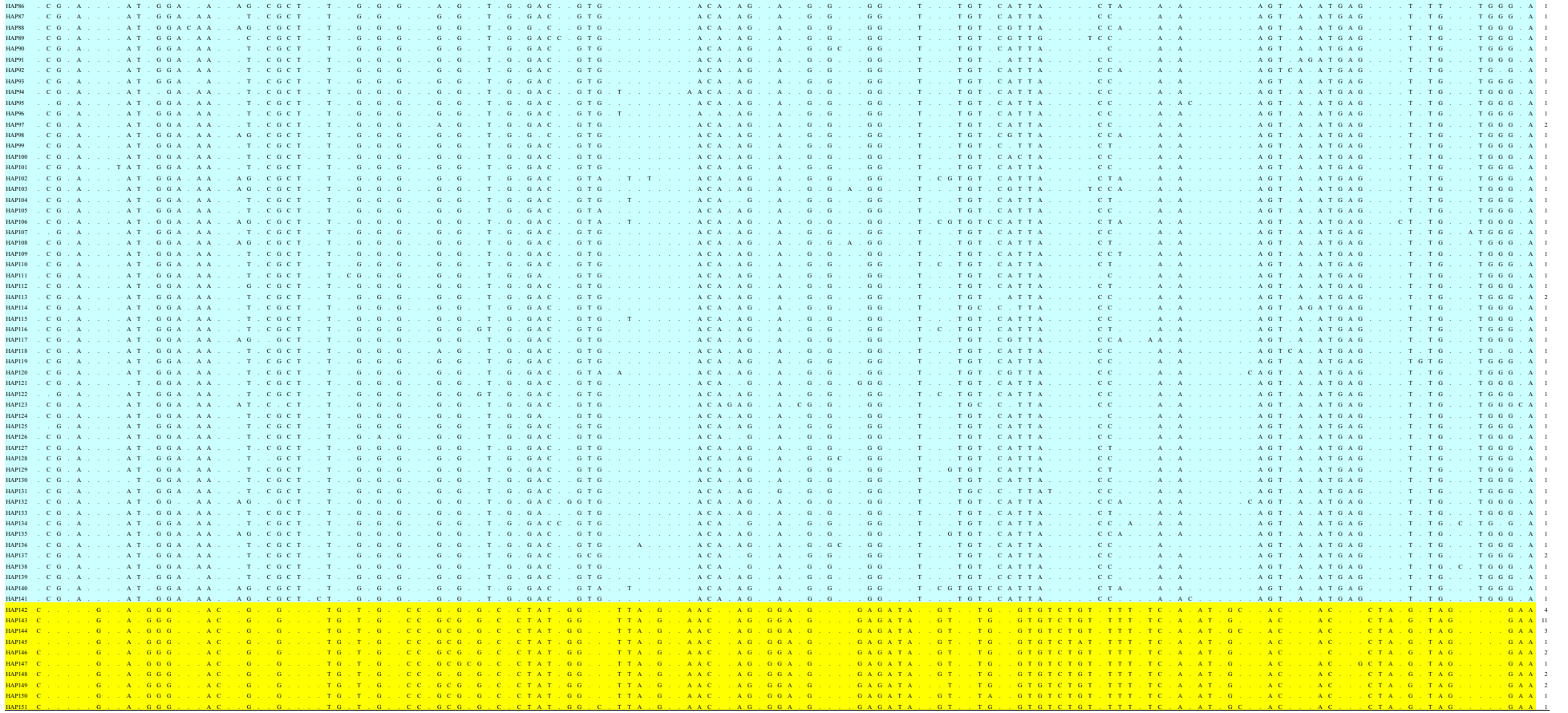
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
GWB	1	1	1	6	1	1	1	1	1																			
BH				3						3	1	1	1	1	2													
RB				7						3				1		1	1	1	1	1	1	1	2	1	1	1	1	1
GR				4						6				4				1									1	
PC				4		1				4																	1	
BS				6						5				4													1	
BV				7		1				5				1								2					1	
MSM				5		1				1																		
MRL				1																								
MO																												
CC																												
LO																												
Total	1	1	1	43	1	3	1	1	1	27	1	1	1	11	2	1	1	2	1	1	1	3	2	1	1	1	5	1

	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55
GWB																											
BH																											
RB																											
GR	1	1	1	1	1																						
PC						1	2																				
BS								1	1	1	1	2	1	1	1												
BV																1	1	2	1								
MSM																				1	1	1	1	1	1	1	1
MRL																											
MO																											
CC																											
LO																											
Total	1	1	1	1	1	1	2	1	1	1	1	2	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1

	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81
GWB																										
BH																										
RB																										
GR																										
PC																										
BS																										
BV																										
MSM																										
MRL	1	1	4																							
MO			7	8	1	1	1	1	1	1	1	1	1	1	1											
CC			3	1										1		1	1	1								
LO			6	2										2					1	1	1	1	1	1	1	1
Total	1	1	20	11	1	1	1	1	1	1	1	1	1	4	1	1	1	1	1	1	1	1	1	1	1	1

Annexe I. 3 : *Owenia fusiformis* . Position des sites variables pour les 151 haplotypes du mtCOI. Clade 1 (62 haplotypes); Clade 2 (79 haplotypes, en bleue); Clade 3 (10 haplotypes, en jaune). Jolly et *al* . (accepté)





Annexe I. 4 : *Pectinaria koreni* . Position des sites variables pour les 87 haplotypes du mtCOI pour le Clade 1. *Jolly et al.* (accepté).

	8 1 3 4 6 8 9 1 1 1 1 1 1 1 1 1 1 1 2 2 2 2 2 2 2 2 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 4 4 4 4 4 4 4 4 4 4 4 4 4 4 5 5 5 5 5 5 5 5 5 5 5 5 5 5	
	1 2 4 5 6 8 1 2 3 3 4 4 5 6 7 7 9 1 1 2 4 5 6 7 8 9 0 0 1 2 2 3 4 5 5 6 6 7 7 8 9 9 0 1 1 1 1 2 3 3 5 5 6 6 7 8 8 8 9 1 1 2 3 3 3 5 7 7 7 7 8 9 9 9	
	3 8 1 4 3 9 5 7 0 1 5 5 8 7 3 7 9 1 7 6 2 8 4 0 6 5 8 7 3 6 2 5 4 5 0 5 8 1 0 1 3 6 2 1 4 8 9 1 4 3 5 6 7 1 2 8 7 0 1 6 4 2 3 5 8 4 0 3 6	
HAP01	T A A G T G A A C A T T G A C A T G T A A C G A T T A A C A A T G T A A C T T A G G C A T T A T T A G G A G C T G G A T T A T A A G T C A T C C A G A A	1
HAP02	. . . . . C . . G . . . . C . . . A . . . . C . G . . . . . G . . . . . A . . A . . . . . G	1
HAP03	. . . . . C . . . . . C . . . . . G . . . . A	1
HAP04	. . . . . C . . . . . C . . . . . G	81
HAP05	. . . . . C . . G . . . . C G . . A . . . . G . . . . . G . . . . . A . . A . . . . . G	1
HAP06	. . . . . C . . . . . C . . . . . G . . . . A . . A	4
HAP07	C . . . . . C . . . . . C . . . . . G	2
HAP08	. . . . . C . . . T . . . C . . . . . G . . . . A	1
HAP09	. . . . . C . . . . . C . . . . . G . . . . . G	1
HAP10	. . . . . C . . G . . . . C . . . A . . . . G . . . . . G . . . . . A . . A . . . . . G	38
HAP11	. . . . . C . . . . . C . . . . . G . . . . G	1
HAP12	. . . . . T . C . . G . . . . C . . . A . . . . . . A . . . G . . . . G . . . . . A . . A . . . . . G	1
HAP13	. . . . . C . . . . . C . . . A	1
HAP14	. . . . . C . . . . . C G . . . . . G	15
HAP15	. . . . . C . . . . . C . . . . . G . . . . A	2
HAP16	. . . . . C . . G . . . . C G . . . . . G . . . . G . . . . T	1
HAP17	. . . A . . . . . C . . G . . . . C . . . A . . . . G . . . . . G . . . . . A . . A . . . . . G	1
HAP18	. . . . . C . . . . . C . . . . . G . . . . A	2
HAP19	. . . . . C . . . . . C . . . . . G C	1
HAP20	. . . . . C . . . . . C G . . . . C . G . . . . A	1
HAP21	. . . . . C . . G . . . . C . . . A . . C . . . . G . . . . . G C . . . . . A . . A . . . . . G	1
HAP22	. . . . . C . . . . G . . C . . . . G . . . . G	3
HAP23	. . . . . C . . . . . C . . . A . . . . G	3
HAP24	. . . . . C . . . . . C . . . . G . . C	1
HAP25	. . . . . C . . G . . . . C . . . A . . . . G . . . . . G . . . . . A . . A . . . . . G . . . . G	1
HAP26	A . . . . . C . . G . . . . C . . . A . . . . G . . . G . . . G . . . . . A . . A . . . . . G	1
HAP27	. . . . . T . C . . G . . . . C . . . A . . . . G . . . . . G . . . . . A . . A . . . . . G	5
HAP28	. . . . . C . . . . . C . . . . G . . . . G	2
HAP29	. . . . . C . . . . G . . C . . . . G . . . . . G	1
HAP30	. . . . . A . . . . C . . G . . . . C . . . A . . . . G . . . . . G . . . . . A . . . . . G	1
HAP31	. . . . . C . . G . . . . C . . . A . . . . G . . . . . G . . . . A . . A . . A . . . . . G	1
HAP32	. . . . . C . . G . . . . C . . . A . . . . G . . . . . G . . . . . A . . A . . . G . . . . . G	1
HAP33	. . . . . C . . . . . C G . . . . G T	1
HAP34	. . . . . C . . . . . C . . . . A . . . . . G	1
HAP35	. . . . . C . . . . . C . . . . . G . . . . A . .	3
HAP36	. G . . . . . C . . . . . C . . . . . G	1
HAP37	. . . . . C . . . . . C G . . . . C . G . . . . G	1
HAP38	. . . . . T . C . . G . . . . C . . . A . . . . G . . . . . G . . . . . A . . A . . . . . C . . G	1
HAP39	. . . . . C . . . . . C . . . . . G . . . . A . . A . . . . . C	1
HAP40	. . . . . C . . G . . . . . A . . . . . G . . . . . G . . . . . A . . A . . . . . G	1
HAP41	. . . . . C . . G . . . . C . . . A . . . . . G . . . . . G . . . . . A . . A . . . . . G . . . . A	2
HAP42	. . . . . C . . G . . . . C . . . A . . . . . G . . . . . G . . . . A . . . . . G . . . . A	1
HAP43	. . . . . C . . . . G . . C . . . . G . . . . . C . . . . . G . . . . A	1
HAP44	. . . . . C . . . . . C . . . . . G . . . . A . . G	1
HAP45	. . . . . C . . . . . C . . . . . C . . . . . G	5
HAP46	. . . . . C . . . . . C . . . . . G . . . . T	2
HAP47	. . . . . C . . . . . C G . . . . . G . . . . A . . A	2
HAP48	. . . . . C . . . T . . . C G . . . . . G	1
HAP49	. . . . . G . . C . . G . . . . C . . . A . . . . . G . . . . . G . . . . . A . . A . . . . . G	1
HAP50	. . . . . C . . G . . . . C . . . A . A . . . . . G . . . . . G . . . . . A . . A . . . . . G . . . . A	1
HAP51	. . . . . C . . . . . C . . . A . . . . . . G G	1
HAP52	. . . . A . . . . . C . . . . . C . . . . . G	1
HAP53	. G . . . . . C . . G . . . . C . . . A . . . . . C . G . . . . . G . . . . . A . . A . . . . . G	1
HAP54	. . . . . C . . . . . C . . . . . G . . . . . A . . A C . A	1
HAP55	. . . . . C . . . . . C . . . . G . . . . . G	1
HAP56	. . . . . C . . . . . C . . . . . G . . . . . A . . . . . A	1
HAP57	. . . . . C . . . . . C . . . . . G . . . . . G	2
HAP58	. . . . . C . . G . . . . C . . . A . . . . . G . . . . . G . . . . . A . . A . . . . . G T	1
HAP59	. . . . . C . . G . . . . C . . . A . . . . . G . . . . . G . . . . . A . . A . . A . . . . . G	3
HAP60	. . . . . C . . . . . C . . . A . . . . . G . . . . . A . . A . . . . . G	11
HAP61	. . . . . C . . . . . C . . . . . G . . . G	1
HAP62	. . . . . C . . . . . C . . . T . . . . . C . G . . . . . G . . .	1
HAP63	. . . . . C . . . . . C . . . . . G . . . G . . . . . A . . A	1
HAP64	. . . . . C . . . . . C . . . . . G . . . . A . . . . . A . . G	1
HAP65	. . . . . C . . . . . C	1
HAP66	. . . . . G C . . G . . . . C . . . A . . . . . G . . . . . G . . . . . A . . A . . . . . G	1
HAP67	. . . . . A . . G . . . . C . . . A . . . . . G . . . . . T G . . . . . A . . A . . . . . G	1
HAP68	. . . . . T G C . . . . . C G . . . . . G	1
HAP69	. . . . . C . . G . . . . C . . . A . . . . . G . . . . . G . . G . . . A . . A . . . . . C G	1
HAP70	. . . . . C . A G . . . T C . . . A . . . . . G . . . . . G . . . . . A . . A . . . . . G	1
HAP71	. . . . . C . . . C . . . . . C G . . . . . G	1
HAP72	. . . A . . . . . C . . . . . C . . . . . G	1
HAP73	. . . . . C . . . . . C . G . . . . . G	1
HAP74	. . . . . C C . G . . . . C . . . A . . . . . G . . . . . G . . . . . A . . G . . . . . G	1
HAP75	. . . . . C . . . . . C . . . . . G . . . . . C	1
HAP76	. . . . . C . . . . . C . . . . . G . . . . . A	1
HAP77	. . . . . C . . . . . C . . . . . G . . . . . A	1
HAP78	. . . . . C . . . . . C C . . . . . G	1
HAP79	. . . . . C . . . . . C . . . A . . . . . G . C . . . . . G	1
HAP80	. . . . . C . . G . . . . C . . . A . . . . . A . . G . . . . . G . . . . . A . . A . . . . . G	1
HAP81	. . . . . C . . G . . . . C . . . A . . . . . G . . . . . G . . . . . A . . A . . . . . G . C	1
HAP82	. . . . . C . . . . . C . . . . G . . . . . G	1
HAP83	. . . . . C . . . . . C . . A . . . . . G . . . . . G	1
HAP84	. . . . . C . . . . . C . . . . C . . . G	1
HAP85	. . . . . C . . . . . C . . . . . G . . . . . T	1
HAP86	. . . . . C . . . . . C . . . . A . . G . . . . . T	2
HAP87	. . . . . C . . . . . C . . . . A . . G	1

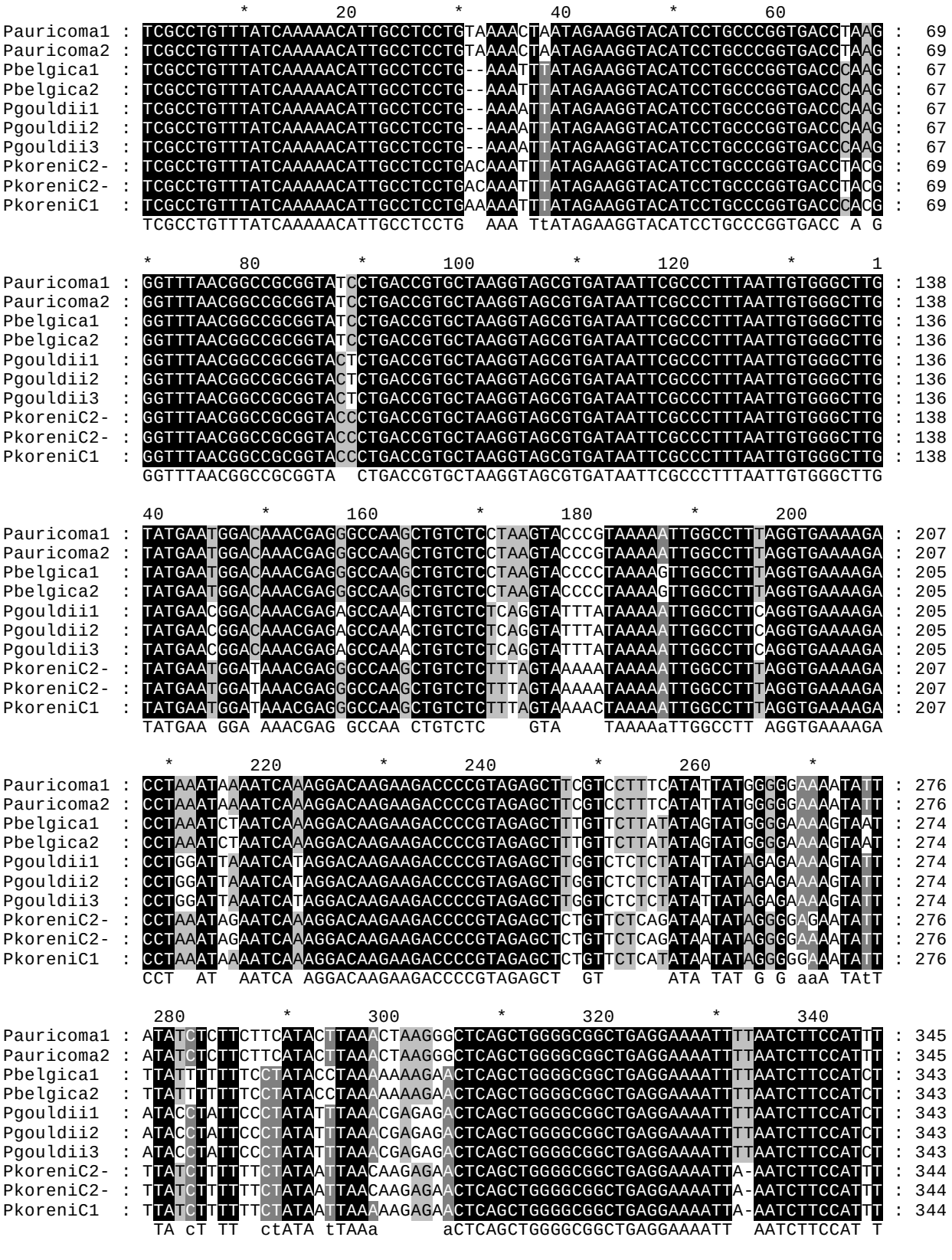
Annexe I. 5 : *Pectinaria koreni* . Position des sites variables pour les 41 haplotypes du mtCOI pour le Clade 2. Jolly *et al.* (accepté).

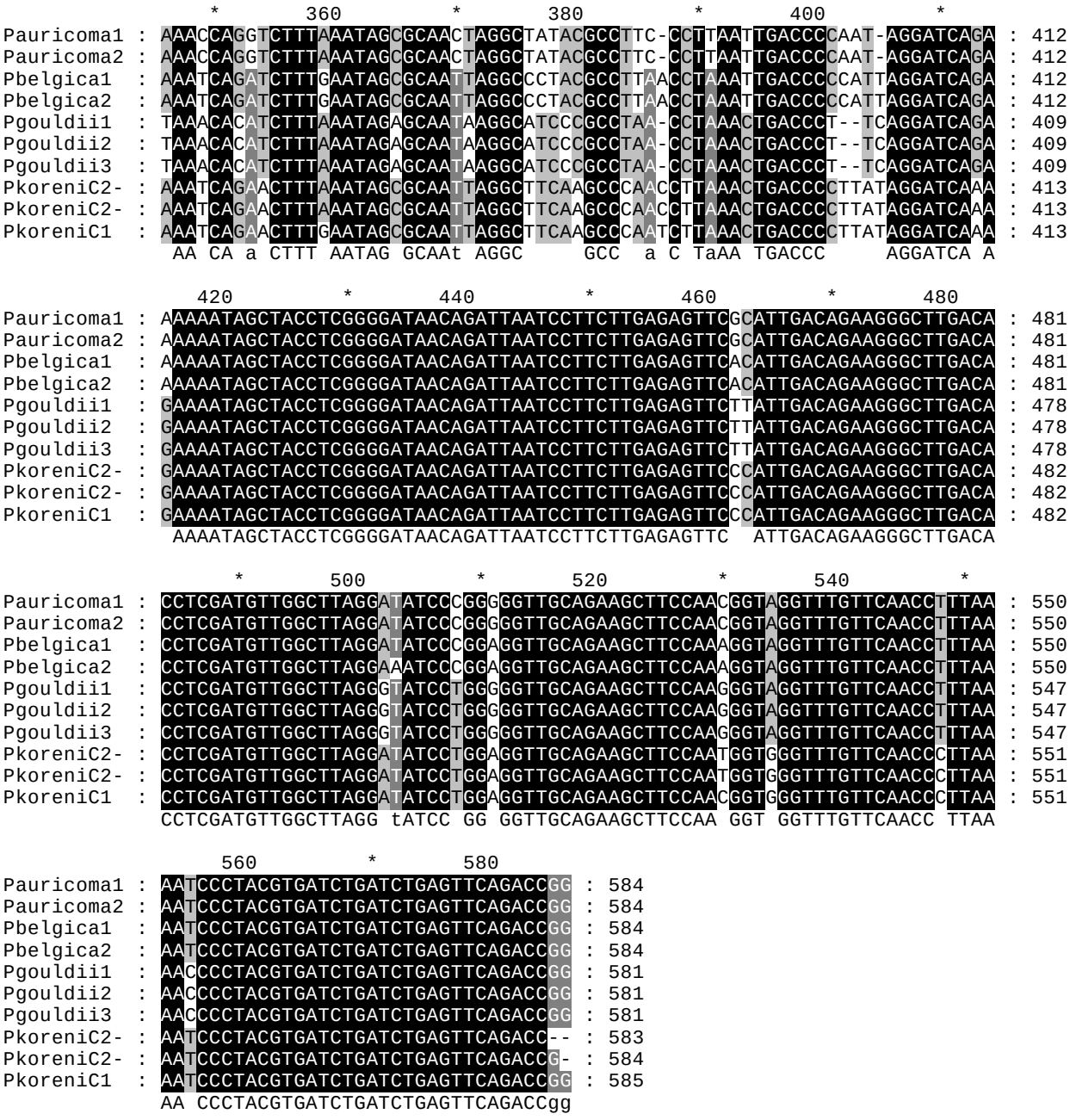
	8	4	4	6	6	7	8	8	1	1	1	1	1	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3	3	4	4	4	4	4	4	4	4	5	5	5	5	5	5	5	
		1	7	2	3	1	0	9	2	4	6	7	9	0	0	0	1	2	3	4	9	9	0	1	2	4	5	7	8	8	9	0	2	4	5	6	6	7	7	0	4	7	7	7	8	9	
									3	9	4	9	7	0	3	6	5	1	9	5	6	9	2	7	3	1	3	4	3	6	2	4	2	6	2	1	4	0	3	3	2	2	5	8	4	0	
HAP01	G	A	T	G	T	T	G	C	G	A	A	C	A	T	C	G	A	G	T	G	A	C	G	G	A	G	T	A	C	A	C	A	A	G	G	A	G	A	A	G	T	G	G	A	A	T	6
HAP02	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	C	.	.	.	G	C	1
HAP03	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	31
HAP04	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	1
HAP05	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	C	.	.	.	.	.	1
HAP06	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	C	12
HAP07	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	1
HAP08	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	C	.	.	.	.	.	1
HAP09	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	G	.	.	.	A	.	.	.	C	.	A	G	.	.	1
HAP10	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	A	.	.	.	1
HAP11	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	C	.	.	.	.	.	1
HAP12	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	C	C	.	.	.	.	1
HAP13	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	C	.	.	G	.	.	1
HAP14	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	1
HAP15	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	C	.	.	.	.	.	1
HAP16	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	1
HAP17	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	C	1
HAP18	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	C	.	.	.	.	.	1
HAP19	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	C	1
HAP20	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	1
HAP21	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	4
HAP22	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	C	.	.	.	.	.	1
HAP23	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	1
HAP24	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	1
HAP25	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	1
HAP26	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	C	1
HAP27	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	1
HAP28	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	1
HAP29	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	A	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	1
HAP30	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	A	.	.	.	.	G	.	.	.	.	.	.	.	.	.	A	C	.	.	.	.	.	1
HAP31	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	C	.	.	.	.	.	1
HAP32	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	C	.	.	.	.	.	1
HAP33	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	G	.	.	1
HAP34	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	1
HAP35	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	C	.	.	.	.	.	1
HAP36	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	1
HAP37	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	G	.	.	.	.	.	C	.	.	.	.	C	1
HAP38	A	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	1
HAP39	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	1
HAP40	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	1
HAP41	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	C	1

Annexe I. 6 : *Pectinaria auricoma* . Position des sites variables pour les 17 haplotypes du mtCOI. Jolly *et al.* (accepté).

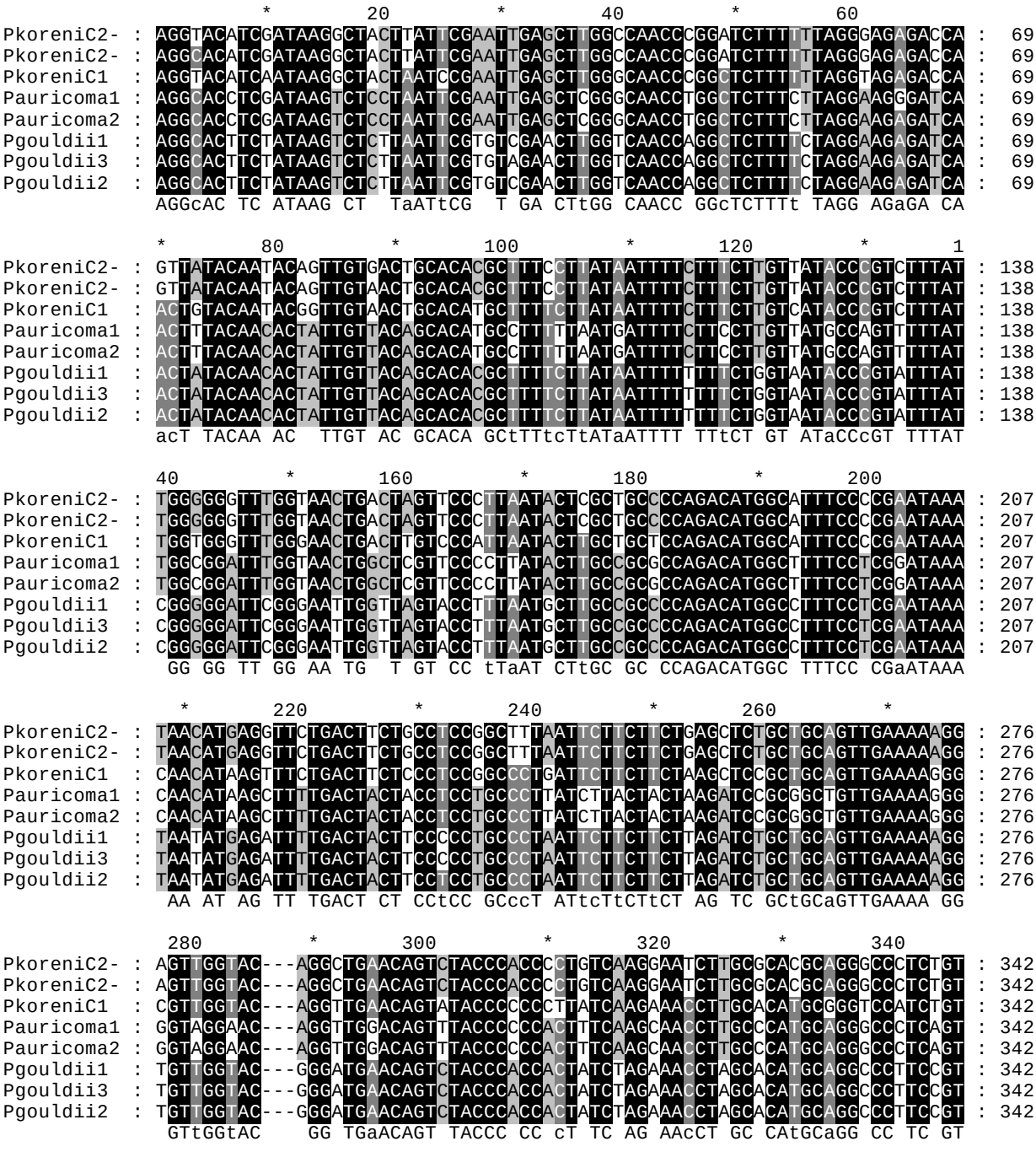
	4	5	6	1	1	1	1	1	2	3	4	4	4	4	4	4	4	5	5	5	5	5	5	
	9	8	1	0	1	6	8	9	7	6	1	1	1	1	3	3	7	0	3	4	4	6	7	
				8	8	6	7	3	7	1	2	5	8	9	3	7	5	2	6	1	7	2	4	
HAP01	G	T	C	A	T	G	G	C	G	G	A	A	A	A	G	C	T	C	C	C	C	C	G	1
HAP02	A	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	T	.	.	3
HAP03	A	.	.	T	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	6
HAP04	A	.	.	.	C	.	A	.	.	.	.	.	.	C	.	.	C	.	.	.	T	.	.	1
HAP05	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	1
HAP06	A	.	.	T	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	T	.	.	2
HAP07	A	.	.	T	.	.	.	.	.	.	.	.	.	.	.	G	.	.	A	.	T	.	.	1
HAP08	A	.	.	T	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	T	.	1
HAP09	A	.	.	T	.	.	.	.	.	A	.	.	.	.	A	.	.	T	.	.	T	.	.	1
HAP10	A	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	2
HAP11	A	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	T	T	.	.	1
HAP12	A	.	T	T	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	T	.	.	1
HAP13	A	.	.	T	.	A	.	.	.	.	.	G	.	.	.	.	.	.	.	.	T	.	A	1
HAP14	A	.	.	T	.	A	.	.	.	.	.	.	G	.	.	.	.	.	.	.	T	.	.	1
HAP15	A	C	.	T	.	A	.	.	.	.	.	.	G	.	.	G	.	.	.	.	T	.	.	1
HAP16	A	.	.	T	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	T	.	.	1
HAP17	.	.	.	T	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	1

Annexe I. 7 : *Pectinaria sp*. Alignement des séquences du gène 16SRNA pour 4 espèces dont les deux clades *P. koreni* C1 et C2 (Jolly *et al*., accepté).





Annexe I. 8 : *Pectinaria sp*. Alignement des séquences non traduites du gène mtCOI pour 3 espèces dont les deux clades *P. koreni* C1 et C2 (Jolly *et al*., accepté).



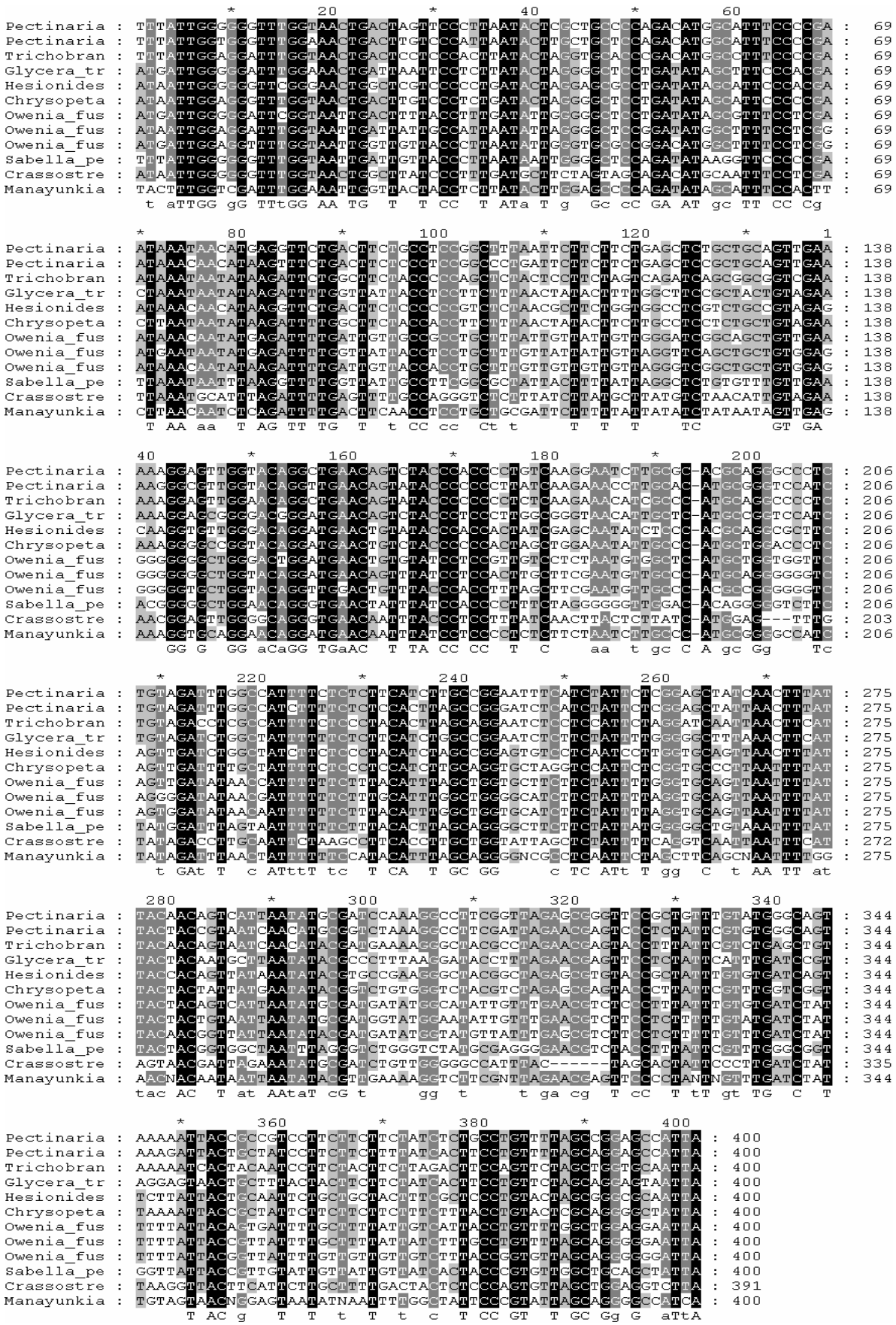
```
                 *       360         *       380         *       400         *
PkoreniC2-  : AGATTTGGCCATTTTCTCTCTTCATCTTGCCGGAATTTCATCTATTCTCGGAGCTATCAACTTTATTAC : 411
PkoreniC2-  : AGATTTGGCCATTTTCTCTCTTCATCTTGCCGGAATTTCATCTATTCTCGGAGCTATCAACTTTATTAC : 411
PkoreniC1   : AGATTTGGCCATCTTTTCTCTCCACTTAGCCGGGATCTCATCTATTCTCGGGGCTATTAACTTTATTAC : 411
Pauricoma1  : AGACCTTGCAATCTTCTCACTTCACTTGGCCGGGATTTCTTCTATTTTAGGGGCTATTAATTTTATTAC : 411
Pauricoma2  : AGACCTTGCAATCTTCTCACTTCACTTGGCCGGGATTTCTTCTATTTTAGGGGCTATTAATTTTATTAC : 411
Pgouldii1   : GGATCTTGCTATTTTTCTCTTCATTTAGCTGGAATCTCATCTATTTTAGGGGCAATCAATTTTATTAC : 411
Pgouldii3   : GGATCTTGCTATTTTTCTCTTCATTTAGCTGGAATCTCATCTATTTTAGGGGCAATCAATTTTATTAC : 411
Pgouldii2   : GGATCTTGCTATTTTTCTCTTCATTTAGCTGGAATCTCATCTATTTTAGGGGCAATCAATTTTATTAC : 411
                GAt T GC AT TT TCtCTtCA tT GC GG AT TCaTCTATT T GGgGC AT AA TTTATTAC

                  420         *       440         *       460         *       480
PkoreniC2-  : AACAGTCATTAATATGCGATCCAAAGGCCTTCGGTTAGAGCGGGTTCCGCTGTTTGTATGGGCAGTAAA : 480
PkoreniC2-  : AACAGTCATTAATATGCGATCCAAAGGCCTTCGGTTAGAGCGGGTTCCGCTGTTTGTATGGGCAGTAAA : 480
PkoreniC1   : TACCGTAATCAACATGCGATCTAAAGGCCTTCGATTAGAACGAGTACCTCTATTCGTGTGGGCAGTAAA : 480
Pauricoma1  : AACAGTAATTAATATACGATCAAAAGGCCTACGCTTGGAGCGAGTCCCTTTATTTGTCTGGGCAGTAAA : 480
Pauricoma2  : AACAGTAATTAATATACGATCAAAAGGCCTACGCTTGGAGCGAGTCCCTTTATTTGTCTGGGCAGTAAA : 480
Pgouldii1   : TACAGTGATCAACATGCGATCAAAGGGACTACGACTAGAACGTGTTCCTCTATTTGTGTGAGCAGTAAA : 480
Pgouldii3   : TACAGTGATCAACATGCGATCAAAGGGACTTCGACTAGAACGTGTTCCTCTATTTGTGTGAGCAGTAAA : 480
Pgouldii2   : TACAGTGATCAACATGCGATCAAAGGGACTACGACTAGAACGTGTTCCTCTATTTGTGTGAGCAGTAAA : 480
                ACaGT AT AA ATgCGATC AA GG CT CG  TaGA CG GT CCtcTaTTtGT TG GCAGTAAA

                     *       500         *       520         *       540         *
PkoreniC2-  : AATTACCGCCGTCCTTCTTCTTCTATCTCTGCCTGTTTTAGCCGGAGCCAT-TACTATGCTCCTGACTG : 548
PkoreniC2-  : AATTACCGCCGTCCTTCTTCTTCTATCTCTGCCTGTTTTAGCCGGAGCCAT-TACTATGCTCCTGACTG : 548
PkoreniC1   : GATTACTGCTATCCTTCTTCTTTTATCACTTCCTGTTTTAGCAGGGGCCAT-TACTATACTCCTAACTG : 548
Pauricoma1  : AATTACAGCTATTCTTTTTACTTCTTTCCCTGCCAGTCCTTGCTGGGGCAAT-CACTATACTGTTAACAG : 548
Pauricoma2  : AATTACAGCTATTCTTTTTACTTCTTTCCCTGCCAGTTCTTGCTGGGGCAAT-CACTATACTGTTAACAG : 548
Pgouldii1   : AATCACAGCTATTTTTACTTTTTACTCTCTCTCCCCGTATTAGCTGGGGCAAT-CACAATACTTCTCACCG : 548
Pgouldii3   : AATCACAGCTATTTTTACTTTTTACTATCTCTCCCCGTATTAGCTGGGGCAAT-CACAATACTTCTCACCG : 548
Pgouldii2   : AATCACAGCTATTTTTACTTTTTACTATCTCTCCCCGTATTAGCTGGGGCAAT-CACAATACTTCTCACCG : 548
                aAT AC GCtaT  T cTt T cT TC CT CC GT tTaGC GGgGC AT  AC ATaCT cT AC G

                   560         *       580         *
PkoreniC2-  : ACCGTAACTTAAATACCTCATTCTTCGACCCGGCGGGAGGGGGAG : 593
PkoreniC2-  : ACCGTAACTTAAATACCTCATTCTTCGACCCGGCGGGAGGGGGAG : 593
PkoreniC1   : ACCGTAATTTAAACACCTCATTCTTCGATCCAGCAGGGGGAGGAG : 593
Pauricoma1  : ACCGAAACCTTAATACATCTTTCTTTGACCCTGCAGGCGGGGGGG : 593
Pauricoma2  : ACCGAAACCTTAATACATCTTTCTTTGACCCTGCAGGCGGGGGGG : 593
Pgouldii1   : ACCGTAATTTAAATACTTCTTTTTTTGACCCAGCAGGAGGGGGGG : 593
Pgouldii3   : ACCGTAATTTAAATACTTCTTTTTTTGACCCAGCAGGAGGGGGGG : 593
Pgouldii2   : ACCGTAATTTAAATACTTCTTTTTTTGACCCAGCGGGAGGGGGAG : 593
                ACCGtAA tTaAAtAC TC TT TT GAcCC GC GG GGgGG G
```

Annexe 1.9. Alignement des séquences non traduites du gène mtCOI pour 9 espèces dont les deux clades de *P. koreni* (C1 et C2) ainsi que les trois clades d'*O. fusiformis* (C1, C2 et C3).



CHAPITRE II

IDENTIFICATION DES PHENOMENES CONTEMPORAINS AGISSANT SUR LA STRUCTURE DES POPULATIONS DE *PECTINARIA KORENI* A MACRO ET MESO ECHELLES.

Introduction

L'intégration de plusieurs échelles spatio-temporelles et l'utilisation de nombreux marqueurs moléculaires (avec des taux de mutation différents) sont nécessaires si l'on veut comprendre la part relative de l'histoire évolutive et des phénomènes contemporains dans la mise en place de la structure actuelle des populations au sein d'une lignée évolutive et son maintien au cours du temps dans un milieu fragmenté et parfois instable (la métapopulation au sens de Botsford, 1994).

Si le polymorphisme enzymatique a été largement utilisé pour fournir des marqueurs co-dominants permettant l'identification des flux géniques dans les populations marines, sa neutralité face à certains effets environnementaux (notamment la température) a été de nombreuses fois contestée (Somero, 1995 ; Jollivet *et al*., 1995 ; Rand *et al*., 2002). L'hétérogénéité de l'habitat a aussi été reconnue comme ayant des effets diversifiants sur certains polymorphismes enzymatiques (Mitton & Grant, 1984 ; Hilbish & Koehn, 1985) ou balancés, en milieu homogène (Karl & Avise, 1992). De plus, son faible taux d'évolution fait que ce marqueur intègre la signature des flux géniques passés, et ne permet donc pas de quantifier finement les échanges actuels entre populations. L'utilisation de gènes mitochondriaux quant à lui, ne permet d'avoir qu'une vision restreinte des flux de gènes car il n'intègre que la lignée maternelle. L'apport de marqueurs neutres co-dominants hautement polymorphes tels que les microsatellites et les marqueurs SNP (Single Nucleotide Polymorphisms) semble beaucoup plus intéressant pour l'étude de la dispersion larvaire,

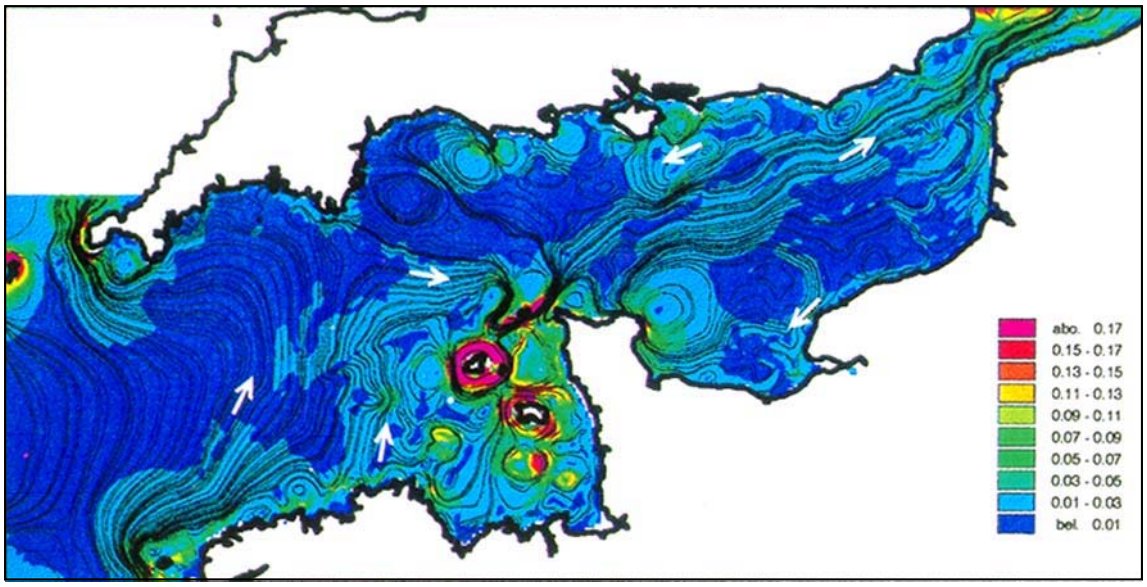


Figure 17. Trajectoires à long terme des masses d'eau en fonction de l'hydrodynamisme de marée (Salomon & Breton, 1993). Ce modèle a été validé par des données empiriques comme le suivi des radioéléments à partir de la Hague (Guegueniat *et al*., 1993).

surtout dans les zones géographiques ayant connu les effets des dernières glaciations. En effet, bien que l'impact du dernier maximum glaciaire (LGM) sur la faune de l'Atlantique Nord Est reste à quantifier, la colonisation des zones côtières autour des îles Britanniques par *Pectinaria koreni* est un phénomène récent qui, au vu de la littérature, s'est déroulé progressivement au cours des derniers 13 000 ans, et encore plus récemment en Manche, puisque ce chenal n'existe que depuis l'ouverture catastrophique du détroit du Pas-de-Calais, il y a -10 000 à -8 000 ans (Smith, 1989).

La Manche constitue un vaste plateau continental par lequel transitent les eaux de l'Océan Atlantique vers la Mer du Nord. C'est une mer mégatidale caractérisée par un hydrodynamisme intense (Figure 15, Salomon & Breton, 1993), avec des eaux entrant en Manche à partir de la dérive Nord Atlantique, des tourbillons cantonnés le long des baies, des fronts hydrodynamiques (Mer d'Iroise, Pointe du Cotentin) et un fort hydrodynamisme séparant les côtes françaises des côtes anglaises en Manche orientale. Les vitesses maximales des courants sont observées au niveau des rétrécissements locaux de la Manche (la presqu'île du Cotentin, le Pas de Calais). On constate une diminution progressive de la vitesse résiduelle du courant, du centre de la Manche vers les baies et les estuaires des côtes françaises et anglaises. Les populations de *P. koreni* en Manche forment un groupe de populations qui peut échanger des gènes par l'intermédiaire d'une phase larvaire pélagique de relativement longue durée. L'apport de la modélisation à l'étude du processus de transport larvaire par forçage hydrodynamique est essentiel mais ne permet pas de retracer les échanges efficaces entre populations. Son utilisation associée à la quantification des flux géniques passés et présents, permettrait peut être d'y voir plus clair quant au rôle des courants et du forçage météorologique sur le degré de connectivité des populations.

Dans ce chapitre, une première question est de savoir si le groupe « Manche » de *P. koreni* (clade 1) est actuellement fermé aux apports externes (provenant de la Mer du Nord et/ou de la Mer d'Irlande via la pointe de Cornouaille). Pour répondre à cette question, nous

avons analysé la structure génétique de *P. koreni* (clade 1) à l'aide de 4 locus microsatellites pour distinguer les flux passés et contemporains en confrontant les données génétiques aux flux larvaires simulés à l'échelle de la Manche à partir du modèle de Salomon & Breton (1993) et des caractéristiques biologiques et écologiques de *P. koreni*.

Lack of strong genetic structure in the polychaete tubeworm *Pectinaria koreni* (Malmgren) around the British Isles inferred from highly polymorphic microsatellite markers.

MT Jolly, C Ellien, F Gentil, Guyard P, F Viard, E Thiébaut and D Jollivet

En preparation

Abstract

In terms of conservation and sustainability of marine habitats, it is essential to distinguish between contemporary patterns of connectivity and the historic processes which have led to the present day distribution of marine populations. Coupling differently evolving genetic makers and mesoscale hydrodynamic modelling of larval dispersal clearly provides means of drawing and comparing connectivity at different time scales. With respect to the historic colonisation pathways around the British Isles previously inferred from mitochondrial DNA, we use four highly polymorphic microsatellite loci to trace more recent patterns of connectivity among natural populations of *Pectinaria koreni*, a non commercialised species characterised by a disjunct distribution and a bentho-pelagic life cycle. We test particularly the degree to which English Channel populations are connected to those from the Irish and North Seas, in relation to the feasibility of larval exchanges respectively through the western and eastern approaches of the English Channel. While the lack of a strong genetic structure between marine basins is seemingly related to a recent history of colonisation of the seas around the British Isles, connectivity was oriented from the Irish Sea towards the southern North Sea via intermediate populations of the eastern English coast. Genetic exchanges with the English Channel were greater at its north-eastern entrance, proceeding seemingly via the south-eastern English coast. The implications of similarities and discrepancies between genetic datasets are discussed in relation to the long term pattern of marine currents around the British Isles and to hydrodynamic simulations of larval transport in the English Channel.

Keywords : gene flow, hydrodynamic modelling, English Channel, Irish Sea

Introduction

In the marine environment, most species of sessile marine invertebrates reproducing externally present largely disjunct distributions, typically assemblages of geographically isolated populations linked together by a dispersive larval stage. For species with such a life cycle, the structure, dynamics and ultimately the stability and persistence of populations are strongly dependant upon the success of the larval stage in reaching distant populations or in replenishing the genitor population (Gaines & Bertness, 1993; Bertness *et al*., 1996; Botsford, 2001; Caley *et al*., 1996). Essentially, factors affecting larval dispersal and the benthic recruitment phase include advective hydrodynamic processes caused by tide and wind-induced currents, oceanic boundaries (fronts and eddies) and the long-term patterns of ocean currents, reproductive success and the duration of the larval phase, and finally the behavior and physiological plasticity of larvae (Sponaugle *et al*., 2002). Significant mortalities may occur at all stages of development from larvae to newly settled recruits and adults, thus, for dispersal to be effective in producing the next generation, larvae need to survive in the plankton, recruit onto suitable substrates, reach mature adulthood and reproduce. Although the regulation of larval dispersal by physical circulation is of paramount importance, still little is known because of the difficulty of accurately delineating breeding populations and measuring dispersal in the marine environment (but see Thorrold *et al*., 2002). Hydrodynamic modelling studies of larval transport may provide means of recognising patterns of larval movement and the dispersal potential of larvae (Tremblay et al., 1994; Cowen et al., 2000; Barnay et al., 2003; Ellien et al., 2004). However, quantifying the "effective" connectivity of marine populations requires additionnal indirect methods such as population genetics and population dynamics to validate such models. Recent studies have shown that coupling genetics and fine-scale physical oceanographic modelling may clearly improve our understanding of larval dispersal and its impact on population dynamics (Gilg & Hilbish, 2003).

The occurrence of a dispersive larval stage should insure a higher degree of connection and gene flow between distant populations (Hunt, 1993; Borsa *et al*., 1994; Palumbi, 1996) and thus a longer persistence of populations at geological time scales (Scheltema, 1978; Jablonski & Lutz, 1983). However, while well connected populations are always genetically homogeneous, the reverse is not always true. Such a genetic homogeneity may sometimes be linked to hidden historical effects of past colonisation and remnant ancestral polymorphism associated with selective processes which may blur the effects of presently restricted gene flow (Fauvelot & Planes, 2002) and could lead to erroneous predictions about the real connections among marine populations. While most genetic analyses have failed to reveal significant levels of population differentiation for species with broad larval dispersal in the absence of biogeographic barriers (Palumbi, 1994), a number of studies on bentho-pelagic species have reported genetic differentiation at relatively small spatial scales (Burton, 1983; Hedgecock, 1986; David *et al*., 1997; Todd *et al*., 1998; Jolly *et al*., 2003). This is mainly due to the fragmented nature of the habitat, particular oceanographic conditions and/or larval behaviour favouring local retention, selective mortality of migrants or the model of migration accross populations (i.e. island vs stepping-stone models). While the exchange of only one effective migrant per generation may be sufficient to counter balance genetic differentiation caused by drift or weak selection (Hartl & Clark, 1989), larval retention over many generations or hydrodynamic barriers may set up significant levels of phylogeographic structure (Taylor & Hellberg, 2003).

At the scale of the English Channel and the Irish Sea, strong tidal amplitudes and wind-induced currents are key factors controlling larval dispersal (Lagadeuc, 1992a; Thiébaut *et al*., 1994; Ellien *et al*., 2000, Ellien *et al*., 2004). In addition, the distribution of benthic populations is strongly dependant upon the distribution of sediments (i.e. coarse to muddy-fine sands), which in turn is a function of local hydrodynamism. Populations inhabiting

muddy-fine sediments are therefore characterised by a highly fragmented distribution, in baies and estuaries where local hydrodynamism is relatively weak.

Species such as the polychaete tubeworm *Pectinaria koreni* (Malmgren) constitutes an ideal biological model to study connectivity among disjunct and non-exploited marine populations. The species is widely distributed in the North East Atlantic Boreal region (Holthe, 1977) and both its bentho-pelagic life cycle and its considerable abundance in big aggregates have made it the subject of numerous studies, ranging from its local population dynamics in the eastern Irish Sea (Eagle, 1973, 1975) and English Channel (Elkaim & Irlinger, 1987; Irlinger *et al*., 1991; Lambert, 1991; Thiébaut *et al*., 1997), its patterns of potential larval dispersal in relation to hydrodynamic processes in the eastern English Channel (Lagadeuc 1992a, b; Ellien *et al*., 2000; Ellien *et al*., 2004). These studies have revealed that *P. koreni* is a semelparous species with a life span of 12-18 months with a 2 weeks pelagic larval stage and a seasonal reproductive period which takes place mostly in April-June with several spawning events (Irlinger *et al*., 1991, Lambert, 1991, Lagadeuc 1992b). Hydrodynamic 2-D modelling has highlighted the dominant role of wind-induced currents on the intra- and inter-annual patterns of larval dispersal, in relation to the variability in meteorological conditions over the species' larval life span (Ellien *et al*., 2000; Ellien *et al*., 2004). While it remains that larval exchanges take place between neighbouring populations along the French coasts of the English Channel, the influence of larval vertical behaviour on larval dispersal in some areas may pose a problem with the use of a 2-D model. Molecular genetic markers may help to avoid such a bias by capturing the signature of effective larval dispersal. While allozymes and mitochondrial DNA detected a deep phylogeographic break between two lineages of this species along the north coast of Brittany, one in the Atlantic (clade2), the other in the English Channel and Irish Sea (clade 1) (Jolly *et al*., 2005, Jolly *et al*., submitted). A lack of genetic structure was detected in the English Channel (Jolly *et al*., 2005) and a pattern of isolation by distance was observed with the Irish Sea.

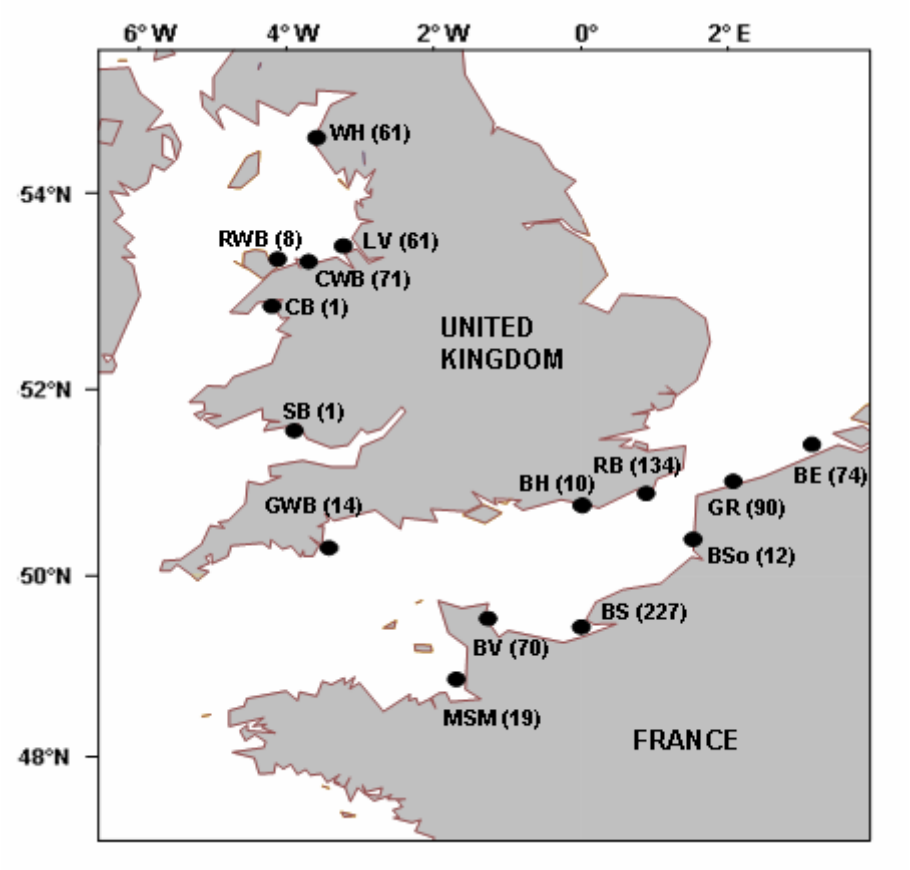


Figure 1. Study area and locations of sampled populations of *Pectinaria koreni*. BS: Baie de Seine; BV: Baie des Veys; RB: Rye Bay ; BH : Beachy Head ; BSo: Baie de Somme ; MSM : Baie du Mont Saint Michel ; GWB : Great Western Bay ; GR : Gravelines; BE: Belgium coast; LV: Liverpool Bay; WH: Whitehaven; CWB: Colwyn Bay; RWB: Red Wharf Bay; CB: Cardigan Bay; SB: Swansea Bay.

This lack of structure could be due to either recent colonisation of the English Channel (within the last 10 000 years) coupled with large population sizes and/or balancing selection on enzyme and mitochondrial loci. Thus, highly polymorphic neutral microsatellite loci are potentially more suitable in inferring contemporary gene flow. In the English Channel, such co-dominant markers may be also useful in correlating the degree of population genetic connectivity to patterns of potential larval dispersal predicted by hydrodynamic modelling.

We present allele frequency data at four highly polymorphic microsatellite loci (Weinmayr *et al*, 1999) for populations of a lineage of *Pectinaria koreni* (clade 1, see Jolly *et al*., 2005) inhabiting the English Channel, Irish Sea and North Sea (Jolly *et al.*, submitted) and further investigate meso-scale patterns of population connectivity. At the scale of the English Channel, we attempt to relate patterns of genetic differentiation and multilocus genotype classification in populations of *P. koreni* to larval trajectories predicted from the 2-D hydrodynamic modelling of its 15 days dispersive larval stage. Finally, we compare our results with those obtained from a previous study of mtDNA.

Material and methods

Sampling for genetic analyses

A total of 856 individuals of the polychaete *Pectinaria koreni* were sampled from 13 populations distributed by dredging bays and estuaries (Figure 1). Sampling was conducted (1) in March 2000 along both coasts of the English Channel (PECTGENE cruise; Baie de Seine = BS, Baie des Veys = BV, Baie de Somme = BSo, Gravelines = GR, Rye Bay = RB, Beachy Head = BH and Great Western Bay = GWB), (2) in July 2003 from the Belgium coast of the North Sea (BE) and (3) in June 2004 from the Irish Sea (PECTIRL cruise; Whitehaven = WH, Liverpool Bay = LV, Colwyn Bay = CWB, Red Wharf Bay = RWB, Cardigan Bay = CB and Swansea Bay = SB). The population of the Mont Saint Michel (MSM) were sampled intertidally in April 2001. Although most samples come from one single position at a given

locality, populations from BS consisted of three discrete sampling locations and those from BV and RB consisted of two discrete sampling locations. Despite considerable sampling effort, samples from CB and SB unfortunately consisted of one individual each and were specifically used in individual assignment testing, as were four individuals obtained from the Gullmarsfjiord (GUL) in Sweden.

Microsatellite genotyping

The individuals' genotypes were screened over four highly polymorphic microsatellite loci (PKGT1, PKAT/GT1, PKAT/GT2 and PKAT/GT4) isolated by Weinmayr *et al*. (1999).

DNA extraction was performed using a CTAB (Cetyl Trimethyl Ammonium Bromide) extraction procedure according to Jolly *et al.* (2003) while slightly different PCR conditions were applied for the amplification of each microsatellite loci. Amplifications were carried out using a PTC200^{TM} thermocycler (MJ Research): (1) an initial denaturation step at 94°C for 4min, (2) 38 cycles of denaturation at 94°C for 1 min, annealing for 40 s (PKAT/GT1-2-4 at 58°C and PKGT1 at 53°C) and elongation at 72°C for 50 s, (3) a final elongation at 72°C for 10 min. PCR reactions were performed into a 10 µl reaction volume consisting of 1xPCR buffer (supplied with polymerase enzyme); $MgCl_2$ at a concentration of 1.5mM (PKGT1) or 2.2mM (PKAT/GT1-2-4); 0.2mM dNTP; 0.4µM of forward and reverse primers; 0.01 $\mu g.\mu l^{-1}$ T4gene32 protein; 0.5 U of High Fidelity *Taq* polymerase (ABgene) (PKAT/GT1-2-4) or 0.5 U Thermoprime Plus *Taq* polymerase (ABgene) (PKGT1); 1µl of a 50ng CTAB-extracted genomic DNA. For each locus, one of the primers was labelled with IR^2-700 or IR^2-800 infra-red fluorescent dye for genotyping. The PCR products were run on a 6% polyacrylamide/ 7 M urea sequencing gel, using an automated DNA sequencer (Li-Cor, model 4200^{TM}). For each microsatellite loci, alleles were labelled according to their size.

Statistical analyses

Genetic diversity as summarised by the observed heterozygosity (H_o) and the expected heterozygosity (H_{NB}- Nei, 1987), the number of alleles per locus per population (N_O) and the allelic richness (R_S), were estimated using FSTAT 2.9 software (Goudet, 1995), which was also used in comparisons of genetic diversity between *a priori* defined groups. Standard deviations associated with gene diversity values were estimated using resampling techniques in GENETIX 4.03 (Belkhir *et al.*, 2002). Tests of genotypic disequilibrium between each pair of loci in each population were performed using Fisher's exact tests in GENEPOP 3.4 (Raymond & Rousset, 1995). Deviations from Hardy-Weinberg equilibrium were also examined for each population and each locus by calculating Wright's fixation index F_{IS} as estimated by Weir & Cockerham's (1984) θ and using the permutation test implemented with GENETIX 4.03 software. The genetic structure over all the samples was analysed using the same software, by calculating Wright's F_{ST} statistics (Wright, 1969); global and pairwise F_{ST} was estimated using Weir & Cockerham's (1984) θ. All tests of genetic differentiation were performed with GENETIX 4.03. The permutation approach implemented in this software was preferable to exact tests based on the identity in allelic distributions, because our highly polymorphic microsatellite loci produced contingency tables with a low number of observations in each genotypic class. Significance levels were corrected for multiple tests using the sequential Bonferroni procedure described by Rice (1989). Isolation by distance over the whole study area and within the English Channel only, was tested with 1000 permutations using the Mantel test (Mantel, 1967) implemented in GENEPOP 3.4 (Raymond & Rousset, 1995).

Individual assignment tests were performed with GENECLASS v2 (Piry *et al*., submitted) using Rannala & Mountain's (1997) criterion and Paetkau *et al* (2004)'s simulation algorithm with 10 000 simulated individuals. Self assignments were first carried out using all our potential reference populations (populations where N> 60), to estimate whether a sampled

individual is more likely to originate from its own sampling location or from another reference population. Localities where only a few samples were obtained were viewed as putative sinks (BH, BSo, GWB, MSM, RWB, SB, GUL) and individuals were assigned to reference populations according to their multiple genotypes, to identify potential source populations.

Hydrodynamic modelling of larval dispersal

The numerical model used is a 2-D hydrodynamic Lagrangian model of the English Channel described in detail by Salomon & Breton (1991, 1993). Briefly, the hydrodynamic model computes instantaneous and residual velocities, as well as the trajectories for different tide and wind conditions, by solving depth integrated hydrodynamic equations using an alternate-direction-implicit finite difference method. It takes into account the two major tidal components on the North-western European continental shelf, M2 and S2, whose combination gives a semi-diurnal tide, and assumes a uniform surface wind stress over the whole area. The coefficient of turbulent diffusion is calculated according to Elder's law. Lagrangian residual velocities and coefficients of turbulent diffusion obtained from the hydrodynamic model were used to simulate the transport and the mixing of planktonic larvae by solving an advection-diffusion equation.

This model was applied by Ellien *et al*. (2000), Ellien *et al.* (2004) and by Barnay *et al*. (2003) to simulate the larval dispersal of respectively *Pectinaria koreni* in the eastern English Channel, and *Owenia fusiformis* at the scale of the English Channel. While the model does not include the effects of pressure gradients due to changes in the density structure of the water and larval behaviour, Ellien et al. (2004) reported a broad agreement between predicted larval dispersal and field data for *Pectinaria koreni* in the Baie de Seine (BS), suggesting that this depth-averaged model is efficient in accurately predicting larval dispersal directions and distances.

From a compilation of historical records on the distribution of *Pectinaria koreni* in the English Channel (Holme, 1950, 1953; Probert, 1975; Thiébaut et al., 1997; Newell et al., 2001; Desroy et al., 2003; Dauvin et al., 2004; authors, pers. obs.), 15 discrete populations of *P. koreni* were identified in muddy fine sediments of the English Channel (see Figure 2, MSM = Mont Saint Michel, BV= Baie des Veys, BS = eastern Baie de Seine, DP = Dieppe, Bso = Baie de Somme, BC = Baie de Canche, GR = Gravelines, FO = Folkestone area RB = Rye Bay; PB = Pevensey Bay, BH = Beachy Head, PoB = Poole Bay, GWB = Great Western Bay, PS = Plymouth Sound, SaB = St. Austell Bay). The quantity of injected larvae per population were calculated from estimated adult densities assuming a sex ratio of 1:1 (Elkaïm & Irlinger, 1987) and a mean female fecundity of 65000 ovocytes (Ellien et al., 2004) (Table 1). The adult habitat was restricted to areas of muddy-fine sands, favourable for the development of settlers. The habitat is distributed according to the distribution of those sediments in bays and estuaries of the English Channel (Larsonneur et al., 1982). As *Pectinaria koreni* exhibits a patchy distribution (Thiébaut et al., 1997), spawning areas were defined as a subset of the adult habitat (i.e. areas of highest densities).

Larval dispersal was simulated over a period of 15 days as estimated from *in situ* field observations in the eastern Baie de Seine (Lagadeuc & Retière, 1993). All simulations were made considering an average tide with constant south-western and north-eastern wind speeds of 6 $m.s^{-1}$ (moderate winds) and 12 $m.s^{-1}$ (strong winds and gales). An average tide was considered since the lunar tidal cycle has no significant effect on the simulated larval dispersal (Ellien *et al*., 2004). The two wind directions chosen correspond to the winds prevailing in the English Channel during *P. koreni*'s reproductive period (i.e. May to September). Moderate and strong winds allowed us to separate the effect of average and extreme environmental conditions on larval dispersal. Larval mortality was set to zero since our main concern was the spatial scale of dispersal. At the end of each simulation, we calculated for each population (1) a retention rate (i.e. the ratio between the number of

Table 1. *Pectinaria koreni*. Characteristics of the different populations in the English Channel used for simulations of larval dispersal.

Population	Size of adult habitat (km^2)	Average adult density (ind. m^{-2})	Size of spawning area (km^2)	Larval release
Baie du Mont Saint Michel	65.4	0.22	13.8	$4.7.10^{11}$
Baie des Veys	165.3	18.4	34.4	$0.99.10^{14}$
Eastern Baie de Seine	544.1	27.1	34.4	$4.79.10^{14}$
Dieppe	172.2	3.75	34.4	$2.10.10^{13}$
Baie de Somme	155	0.73	34.4	$3.68.10^{12}$
Baie de Canche	124	1.80	34.4	$7.30.10^{12}$
Gravelines	223.8	3.40	34.4	$2.48.10^{13}$
St. Austell Bay	58.5	0.06	17.2	$1.14.10^{11}$
Plymouth Sound	471.8	0.18	55.1	$2.76.10^{12}$
Great western Bay	1811.5	0.27	68.9	$1.59.10^{13}$
Poole Bay	41.3	0.26	10.3	$3.49.10^{11}$
Beachy Head	79.2	1.68	20.6	$4.32.10^{12}$
Pevensey Bay	151.5	0.84	10.3	$4.14.10^{12}$
Rye Bay	241	4.98	24.1	$3.90.10^{13}$
Folkestone	193.3	1.30	17.2	$8.20.10^{12}$

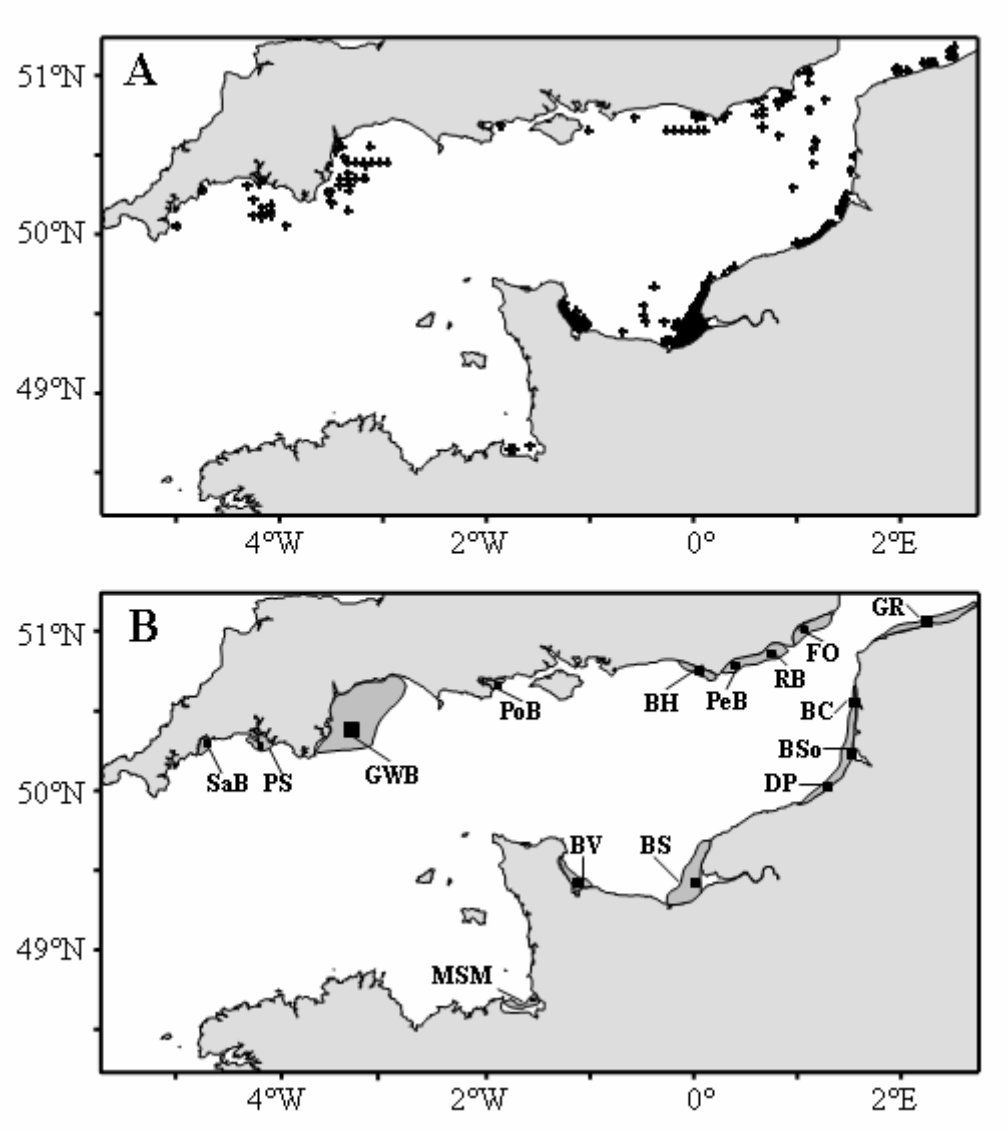


Figure 2. *Pectinaria koreni* distribution in the English Channel. (A) Compilation of historical records (Holme, 1950, 1953; Probert, 1975; Thiébaut et al., 1997; Newell et al., 2001; Desroy et al., 2003; Dauvin et al., 2004; Cabioch, pers. com.; Gentil & Thiébaut, pers. obs.). (B) Location of adult habitat (i.e. grey zone) and spawning area (i.e. square) for each population considered for larval dispersal modelling. MSM : Baie du Mont Saint Michel; BV: Baie des Veys; BS: eastern Baie de Seine; DP: Dieppe; BSo: Baie de Somme; BC: Baie de Canche; GR : Gravelines; FO: Folkestone area; RB: Rye Bay; PB: Pevensey Bay; BH : Beachy Head; PoB: Poole Bay; GWB : Great Western Bay; PS: Plymouth Sound; SaB: St. Austell Bay.

Table 2. *Pectinaria koreni*. Genetic diversity (H_{NB}) and observed heterozygosity (H_O) for each microsatellite loci. Number of alleles (N_{ALL}); Fixation index (F_{IS}) according to Weir & Cockerham (1984).

Locus	Length (bp)	N_{ALL}	H_O	H_{NB}	F_{IS}
PKGT1	191- 354	42	0.2932	0.9252	0.7116*** [0.0226]
PKAT/GT1	246- 438	59	0.5546	0.9605	0.4005*** [0.0226]
PKAT/GT2	230- 400	73	0.5121	0.9623	0.4719*** [0.0226]
PKAT/GT4	242- 394	34	0.7243	0.9408	0.2494*** [0.0226]

injected larvae and the number of larvae retained on the adult habitat of the local population), (2) a colonisation rate (i.e. the ratio between the number of injected larvae and the number of larvae transported to distant populations) and (3) a local supply rate (i.e. the ratio between the number of larvae retained on the adult habitat and the total number of larvae received by the local population). Retention and colonisation rates were fixed to zero when less than 1 ind. m^{-2} were found in any population at the end of the simulation process.

Results

Genetic diversity of *P. koreni* (clade 1)

Testing for genotypic disequilibrium did not reveal any significant association of alleles between any of our four diploid loci, which shows the independent information provided by our four loci. The 4 microsatellite loci exhibited a very high polymorphism (N_{ALL}), ranging from 34 for the locus PKAT/GT4 to 73 for the locus PKAT/GT2 (Table 2), and consequently, gene diversity (H_{NB}) was very high. All loci deviated significantly from Hardy-Weinberg proportions as revealed by the fixation index (F_{IS}) which reflected significant heterozygote deficiencies for all loci. However, these deficiency levels were nearly three-fold lower for the less polymorphic locus PKAT/GT4 (34 alleles) compared to PKGT1 (42 alleles), while intermediate levels were recorded for the two loci showing the highest number of alleles (PKAT/GT1, PKAT/GT2, see Table 2). Such deficiencies may result from the presence of genetically differentiated entities (Walhund effect), and our data also suggests that null alleles occur, maybe because of the high effective population sizes of *P. koreni*. Because of the high degree of polymorphism exhibited by the microsatellite loci, we estimated the minimum sample size required to cover most, if not all, of the genetic diversity found in populations of *P. koreni*. The number of alleles was plotted as a function of the number of individuals from the Baie de Seine population, our largest sample (N > 220). A minimum of 75 individuals are

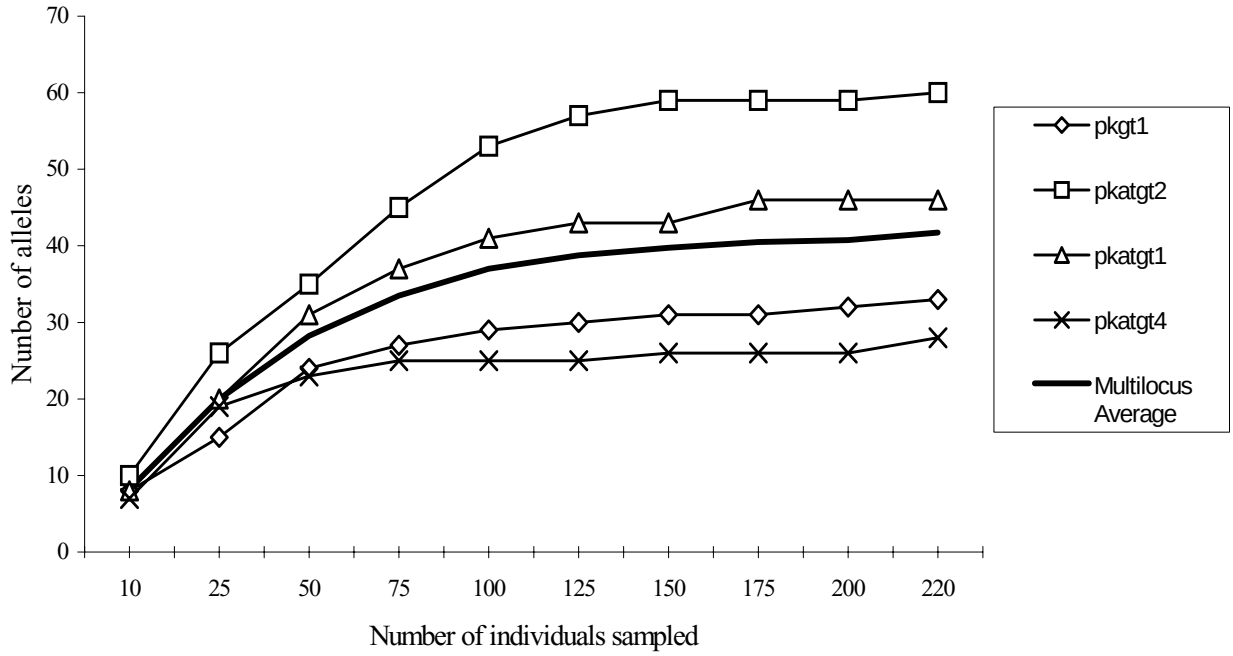


Figure 3. *Pectinaria koreni*. Evolution of genetic diversity with increasing sample sizes for microsatellite loci (PKGT1, PKAT/GT1, PKAT/GT2, PKAT/GT4).

needed to reach 85% of the total genetic diversity overall loci (Figure 3), whereas samples consisting of less than 20 individuals do not cover at most half of the total genetic diversity.

Gene diversity (H_{NB}) was high for all populations (Table 3), but samples consisting of less than 20 individuals displayed biased estimates of genetic diversity (see Figure 3) and were consequently removed from the dataset when comparing gene diversities and estimating F-statistics. Comparisons between geographic groups (i.e. the English Channel, the Irish Sea and the southern North Sea) revealed a significant difference in both gene diversity (P= 0.042) and allelic richness (P = 0.041) (Table 4). This was attributable to the Irish Sea group which showed a significant reduction in genetic diversity (H_{NB} = 0.956, R_S = 26.779; P_{HNB}= 0.003 and P_{RS} = 0.012) compared to the English Channel (H_{NB} = 0.962; R_S = 29.505). This difference was however not observed when populations of the English Channel were pooled together with those from the southern North Sea (P_{HNB} = 0.444; P_{RS} = 0.069).

Genetic structure at the scale of the English Channel and Irish Sea

To identify broad scale patterns of genetic structure we used populations presenting a large number of individuals (60 < N < 227, see Figure 3). While significantly lower levels of intra-population heterogeneity (F_{IS} = 0.387; P = 0.0076) were observed in the Irish Sea (Table 4), all populations deviated significantly from Hardy-Weinberg expectations (F_{IS} = 0.4574; P < 0.001). Globally, population differentiation was very shallow but highly significant (F_{ST} = 0.0042; P < 0.001) and no differences were observed neither between the 3 basins (P = 0.294) nor between the Irish Sea and the English Channel (P = 0.142). In addition, there was no significant correlation between pair-wise matrices of genetic and geographic distance at the scale of the study area (Mantel test, P= 0.099). With the exception of BV and GR, all other populations of *P. koreni* within the eastern English Channel were genetically similar (0.0001 < F_{ST} < 0.0003). Moreover, both French (GR) and English coasts (RB) were seemingly connected in the vicinity of the Dover Strait (Table 5). The population from the Belgium coast

Table 3. *Pectinaria koreni*. Genetic diversity and heterozygosities for each sampled population. N: number of individuals; N_O: average number of alleles; H_O= observed heterozygosity; H_{NB}= non biased expected heterozygosity; R_S= allelic richness based on a minimum sample size of 6 diploid individuals (* indicates bias by low sample size). Sample locations: Baie de Seine (BS), Baie des Veys (BV), Rye Bay (RB), Beachy Head (BH), Baie de Somme (Bso), Mont Saint Michel (MSM), Great Western Bay (GWB), Gravelines (GR), Belgium (BE), Liverpool (LV), Whitehaven (WH), Colwyn Bay (CWB), Red Wharf Bay (RWB).

	English Channel							**North Sea**		**Irish Sea**			
	BS	BV	RB	BH	BSo	MSM	GWB	GR	BE	LV	WH	CWB	RWB
N	227	70	134	10	12	19	14	90	74	61	61	71	8
N_O	42	33	38	10	14	16	13	33	31	28	31	31	10
H_O	0.4699 [0.1826]	0.4772 [0.1838]	0.5059 [0.2116]	0.4111 [0.3748]	0.6736 [0.2361]	0.5392 [0.1396]	0.4350 [0.294]	0.5224 [0.2381]	0.5514 [0.2263]	0.5852 [0.2215]	0.5407 [0.1506]	0.6299 [0.1574]	0.6205 [0.2218]
H_{NB}	0.9604 [0.0165]	0.9604 [0.0143]	0.9597 [0.0124]	0.9143 [0.0298]	0.9378 [0.0271]	0.9334 [0.0243]	0.9331 [0.032]	0.9542 [0.0189]	0.9562 [0.0168]	0.9535 [0.0151]	0.9518 [0.0230]	0.9522 [0.0215]	0.9375 [0.0506]
R_S	9.80	9.79	9.75	7.64*	8.9*	8.74*	8.47*	9.55	9.58	9.53	9.49	9.351	8.72*

Table 4. *Pectinaria koreni*. Comparison of genetic diversity and genetic differentiation between groups of populations from (A) the English Channel, (B) the North Sea and (C) the Irish Sea; R_S= allelic richness based on a minimum sample size of 40 diploid individuals; H_{NB}= non biased expected heterozygosity, F_{IS}= within-population differentiation, F_{ST}= within-group genetic structuring. The P-values for group comparisons were obtained after 10 000 permutations.

	R_S	H_{NB}	F_{IS}	F_{ST}
A. English Channel (BS, BV, RB)	29.505	0.962	0.498	0.002
B. North Sea (GR, BE)	27.349	0.958	0.441	0.007
C. Irish Sea (WH, LV, CWB)	26.779	0.956	0.387	0.006
P-value (A/B/C)	0.041	0.042	0.095	0.294
P-value (A/C)	0.012	0.003	0.0076	0.142

Table 5. *Pectinaria koreni*. Above diagonal: significant pairwise F_{ST} values from Weir & Cockerham (1984). Below diagonal: P-value associated test of significance performed with GENETIX 4.02 (Belkhir *et al.*, 1996) with 1000 permutations. N= number of individuals genotyped. In bold are values of pairwise genetic differentiation for which statistical significance disappears after sequential Bonferroni correction.

	English Channel			North Sea		Irish Sea		
	BS	BV	RB	GR	BE	LV	WH	CWB
N	227	70	134	90	74	61	61	71
BS	-	0.0003	0.0021	0.0001	0.0032	0.0026	0.0032	0.0041**
BV	0.401	-	0.0037	0.0056***	0.0068***	0.0080***	0.0039	0.0076***
RB	**0.012**	**0.005**	-	0.0023	0.0038	0.0043	0.0069***	0.0060***
GR	0.455	0.000	**0.046**	-	0.0066***	0.0069**	0.0069**	0.0095***
BE	**0.006**	0.000	**0.007**	0.000	-	0.0038	0.0031	0.0004
LV	**0.031**	0.000	**0.006**	0.001	**0.042**	-	0.0053	0.0022
WH	**0.027**	**0.033**	0.000	0.001	0.063	**0.016**	-	0.0101***
CWB	0.001	0.000	0.000	0.000	0.377	0.117	0.000	-

*P<0.05, **P<0.01, ***P<0.001

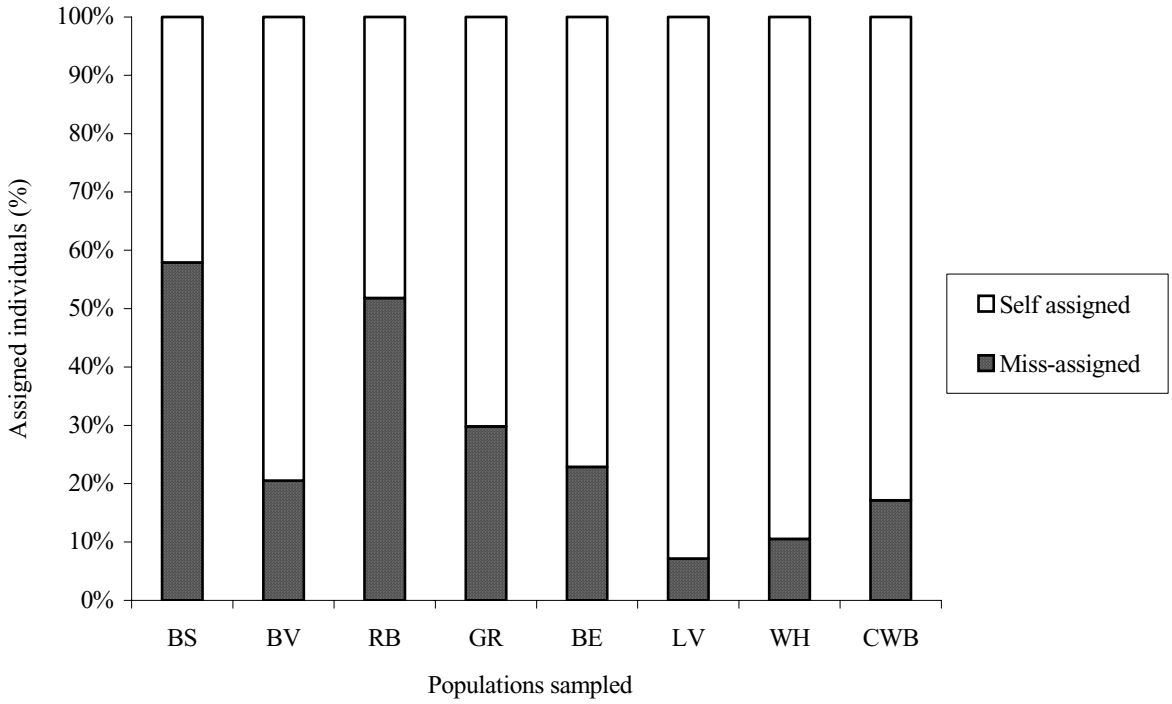


Figure 4. *Pectinaria koreni.* Proportions (%) of correctly assigned individuals by direct self assignments (no simulations), based on the Bayesian method of Rannala & Mountain (1997) implemented in Geneclass v.1 (Cornuet *et al*., 1999).

(BE) was genetically more similar to the Irish Sea populations and to RB and BS than to GR and BV. Conversely, Gravelines was differentiated from all Irish Sea and Belgium coast populations. The highest level of differentiation (F_{ST} = 0.0101, P < 0.001) was found within the Irish Sea, between WH and CWB situated further south, just below LV with which it is genetically similar. Note also that CWB was significantly differentiated from all populations of the English Channel.

Individual assignements testing

Direct self-assignment of 728 individuals from 8 populations (BS = 227; BV = 70; RB = 134; GR = 90; BE = 74; LV = 61; WH = 61; CWB = 71) were performed using the Bayesian method of Rannala and Mountain (1997) implemented in Geneclass v.1 (Cornuet *et al*., 1999). This method revealed a high proportion of self assignments, especially among populations of the Irish Sea (85 %, see Figure 4). Among the English Channel populations, self assignments were the highest in BV (80 %) while they were the lowest in BS (42 %). To test the likelihood of those assignments, we performed simulated self-assignments. Overall, only 20% of all individuals had a significant probability of being assigned to their sampling location (Table 6). In addition, approximately 63% and 45% of the individuals sampled in the English Channel and the Irish Sea were assigned respectively to their own basin, whereas only 14 % of the individuals from BE were self-assigned with confidence. These overall results are in good agrement with those obtained with pairwise analyses of genetic differentiation: (1) a greater proportion of individuals sampled in BE were assigned to populations of the Irish Sea (approx. 50 %), and (2) individuals sampled in the English Channel were more likely to have originated from populations within their own basin. Although there were more individuals assigned within or close to their geographic sampling location, no significant isolation-by-distance was found (Mantel test, P = 0.719). This is due to the lack of any sharp genetic structure between basins. Our results indicate that the

Table 6. *Pectinaria koreni*. Proportion of individuals sampled in a population showing more than 5% probability of being classified in each of the populations (number of individuals). Numbers in bold are the proportion of individuals classified into their sampling location after 10 000 simulations using Rannala & Mountain's (1997) criterion with Paetkau *et al*'s (2004) simulation algorithm. Numbers surrounded in black represent assignment values that are equal or superior to those values representing self-assigned individuals (in bold).

	Classified in								Not classified
	English Channel			North Sea		Irish Sea			
Sampled in	BS	BV	RB	GR	BE	LV	WH	CWB	
BS (227)	**0.1718**	0.1365	0.1013	0.1542	0.1013	0.1145	0.1365	0.0793	0.0044
BV (70)	0.1143	**0.1857**	0.2143	0.1286	0.0571	0.0857	0.1143	0.0857	0.0143
RB (134)	0.0746	0.1642	**0.2910**	0.1567	0.0896	0.0672	0.0896	0.0672	0
GR (90)	0.2222	0.1111	0.1222	**0.2111**	0.1111	0.0556	0.0889	0.0778	0
BE (74)	0.1216	0.0135	0.1351	0.0946	**0.1351**	0.1081	0.1351	0.2568	0
LV (61)	0.1311	0.0819	0.0656	0.0819	0.1475	**0.2295**	0.1475	0.0984	0.0164
WH (61)	0.1639	0.0819	0.0328	0.1148	0.1475	0.1639	**0.1967**	0.0656	0.0164
CWB (71)	0.0704	0.0845	0.1127	0.0845	0.2113	0.1549	0.0986	**0.1831**	0

Table 7. *Pectinaria koreni*. Proportion of individuals sampled in a putative "sink" population showing more than 5% probability of being classified in a particular reference population after 10 000 simulations using Rannala & Mountain's (1997) criterion and Paetkau *et al*'s (2004) algorithm. Numbers in bold represent the largest proportion of individuals classified into a population.

	Classified in								Not classified
	English Channel			North Sea		Irish Sea			
Sampled in	BS	BV	RB	GR	BE	LV	WH	CWB	
BH (10)	0	**0.3**	0	0	0.1	0	0.2	0.1	**0.3**
BSo (12)	**0.3333**	0	0	0	0.0833	0.0833	0.1667	0.1667	0.1667
MSM (19)	**0.2105**	0	0.0526	0.0526	0.1579	0.1053	**0.2105**	0.1579	0.0526
GWB (14)	0.0714	0.2143	0	0	0	**0.4286**	0.1428	0.0714	0.0714
RWB (8)	0	**0.25**	0	0	0.125	**0.25**	0.125	0	**0.25**
GUL (4)	0	0	0	0	0	**0.5**	0.25	0	0.25
SB (1)	0	0	0	0	0	0	0	**1**	0
CB (1)	0	0	0	0	0	0	0	0	**1**

southern North Sea populations are probably situated at a cross-road between dispersing larvae coming from populations situated along the English coast of the North Sea which may exchange more genes with those of the Irish Sea than with populations from the English Channel.

Table 7 shows the results of simulated individual assignments for putative sink populations where only a few individuals were sampled ($1 < N < 20$). Overall, 12.7 % of the individuals from putative sinks the English Channel were miss-assigned and, of those assigned succesfully, 29.3 % were assigned to the English Channel and only 11 % to the North Sea while 47 % were classified among reference populations of the Irish Sea. However, nearly all individuals classified within the English Channel were more likely to have originated in BV and BS. Further interesting observations can be made: (1) the genotypes of the individuals from the western English Channel (GWB), the Bristol Channel (SB) and Sweden (GUL) were more likely to be found among populations of Irish Sea than among those from the English Channel, (2) BH close to Rye Bay, is more likely to receive migrants from BV, and (3) individuals from the BSo and from MSM are more likely to have originated from BS.

Hydrodynamic modelling of larval transport in the English Channel

For each population, larval dispersal patterns, and consequently larval retention and colonisation rates were strongly influenced by meteorological conditions. Under moderate wind (i.e. 6 m. s^{-1}), larval retention rate was highly variable among populations, from 0 % at GR to about 75 % at GWB for a NE wind, and from 0 % at PB to 88.37 % at GWB for a SW wind (Table 8). Colonisation rates did not generally exceed 1 % of the larval release and wer lower than retention rates except for 7 populations: PB and RB whatever the wind direction, BSo, GR and FO for a NE wind, and BC and BH for a SW wind. While the relative importance of local *versus* external larval imports fluctuated among populations, most of

Table 8. *Pectinaria koreni*. Modelled retention, colonisation and local supply rates (in %) for the different populations in the English Channel according to wind directions.

Population	NE wind						SW wind					
	6 $m.s^{-1}$			12 $m.s^{-1}$			6 $m.s^{-1}$			12 $m.s^{-1}$		
	Ret.	Col.	Loc. supply	Ret.	Col.	Loc. supply	Ret.	Col.	Loc. supply	Ret.	Col.	Loc. supply
Baie du Mont Saint Michel	16.34	0	100	0.26	0	100	12.35	0	100	2.46	0	100
Baie des Veys	3.13	0	99.999	1.64	0	22.16	3.58	0	100	3.54	$3.44.10^{-6}$	100
Eastern Baie de Seine	21.77	$5.08.10^{-6}$	100	$1,3.10^{-2}$	1.18	13.97	31.35	$1.86.10^{-13}$	100	1.23	$4.69.10^{-5}$	99.999
Dieppe	21.48	$9.82.10^{-5}$	81.03	0.33	1.80	4.98	24.38	11.76	99.92	0.76	22.86	99.64
Baie de Somme	8.20	28.74	54.93	0.11	8.39	0.36	27.00	10.35	28.87	6.24	11.17	11.09
Baie de Canche	13.38	3.41	52.82	$3,2.10^{-3}$	10.55	0.08	1.90	2.15	13.13	0.20	0.35	0.67
Gravelines	$2,3.10^{-4}$	1.29	100	$5.87.10^{-9}$	6.57	100	4.78	$3.05.10^{-11}$	87.94	0	0	-
St. Austell Bay	4.27	$7.85.10^{-8}$	23.59	$8,72.10^{-2}$	0	0.23	22.76	2.23	99.996	$6,86.10^{-2}$	4.38	100
Plymouth Sound	13.55	0.57	99.999	$2,17.10^{-3}$	1.57	0.01	17.73	0.09	99.48	$7,78.10^{-5}$	56.09	0.045
Great Western Bay	75.00	$2.51.10^{-7}$	99.999	$1,20.10^{-2}$	3.77	99.78	88.37	$6.92.10^{-7}$	99.98	0.50	0.16	4.84
Poole Bay	10.68	0	100	1.75	1.37	99.99	11.26	3.8910^{-8}	100	1.56	$1.07.10^{-7}$	99.999
Beachy Head	5.33	$1.17.10^{-3}$	66.85	0.1	$1.57.10^{-5}$	0.32	1.80	2.11	100	0.19	9.12	99.999
Pevensey Bay	0.48	1.65	0.47	$1,29.10^{-2}$	1.85	1.31	$1,96.10^{-2}$	24.54	0.99	$1,72.10^{-4}$	0.72	0.02
Rye Bay	0.63	10.67	24.11	$2,32.10^{-4}$	2.56	1.87	0.49	10.00	34.42	$6,83.10^{-3}$	0.16	2.44
Folkestone	0.13	17.46	26.47	$3,02.10^{-2}$	4.52	99.999	2.99	$1.56.10^{-3}$	5.68	$2,60.10^{-4}$	$7.89.10^{-12}$	$8.00.10^{-3}$

them were mainly self-sustained except for PB, RB and FO for both wind directions, SaB for a NE wind, and BSo and BC for a SW wind.

Relative to the case with moderate wind conditions, retention rates decreased for all populations under strong winds (i.e. 12 m. s^{-1}) while colonisation rates increased for most of them (Table 8). Consequently, larval supply from distant populations generally exceeded local supply although larval retention remained the dominant process for replenishment in 7 populations for a strong SW wind (e.g. BS, PoB) and in 5 populations for a strong NE wind (e.g. GWB, GR).

Although both the direction and the spatial scale of larval dispersal predicted by the model displayed large variations among populations according to the prevailing meteorological conditions during larval life span, the strength of long-term connectivity among local populations over several generations can nevertheless be drawn (Figure 5). Under average winds, all populations except for MSM were connected to their neighbouring populations. Despite increased current velocities, most exchanges occurred in the vicinity of the Dover Strait where geographical distances between populations are shorter. For example, larvae released from Baie de Canche were able to colonise up to 6 distant populations while this bay could receive larvae from up to 7 different populations. Under strong winds, dispersal distances increased and the degree of connectivity between populations was modified (Figure 5). Under such conditions, larval exchanges among populations located along the French coasts of the eastern English Channel were strengthened and so were those along the English coast with exchanges linking populations of the western and eastern English Channel, via Poole Bay. Under such extreme meteorological conditions, all populations, with the exception of MSM, were directly or indirectly interconnected to each other although exchanges between the French and English coasts remained restricted to the vicinity of the Dover Strait.

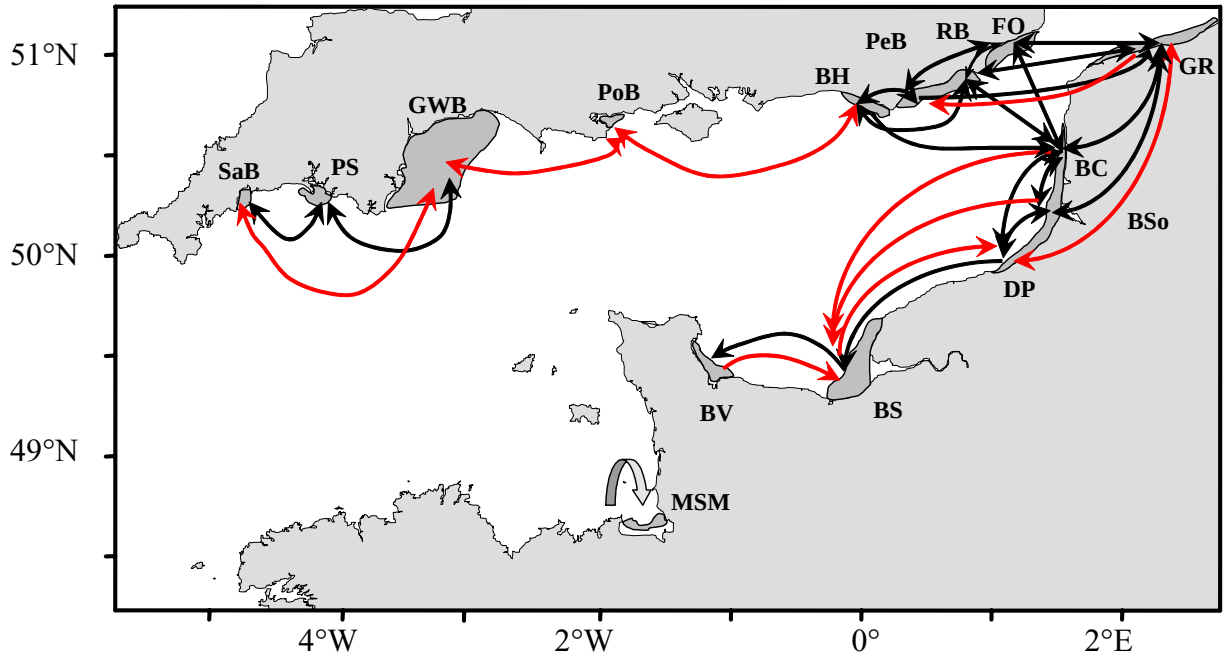


Figure 5. *Pectinaria koreni*. Synthesis of hydrodynamic-based modelling of potential larval dispersal and exchanges in the English Channel under moderate wind conditions (i.e. 6m.s^{-1}; black arrows) or strong wind conditions (i.e. 12m.s^{-1}; red arrows).

Discussion

Large scale comparison of genetic diversity and genetic structure between marine basins

Levels of genetic diversity were high in all populations, although both heterozygosity and allelic richness were significantly lower in the Irish Sea compared to the English Channel, the southern North Sea populations showing intermediate levels. While such lower genetic diversity in the Irish Sea might be indicative of recently founded populations, it may also have resulted from either a bottleneck event following the colonisation of the area during the Holocene, or from local extinction-recolonisation events affecting the "within-basin" effective population size. The scenario of a founder event following dispersal from a northern glacial refugium after the Last Glacial Maximum (LGM) might fit better with the low levels of mtCOI haplotype diversity already detected in the Irish Sea for *P. koreni* (Jolly *et al*., submitted) and the lobster *Nephrops norvegicus* (Stamatis *et al*., 2004; the authors rejected the hypothesis of over-exploitation of stocks). Turning things differently, this also suggests that the English Channel was either, colonised from a more diverse source of individuals, or that populations within, are receiving genes from more differentiated sources than the Irish Sea populations.

There is a lack of genetic structuring at the scale of the whole study area (Irish Sea, English Channel and southern North Sea). Most pairwise values of genetic differentiation (even when significant) were low and overall F-statistics did not reveal any significant differences in genetic structure between marine basins. Such low values of genetic differentiation may be partly explained by the fact that the species has discrete generations and considerable population sizes (Eagle, 1973, 1975; Lambert, 1991). While assignment testing might have been less powerful in detecting the source of migrants because of low levels of differentiation between reference populations and the use of only four loci, both F-statistics and individual genotype assignments provides interesting points to discuss in relation to the long term patterns of marine currents around the British Isles: (1) samples from

Figure 6. Maps showing (a) the general current circulation within our study area (Turrell, 1992; Pingree, 1980; Pingree and Le Cann, 1989; Brown *et al*., 2003); (b) the inferred patterns of gene flow given by our genetic data; restrictions on gene flow based on frequency data are illustrated by double bars; assignment-based gene flow pattern are indicated by black arrows (proportion > 15%); other potential patterns are indicated by broken red arrows.

GUL were all, but one individual, classified in the Irish Sea, (2) the Belgium coast population is more strongly linked to populations of the Irish Sea than to those of English Channel, (3) there is a connection between the Irish Sea and the western approaches of the English Channel (i.e. GWB), (4) individuals from BV might be able to disperse towards BH and RB, and *vice versa*, (5) considerable gene flow between the BS, BSo and GR, (6) gene flow between RB and BE. We will discuss these results in turn, in relation to Figure 6.

The first result is not unexpected because of the comparatively weaker long term influx of water coming in through the Dover Strait than that brought in by Atlantic water into the northern North Sea (Turrell *et al*., 1992), and because the analysis of the ^{137}Cs distribution of the nucleotides released from the Sellafield nuclear reprocessing plant (Irish Sea) are mainly transported north towards the North Sea. The second result however is surprising because the water flowing out of the English Channel into the North Sea follows the Belgium and Dutch coasts up to Denmark (Breton & Salomon, 1995; Bailly du Bois & Dumas, 2005), and because the Atlantic water entering the North Sea deviates essentially towards the East at the level of East Anglia, while staying separate from the Channel water (Figure 6). A strong inter-annual variability of the water circulation at the northern entrance of the English Channel may modify this pattern by making it possible for North Sea water to enter into English Channel (Otto *et al.*, 1990). This suggests that both Belgium and north-eastern Channel populations may be connected to the Irish Sea through intermediate populations situated in baies and estuaries along the eastern coast of England and Scotland. However, while the predominant flow through the Dover Strait is from west to east (Figure 6), net fluxes may be close to zero in Spring (Turell *et al*., 1992), thereby dampening the exchanges between GR and BE.

Concerning whether a dispersal pathway exists between the Irish Sea and the western English Channel, through the Celtic Sea front is unnclear. Despite considerable sampling effort, no populations were sampled on the west and south coast of Wales. Either those populations were missed, or both the Celtic Sea and western English Channel are so poorly

supplied in larvae that no stable populations actually exist. During the reproductive season of *P. koreni*, the advent of summer thermal stratification in the Celtic Sea and the associated flow fields induced by the formation of the Celtic Sea front may reduce larval exchanges between the Celtic and Irish Seas (Brown *et al*., 2003). Together with this oceanic front, a westward flow across Saint George's Channel is directed south into the Celtic Sea and west along the Irish coast. Larvae dispersing from the Irish Sea have therefore more chances of being "lost" than to recruit onto favourable substrates within the Bristol Channel. Combining genetic markers with a 2D model of coastal circulation along the coasts of Cornwall, Gilg & Hilbish (2003) suggested that larval transport was mainly directed from the English Channel to the Celtic Sea. From observations of the seasonal circulation associated with the Celtic Sea front, Brown *et al*. (2003) also described a north eastwards flow along the Cornish coast which would make it easier for larvae to disperse in such direction than otherwise. Despite all the observations made above, our genetic data indicates that the multilocus genotypes of the individual from SB and most of those from GWB (south western England) are more likely to be found among Irish Sea populations. Brown *et al*. (2003) do indicate that a degree of exchange through the Saint George's Channel is to be expected, facilitated by the intense mixing and large tidal excursions at the shallow margins of the eastern part of the Channel. In addition, about 1% of the nucleotides released from the Sellafield nuclear plant into the Irish Sea, enter the western English Channel (Bailly du Bois & Guéguéniat, 1999). These observations imply that N-S exchanges might happen but very occasionally.

Our data also suggests that populations are well connected within the central portion of the English Channel (figure 6). In particular, (1) a larger proportion of individuals from BV (french coast) were assigned to RB (south english coast) than they were to their own population, and (2) individuals from GR were assigned preferentially to the BS and to their

own population. These results need to be discussed in relation to hydrodynamic modelling of larval transport and to past signatures of gene flow.

Correlation between gene exchanges and modelled larval transport in the English Channel

Both convergent and conflicting results appear when comparing effective dispersal patterns with potential larval dispersal as predicted by hydrodynamic modelling.

Most results do however converge: 1) there is a strong connection between BS and BV, 2) gene flow estimates along the French coast are concordant with dispersal under relatively strong wind action (9 to 12 $m.s^{-1}$) especially north-eastern winds, but only when taking into account the intermediate populations along the Pays de Caux (DP, BSo, BC), 3) extensive dispersal occurs across the Dover Strait between GR and RB. These results are also in accordance with previous studies on natural populations of *Ostrea edulis* (Launey, 1998) and the invasive *Crepidula fornicata* (Dupont *et al.*, 2003), that indicate genetic homogeneity along the same 230 km stretch of coastline separating the BS from GR. In *P. koreni*, assignment tests reveal that gene flow is probably unidirectionally oriented from BS towards BSo and GR (see Figure 6), which is in accordance with the coastal route taken by radiolabelled water discharges from the nuclear reprocessing plant at La Hague (Bailly du Bois & Guéguéniat, 1999). The lack of genetic differentiation between GR and RB and the fact that larvae can potentialy migrate across the Dover Strait under both moderate and strong winds, indicates that large genetic exchanges take place in this area despite the presence of a frontal zone between both French and English coasts (Brylinski, 1986; Brylinski *et al*., 1988). These exchanges may be facilitated by the residual eddy developping in April - July during the reproductive season of *P. koreni* (“FluxManche” programme), while dampening gene flow between Gravelines and Belgium.

Strong discrepancies also exist between genetic data and hydrodynamic modelling, which may have been induced by a combination of inherent modelling problems, a low number of gene loci screened, a recent history of colonisation of the English Channel resulting in genetic homogeneity, and the effects of extinction-recolonisation dynamics: (1) certain larval fluxes between the oriental and occidental provinces of the English Channel need very improbable meteorological conditions (i.e. between RB and GWB) and (2) dispersal across the central portion of the English Channel is not possible in the light of hydrodynamic modelling (i.e. between BS and MSM; between BS-BV and RB-BH) while genetic data indicates the contrary. The analysis of the pattern of radionuclide distribution further suggests that passive transport from the Cotentin towards the Isle of Wight is very improbable (Bailly du Bois & Guéguéniat, 1999) or that it would require a much prolonged larval life-span (see Breton & Salomon, 1995; Bailly du Bois & Dumas, 2005).

While the model accurately predicted the general pattern of larval dispersal throughout the studied region, the discrepancies observed with genetic data may be explained by some inadequacies inherent with our modelling study: (1) the model does not taken into account the precise spawning and settlement dates, the exact location of the genitor populations and the variability in meteorological conditions over larval life-span and therefore it cannot predict specific dispersal events, (2) small intermediate/ transient populations along both coastlines may be present but not detected (e.g. along the Cotentin and the southern English coasts), and could act to decrease the distance to travel by larvae between neighbouring populations, (3) the model does not include larval behaviour and baroclinic circulation which may affect dispersal distances, and (4) in the less rich waters of the open sea, larvae may delay metamorphosis thereby maybe increasing larval life-span more than only two weeks.

While Lagadeuc & Retière (1993) estimated larval life to last about 15 days from *in situ* observations (i.e. in favourable conditions), Cazaux (1981) gave an estimate of 58 days based on the rearing of larvae (i.e. in unfavourable aquaria conditions). Such a wide difference could

suggest that the larva could delay its metamorphosis, thereby enhancing its potential for effective dispersal.

While it is not clear whether dispersal across the central portion of the English Channel occurs, genetic homogeneity may be maintained indirectly. Our simulation results suggest that even with a 15 days larval stage, over the longer term (i.e. a few generations), the proportion of allochtonous larval supply to each population is not negligeable and comparison with genetic data indicate that migration is not related to geographic distance within the English Channel. Episodic larval dispersal events could be responsible for maintaining genetic homogeneity between populations especially since the exchange of only one effective migrant per generation may be sufficient to counter genetic differentiation caused by drift or weak selection. While larval dispersal probably depends on wind-induced patterns of marine currents during the reproductive period of *P. koreni* and larval behaviour in relation to environmental conditions, the lack of genetic structure might also be reminiscent of historically driven patterns of gene flow associated with the recent colonisation of the English Channel (within the last 10 Kya).

Mitochondrial DNA versus microsatellite markers reveals complementary histories

Limited hydrodynamic exchanges between the English Channel and the Celtic Sea, and the lack of a strong genetic structure in *P. koreni* (clade 1) around the British isles suggests a very recent history of colonisation (within the last 10 Kya according to Palaeo data, see Lambeck, 1997) of the seas surrounding the British Isles, at least concerning the sampled populations. This may pose a problem when comparing our genetic data with the present long term pattern of marine currents and hydrodynamic modelling within the English Channel, since contemporary patterns of migration may be masked by recent ancestry.

Although known to trace back different time scale, some similarities exist between microsatellites and mtCOI gene sequences (Jolly *et al*., 2005; Jolly *et al*., submitted). Mostly,

these are: (1) a reduction of genetic diversity in the Irish Sea relative to the English Channel, (2) a pattern of gene flow oriented from the Irish Sea in a N-S direction, first towards GWB through the Saint George Channel, second, around the northern British isles into the North Sea and south towards the Belgium coast, (3) unidirectional gene flow across the central portion of the English Channel from BV towards RB (refer to Table 6 and Figure 6). While the above results may be concordant with the history of colonisation of the English Channel (see Jolly *et al*., submitted), in some cases it is also possible that mtDNA actually reflects contemporary patterns to a greater degree than previously thought.

Only a third of the individuals sampled in English Channel were assigned to the Irish Sea which points to past gene flow, especially since crossing the Celtic Sea front appears unlikely for larvae in the view of hydrographic data (but see Bailly du Bois & Guéguéniat, 1999). Further, the isolation-by-distance observed with mtDNA between the two marine basins (see Jolly *et al*., submitted) is not detected using microsatellites, which suggests that the spatial structure is either masked by contemporary gene flow or by extinction-recolonisation dynamics. By recolonising nearly "empty" habitats at each generation, a better balance is established between migration and colonisation. This would result in low levels of genetic differentiation by limiting the effects of genetic drift especially if metapopulation processes are reinforced by source-sink dynamics and high "within-basin" effective population size. While the reduction of the genetic heterogeneity within populations (F_{IS}) of the Irish Sea relative to those in the English Channel may result from differences in metapopulation structure and processes (synchronous, asynchronous dynamics), the Irish Sea populations are also less diversified at both mtDNA and microsatellites, thus maybe possessing a lower proportion of null alleles which is reflected in weaker F_{IS} values.

Regarding past gene flow, some fluxes revealed by mtDNA are oriented differently to those predicted by hydrodynamic models of water circulation, suggesting that they reflect the historic colonisation pathways of the English Channel after or during glacial meltdown:

mainly (1) strong unidirectional gene flow oriented from GWB towards BH (mtDNA N_m= 280.7; Jolly *et al*., submitted), (2) more limited but significant gene flow oriented from Sweden (GUL) towards the eastern entrance of the English Channel (mtDNA N_m = 15.4). The first, is improbable without the presence of intermediate populations along the English coast and would also require very improbable meteorological conditions, while the second is contrary to the general circulation of the North Sea.

Most patterns of gene flow estimated from mtCOI gene sequences are similar to those found using microsatellites, which have higher rates of evolution and should reveal more contemporary patterns of gene flow. Four microsatellite loci are probably not enough to obtain the desired statistical power to distinguish accurately contemporary larval exchanges with respect to hydrodynamic processes. Nevertheless, the comparison of the genetic data obtained for *P. koreni* with the modelling of its potential larval transport agree relatively well in recognising patterns that may correspond to past gene flow.

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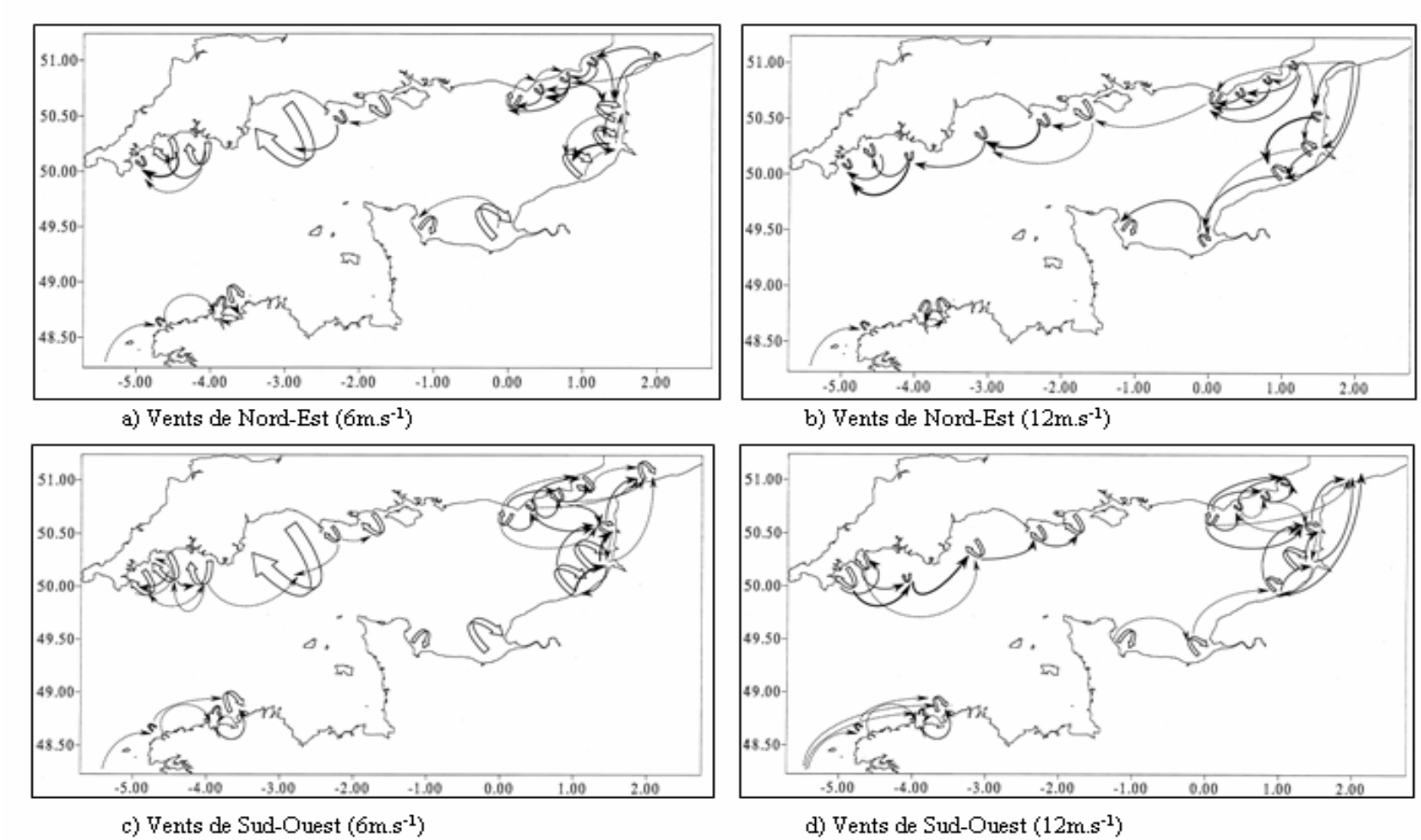
CONCLUSION

Chez *P. koreni* clade 1, nos résultats montrent qu'un flux de gène récent (pendant les derniers 10 000 ans) a existé (ou existe encore) entre les populations de la Mer d'Irlande et de la Manche, avec un passage par la Mer du Nord. La comparaison entre les résultats obtenus avec les marqueurs microsatellites et le marqueur mitochondrial confirme l'hypothèse préalablement posée dans le chapitre 1, celle de 2 voies de colonisation de la Manche, temporellement distinctes. La première aux approches occidentales de la Manche il y a environ 14 000 ans, et la deuxième depuis la Mer du Nord après l'ouverture catastrophique du détroit du Pas-de-Calais, il y a environ 9 à 8 000 ans. Un contact secondaire en Manche entre « pools » génétiquement différenciés pourrait donc expliquer la faible différenciation génétique observée entre la Mer d'Irlande et la Manche. De même, cette remise en contact pourrait être à l'origine d'un effet Wahlund expliquant, en partie (car nos marqueurs présentent des allèles nuls), les forts déficits en hétérozygotes observés avec les marqueurs microsatellites. Malgré une absence notable de différenciation génétique et l'existence de forts flux larvaires le long des côtes picardes, dans le détroit du Pas-de-Calais et des échanges épisodiques de part et d'autre de l'île de Wight, la comparaison des résultats obtenus par l'analyse de la structure génétique et par la modélisation des flux larvaires potentiels souligne certaines inadéquations entre méthodes (notamment la présence de flux trans-Manche ou à la pointe du Cotentin entre la Baie de Seine et la baie du Mont-Saint-Michel). Ces différences pourraient être dues au (1) faible nombre d'individus récoltés le long des côtes Picardes et du Cotentin, (2) à une rétention du polymorphisme ancestral et une faible différenciation génétique associé à la colonisation très récente de la Manche (moins de 10 000 ans), (3) à la rémanence de flux géniques passés, (4) à une sous estimation de la durée de vie larvaire (15 jours *in situ* vs 58 jours *in vivo* ; Cazaux, 1981) ou même (5) à une dispersion d'origine anthropique des larves due à l'augmentation du traffic maritime (e.g. les eaux de ballast).

On peut néanmoins constater le rôle central de la Baie de Seine dans la redistribution des flux géniques en Manche (flux BS-RB ; BS-GWB ; BS-BV). Cette Baie est à l'heure actuelle la plus importante de la Manche orientale et connue pour abriter la population de pectinaires la plus vaste et la plus stable au niveau de l'Atlantique Nord Est.

La structure génétique observée à méso-echelle (Manche) étant le reflet des processus s'établissant à l'échelle locale des populations, il est devient nécessaire de comprendre le fonctionnement de la métapopulation de *P. koreni* clade 1 à l'échelle de cette baie, l'évolution spatio-temporelle des unités reproductrices de pectinaire au sein de cette baie ayant un rôle clé dans la gouvernance des échanges de gènes à l'échelle de la Manche orientale voire occidentale.

ANNEXES
CHAPITRE II



Annexe II. 1 : Flux potentiels de larves de *Pectinaria koreni* par rapport à l'hydrodynamisme et au forçage par le vent en Manche. [Flèches en pointillées: <0,1% des larves émises; flèches en trait plein:>0,1 et <10% des larves émises; flèches en trait plein et gras: >10% des larves émises] (Thiébaut, comm. pers.).

Annexe II. 2 : *Pectinaria koreni.* Fréquences alléliques pour quatre locus microsatellites (PKGT1; PKAT/GT1; PKAT/GT2 et PKAT/GT4). Jolly *et al.* (en préparation).

	POPULATION												
PKGT1	BS	BV	BH	RB	GWB	MSM	PC	GR	BE	LV	WH	RWB	CWB
(N)	154	53	7	99	9	12	9	64	56	45	40	6	50
191	0.0032												
198		0.0189											
200		0.0189		0.0051	0.1111								
202		0.0094											
204	0.0065	0.0377									0.0125		
206		0.0189	0.1429	0.0051									
208		0.0189		0.0202	0.2222								
212	0.0032	0.0566											
214	0.0097	0.0189	0.2857					0.0156	0.0179				
216		0.0283		0.0051									
218	0.0032	0.0377		0.0051	0.0556			0.0156		0.0222			
220	0.0292	0.0283		0.0303				0.0234	0.0179				0.0300
222	0.0097	0.0283		0.0404				0.0156	0.0714	0.0222	0.0250		0.0600
224	0.0227	0.0660		0.0505	0.0556		0.0556		0.0536	0.0222	0.0750		0.0300
226	0.0260	0.0094		0.0202		0.0417		0.0625			0.0250	0.1667	0.0100
228	0.0130	0.0472	0.1429	0.0657		0.0417		0.0156		0.0778			0.0400
230	0.0227	0.0094		0.0354				0.0313	0.0804	0.0222			0.0800
232	0.0292	0.0283	0.1429	0.0152		0.0417		0.0234	0.1071	0.0444	0.0875		0.0200
234	0.0487	0.0094		0.0960	0.1667	0.0833	0.2222	0.0859	0.0982		0.0250		0.0600
236	0.0487	0.0755	0.1429	0.0202	0.1667			0.0156	0.0536	0.0778	0.0500	0.1667	0.0600
238	0.0747	0.0377		0.0505			0.0556	0.0469	0.0714	0.1111	0.0500		0.1600
240	0.0844	0.0660		0.0909		0.2500	0.1111	0.0859	0.0982	0.0556	0.2125		0.0300
242	0.0942	0.0189		0.1061			0.1111	0.1172	0.0893	0.1111	0.0250	0.1667	0.0800
244	0.0617	0.0660	0.1429	0.0354		0.1250	0.1111	0.0703	0.0446	0.0556	0.0375		0.0900
246	0.0714	0.0472		0.0657	0.1111			0.1016	0.0089	0.1111	0.0125	0.0833	0.0500
248	0.0812	0.0943		0.0354	0.1111		0.0556	0.0547	0.0536		0.0875		0.0700
249								0.0078					
250	0.0552	0.0189		0.0253		0.0833		0.0234	0.0179	0.0889	0.0500		0.0500
252	0.0455			0.0606			0.0556	0.1016		0.0444	0.0875		0.0600
254	0.0325	0.0472		0.0404		0.1667	0.0556		0.0179	0.0556	0.0750	0.3333	
256	0.0227			0.0202		0.0417	0.1667			0.0111		0.0833	0.0200
258	0.0195	0.0189		0.0152		0.0417		0.0391		0.0111	0.0250		
260	0.0260					0.0833			0.0268	0.0333	0.0125		
262				0.0101									
264	0.0065	0.0189		0.0051				0.0156	0.0357	0.0222	0.0125		
266	0.0097			0.0101				0.0156					
268	0.0065										0.0125		
274	0.0032			0.0152				0.0078	0.0089				
276	0.0130							0.0078	0.0089				
278	0.0065												
280	0.0097												
354									0.0179				

	POPULATION												
PKAT/GT1	BS	BV	BH	RB	GWB	MSM	PC	GR	BE	LV	WH	RWB	CWB
(N)	222	65	9	128	12	19	12	87	63	56	61	8	64
246								0.0057					
248									0.0079				
270				0.0078									0.0078
286				0.0039									
294				0.0039									
304											0.0082		
318				0.0039									
322				0.0078									0.0078
324	0.0045								0.0079				0.0078
326	0.0023	0.0154			0.0833				0.0317	0.0268			0.0078
328	0.0135	0.0077		0.0117						0.0268	0.0082	0.1250	0.0391
330	0.0158	0.0231		0.0078			0.0417			0.0625	0.0164		
332	0.0023	0.0154	0.1667	0.0039				0.0057		0.0446	0.0246	0.1250	0.0078
334	0.0158	0.0231		0.0195		0.0263			0.0159	0.0179	0.0082		0.0156
336	0.0158	0.0077		0.0117			0.0417	0.0057			0.0328		0.0391
338	0.0135	0.0154		0.0156					0.0079	0.0179			0.0156
340	0.0158	0.0077		0.0234				0.0345	0.0159		0.0082		
342	0.0248	0.0385	0.0556	0.0195	0.1667			0.0057	0.0476	0.0268	0.0082		0.0703
344	0.0428	0.0154	0.0556	0.0391	0.0833			0.0402	0.0397	0.1161	0.0410		0.0703
346	0.0428	0.0769	0.1111	0.0508	0.0417	0.0526	0.0417	0.0172	0.0317	0.0179	0.0328	0.0625	0.0547
348	0.0450	0.0462		0.0586		0.0526	0.0833	0.0287	0.0397		0.0492		0.0078
350	0.0428			0.0547	0.0417	0.0526	0.1250	0.0690	0.0794	0.0536	0.0164		0.0469
352	0.0586	0.0538	0.0556	0.0234			0.0417	0.0115	0.0714	0.0268	0.0820	0.1250	0.0234
353	0.0023												
354	0.0428	0.0462		0.0508	0.1250	0.0263		0.0345	0.0635	0.0625	0.0492	0.1250	0.0313
356	0.0293	0.0769		0.0352	0.0417	0.0789		0.0575	0.0556	0.0446	0.0738	0.1250	0.0313
358	0.0518	0.0462		0.0234	0.0833	0.2368	0.0417	0.0345	0.0476	0.0179	0.0246	0.1250	0.0625
360	0.0405	0.0462		0.0586	0.0417		0.0417	0.0460	0.0238	0.0536	0.0328	0.0625	0.0234
362	0.0360	0.0462	0.1111	0.0273	0.0417	0.0263	0.0417	0.0460	0.0079		0.0492		0.0234
364	0.0473	0.0615		0.0352	0.0417		0.0833	0.0287	0.0476	0.0268			0.0078
366	0.0315	0.0308		0.0391	0.0417		0.0833	0.0632	0.0159	0.0357	0.0164		0.0313
368	0.0338	0.0231	0.0556	0.0469		0.0526	0.0417	0.0345		0.0446	0.0410	0.0625	0.0078
370	0.0338	0.0154	0.1667	0.0469	0.0417	0.0526	0.0417	0.0517	0.0159	0.0804	0.0492		0.0391
372	0.0180	0.0385	0.1111	0.0234		0.0263		0.0345	0.0238	0.0268	0.0656	0.0625	
374	0.0135	0.0154		0.0078	0.0833	0.0526	0.0417	0.0747	0.0556	0.0179	0.0246		0.0234
376	0.0473	0.0154		0.0039		0.0263		0.0517	0.0476	0.0089	0.0246		0.0234
378	0.0338			0.0156		0.0526		0.0172	0.0397	0.0179	0.0164		0.0469
380	0.0158	0.0538	0.0556	0.0234			0.0417			0.0089	0.0328		0.0156
382	0.0203	0.0154		0.0156				0.0172	0.0317	0.0179			0.0391
384	0.0270	0.0154		0.0156		0.0263		0.0115	0.0317		0.0082		0.0313
386	0.0293	0.0154		0.0117		0.1053		0.0230	0.0159	0.0268	0.0246		0.0078
388	0.0113	0.0385	0.0556	0.0156			0.0417	0.0115	0.0079		0.0246		0.0391
390	0.0090	0.0154		0.0313	0.0417		0.0417	0.0230	0.0159	0.0268	0.0328		0.0391
392	0.0090	0.0154		0.0313				0.0115	0.0079	0.0179	0.0082		0.0156
394	0.0068	0.0077		0.0234				0.0172	0.0238	0.0089	0.0082		
396				0.0078			0.0417				0.0082		0.0078
398	0.0068					0.0263		0.0115	0.0079	0.0179			0.0234
400	0.0045					0.0263		0.0230			0.0082		0.0078
402	0.0180			0.0156							0.0082		
404	0.0023	0.0077		0.0039			0.0417		0.0079				
406	0.0023							0.0057			0.0082		
408	0.0068			0.0039					0.0079				
410	0.0090			0.0078				0.0057					
412		0.0077		0.0039									
414				0.0039									
416								0.0172			0.0082		
418				0.0039				0.0230					
426	0.0023										0.0164		
438	0.0023												

	POPULATION												
PKAT/GT2	BS	BV	BH	RB	GWB	MSM	PC	GR	BE	LV	WH	RWB	CWB
(N)	218	63	10	127	12	17	12	88	71	58	60	8	68
230										0.0086			
236	0.0046									0.0086			
238										0.0086			
242	0.0069		0.0500										
252										0.0086	0.0167		
258	0.0023												0.0147
262													0.0074
264											0.0083		
266		0.0238						0.0284					0.0074
268	0.0023			0.0157				0.0341					0.0074
270	0.0115												
272	0.0092			0.0079		0.0294		0.0057	0.0211	0.0345		0.1250	0.0074
274	0.0023							0.0114	0.0211	0.0086	0.0333		
276	0.0161									0.0086			
278	0.0298	0.0159		0.0039				0.0284	0.0493	0.0086			0.0294
280	0.0183	0.0238		0.0079	0.0417			0.0170	0.0141	0.0345	0.0250	0.0625	0.0221
282	0.0252							0.0057	0.0070	0.0086	0.0167		0.0074
284	0.0092	0.0159						0.0341	0.0282	0.0086	0.0167		0.0074
286	0.0252			0.0118		0.0882			0.0423	0.0172	0.0250		0.0294
288	0.0138	0.0159		0.0039	0.0833		0.0833	0.0057	0.0352	0.0345	0.0500		0.0809
290	0.0183			0.0118	0.0417				0.0352	0.0259	0.0333	0.0625	0.0515
292	0.0161	0.0317	0.1000	0.0079	0.0833	0.0294		0.0170	0.0141	0.0172	0.0250		0.0074
294	0.0229	0.0079		0.0394				0.0455	0.0352	0.0517	0.0500	0.1875	0.0368
296	0.0138			0.0079		0.0588		0.0114	0.0352	0.0172	0.0250	0.0625	0.0441
298	0.0344	0.0159		0.0039		0.0294		0.0227	0.0352	0.0690	0.0500		0.0221
300	0.0344			0.0354		0.0294	0.0417	0.0511	0.0211	0.0862	0.0250		0.0074
302	0.0275	0.0079		0.0394	0.0417	0.1176	0.0833	0.0284	0.0141	0.0259	0.0417	0.0625	0.0294
304	0.0252	0.0238		0.0118				0.0341	0.0352	0.0345	0.0167		0.0588
306	0.0138	0.0238		0.0079	0.0833	0.0882	0.0417	0.0227	0.0423	0.0172	0.0333	0.0625	0.0515
308	0.0229	0.0238		0.0236	0.0833		0.2500	0.0398	0.0352	0.0259	0.0750		0.0294
310	0.0183			0.0118	0.0417	0.0882	0.0833	0.0284	0.0141	0.0259	0.0083		0.0147
312	0.0413	0.0159	0.1500	0.0118				0.0284	0.0070	0.0517	0.0583		
314	0.0229	0.0159		0.0236		0.0882		0.0057	0.0141	0.0259	0.0167		
316	0.0275	0.0079	0.1500	0.0630	0.0417			0.0227	0.0634	0.0086	0.0250		0.0294
318	0.0321	0.0317		0.0079	0.1250	0.0294		0.0341	0.0282	0.0431	0.0417		
320	0.0252	0.0714		0.0157				0.0398	0.0070		0.0250		0.0515
322	0.0367	0.0317		0.0512		0.0882	0.0417	0.0341	0.0070	0.0517		0.0625	0.0294
324	0.0115	0.0397		0.0512	0.0833		0.0417	0.0114	0.0070		0.0083		0.0221
326	0.0275	0.0556		0.0276		0.0294		0.0455	0.0141	0.0086	0.0750		0.0147
328	0.0183	0.0317	0.1000	0.0787			0.0417	0.0114	0.0352				0.0221
330	0.0321	0.0159		0.0197				0.0284	0.0211	0.0259			0.0368
332	0.0183	0.0238	0.2500	0.0236			0.0833	0.0341	0.0211	0.0345	0.0167		0.0147
334	0.0092	0.0079	0.0500	0.0276	0.0417	0.0882		0.0284	0.0493		0.0167		0.0074
336	0.0298	0.0159		0.0039	0.0833	0.0294		0.0170	0.0352	0.0172	0.0500		0.0221
338	0.0321	0.0159		0.0079		0.0588	0.0417	0.0114	0.0070	0.0086		0.0625	0.0221
340	0.0138			0.0394					0.0282	0.0259	0.0083		0.0074
342	0.0206			0.0039	0.0417			0.0284	0.0141	0.0172	0.0083		0.0221
344	0.0138	0.0317		0.0315	0.0417			0.0114	0.0141	0.0259		0.0625	0.0221
346	0.0092	0.0556		0.0315	0.0417				0.0211	0.0259		0.0625	0.0074
348	0.0183	0.0476		0.0394			0.0833			0.0086	0.0083		0.0074
350	0.0138	0.0238	0.1000	0.0079						0.0086	0.0083		0.0074
352	0.0092	0.0079		0.0157				0.0170	0.0141				
354	0.0206	0.0397		0.0197		0.0294		0.0341				0.1250	
356	0.0092	0.0079		0.0157				0.0170					
358	0.0069	0.0238		0.0236				0.0227					0.0147
360	0.0092	0.0317		0.0197					0.0141	0.0086			0.0147
362	0.0069			0.0079					0.0070	0.0086			
364	0.0115	0.0317						0.0114			0.0083		
366	0.0138	0.0159		0.0079					0.0141				0.0147
368	0.0046	0.0317	0.0500	0.0079					0.0070		0.0083		

PKAT/GT2 (suite)	BS	BV	BH	RB	GWB	MSM	PC	GR	BE	LV	WH	RWB	CWB
370	0.0046	0.0079		0.0079				0.0057			0.0167		0.0074
372	0.0046			0.0118					0.0070		0.0250		0.0074
374				0.0079									0.0074
376		0.0159		0.0079					0.0070				0.0074
378		0.0159						0.0114					
382	0.0023			0.0079				0.0057					
384				0.0118			0.0417						0.0074
386	0.0023												
390	0.0023												
394							0.0417						
396	0.0046			0.0079									
398	0.0046							0.0114					
400	0.0046												

	POPULATION												
PKAT/GT4	BS	BV	BH	RB	GWB	MSM	PC	GR	BE	LV	WH	RWB	CWB
(N)	220	68	10	131	14	19	12	88	67	52	60	7	65
242									0.0149				
244	0.0023												
264											0.0083		
268											0.0083		
336	0.0023								0.0075				
338	0.0068								0.0672	0.0481	0.0250	0.0714	0.1077
340	0.0250	0.0147		0.0153				0.0170	0.0896	0.0865	0.1250	0.0714	0.0615
342	0.0568	0.0588	0.0500	0.0611	0.0357		0.0417	0.0398	0.1194	0.1058	0.0333		0.1385
344	0.1159	0.1471	0.1500	0.0496	0.1786	0.0526	0.0833	0.0909	0.0672	0.0577	0.1000	0.0714	0.0769
346	0.1045	0.0882		0.0496	0.0714	0.1053	0.2083	0.1534	0.0224	0.0096	0.0417		0.0154
348	0.0455	0.0441		0.0687		0.0789	0.1250	0.0966	0.0522	0.0192	0.0250		
350	0.0386	0.0368		0.0534			0.0417	0.0284	0.0299	0.0769	0.0417	0.0714	0.0385
352	0.0477	0.0294		0.0267	0.0714	0.0263	0.0417	0.0455	0.0224	0.0673	0.0583		0.0154
354	0.0250	0.0221	0.1000	0.0153		0.1053	0.0417	0.0398	0.0299	0.0577	0.0167	0.0714	0.0308
356	0.0182	0.0221	0.0500	0.0420		0.0263		0.0114	0.0448	0.0385	0.0250		0.0231
358	0.0682	0.0441	0.2000	0.0878		0.0526		0.0455	0.0373	0.0481	0.0417	0.0714	0.1000
360	0.0295	0.0294	0.1000	0.0534	0.1786	0.0263	0.0417	0.0341	0.0821	0.0577	0.0417		0.0846
362	0.0318	0.0221		0.0382	0.0357	0.0526		0.0455	0.0672	0.0385	0.0250	0.0714	0.0231
364	0.0591	0.0735		0.0725	0.1071	0.0526		0.0511	0.0522	0.0673	0.0500		0.0538
366	0.0477	0.0809	0.0500	0.0344		0.1316	0.0833	0.0455	0.0149	0.0192	0.0083	0.1429	0.0615
368	0.0500	0.0515	0.1000	0.0611	0.0357		0.1667	0.0341	0.0224	0.0673	0.0667	0.0714	0.0308
370	0.0545	0.0809		0.0305	0.1429	0.0789	0.0417	0.0227	0.0299	0.0577	0.0333		0.0231
372	0.0364	0.0221	0.0500	0.0725	0.1071	0.0263	0.0417	0.0568	0.0522	0.0385	0.0417	0.0714	0.0077
374	0.0205	0.0221	0.0500	0.0573				0.0341	0.0299	0.0096	0.0583	0.0714	0.0462
376	0.0182	0.0294		0.0153		0.0263	0.0417	0.0057		0.0096	0.0333	0.1429	0.0231
378	0.0432	0.0294	0.0500	0.0305	0.0357	0.0526		0.0057	0.0299		0.0167		0.0231
380	0.0273	0.0221		0.0229				0.0398	0.0075		0.0250		0.0154
382	0.0136	0.0074		0.0115		0.0526		0.0227	0.0075	0.0192	0.0250		
384	0.0045	0.0074		0.0153							0.0083		
386	0.0045	0.0074	0.0500	0.0115		0.0263		0.0114					
388		0.0074		0.0038				0.0114					
390								0.0057			0.0083		
392	0.0023					0.0263		0.0057					
394											0.0083		

CHAPITRE III

ETUDE DE LA VARIATION GENETIQUE SPATIO-TEMPORELLE D'UNE METAPOPULATION SOURCE-PUITS DE *PECTINARIA KORENI* : LA BAIE DE SEINE.

Introduction

Pour comprendre les forces micro-évolutives (adaptation locale, stochasticité démographique) agissant sur la structure d'une population, il est nécessaire d'aborder la variabilité spatio-temporelle de la structure génétique à une autre échelle beaucoup plus fine, et de recouper les connaissances accumulées en matière de dynamique des populations et de micro-habitats. Non seulement c'est la taille efficace des populations de géniteurs qui conditionne l'évolution des fréquences alléliques au cours du temps, mais l'action superposée de l'hydrodynamisme induit par la marée et le vent influence les processus dispersifs et donc l'évolution de la structure génétique dans l'espace. Les contraintes locales seront également d'autant plus fortes sur les populations que ces dernières seront disposées en agrégats de taille différente comme c'est le cas chez *Pectinaria koreni* en Baie de Seine. En effet, la micro-hétérogénéité de l'habitat peut alors conduire rapidement à un différentiel dans le succès reproducteur et le synchronisme de la ponte. Les caractéristiques locales de l'habitat (en terme de fragmentation et de qualité du substrat) influencent directement les caractéristiques biotiques (e.g. croissance des individus, densité et taille des agrégats) et écologiques de l'espèce (compétition intra- et inter-spécifique) qui jouent directement sur la fécondité des adultes et la mortalité des premiers stades de vie (jeunes recrues). Dépendant de ces facteurs, des ajustements phénotypiques peuvent engendrer une forte hétérogénéité dans la composition génétique spatio-temporelle.

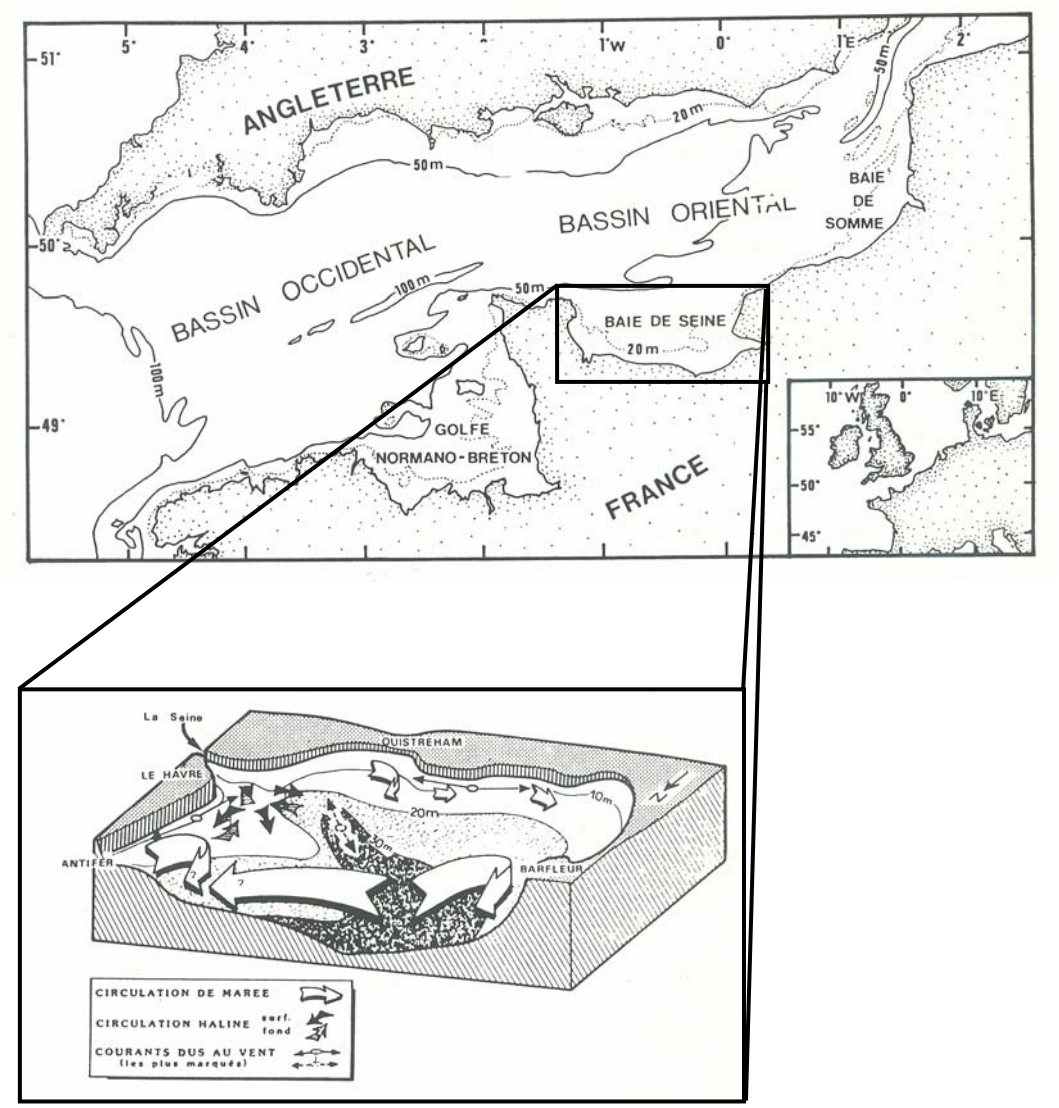


Figure 18. Carte présentant d'une part, la position de la Baie de Seine en Manche orientale, et d'autre part, le schéma de circulation résiduelle moyenne en Baie de Seine (d'après Le Hir *et al*., 1986 et tiré de Lambert, 1991).

Au fil des nombreux travaux entrepris sur les espèces composant les peuplements des sédiments fins envasés de la Baie de Seine, on peut dire que ce site est devenu un observatoire d'étude des mécanismes fins de la dynamique des populations.

La Baie de Seine est située dans la partie sud de la Manche orientale (Figure 18) où elle est délimitée par la presqu'île du Cotentin à l'ouest, par les côtes du Pays de Caux à l'est. Au nord, elle est largement ouverte sur la Manche entre la pointe de Barfleur et le cap d'Antifer. L'hydrodynamisme local en Baie de Seine présente deux composantes majeures que sont la circulation instantanée et la circulation résiduelle, la première étant principalement régie par les courants de marée alors que la seconde dépend plutôt des effets du vent et de la circulation haline au débouché de la Seine (Figure 18). En liaison avec la circulation résiduelle étagée au débouché de la Seine, il existe de forts flux particulaires d'origine terrigène. Lorsque le débit de la Seine est faible au printemps et en été, les apports sédimentaires se font en direction de l'estuaire. A partir des mois de Mai et de Juin, on assiste à un envasement important en face de l'estuaire qui est suivi d'une remise en suspension des particules à partir de la fin de l'automne (Avoine, 1986). Ces conditions hydrodynamiques influencent directement la dynamique des populations de *P. koreni*, à savoir que la phase de renouvellement de la population (recrutements printaniers et estivaux) a lieu lorsque la circulation résiduelle de fond est dirigée vers l'estuaire. Pour compléter ce schéma, il faut noter (1) qu'une phase de recrutement est possible en Automne au cours de laquelle les périodes de perturbations majeures du fond sont fréquents, (2) que le dernier stade larvaire « aulophore » peut se déplacer dans les eaux de fond avant sédentarisation, et que (3) les postlarves et les juvéniles possèdent, suite à leur sédentarisation initiale, des capacités de se déplacer vers un nouvel habitat par des mécanismes de remise en suspension à l'aide d'une voile muqueuse (Lambert, 1991), sur des distances pouvant être de l'ordre du kilomètre (Olivier *et al*., 1996 ; Thiébaut *et al*., 1996). Ce mode de déplacement permet à la post-larve voire au juvénile de pouvoir choisir son habitat et de se regrouper en noyaux (grégarisme).

Pour apprécier les mécanismes jouant sur la structure spatio-temporelle de la métapopulation locale de *P. koreni* en Baie de Seine, nous avons entrepris deux études. La première se concentre sur l'évolution de la structure génétique des noyaux d'adultes reproducteurs au cours de quatre générations successives, ceci afin de déterminer à quel modèle de métapopulation se rapproche *P. koreni,* et définir la meilleure stratégie d'échantillonnage à conduire pour décrire au mieux l'hétérogénéité des populations. Notre seconde étude s'est focalisée sur l'effet du changement de génération (i.e. le renouvellement de la population) dans l'évolution de la structure génétique observée entre adultes et juvéniles, ceci afin de mieux comprendre les effets micro-évolutifs associés à la phase de recrutement.

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ORIGINAL ARTICLE

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Does the genetic structure of *Pectinaria koreni* (Polychaeta: Pectinariidae) conform to a source–sink metapopulation model at the scale of the Baie de Seine?

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Abstract According to a recent demographic survey, the population structure of *Pectinaria koreni* might fit a source–sink metapopulation model at least at a regional scale. Spatial and temporal genetic structure of the populations present in the Baie de Seine (eastern Baie de Seine and Baie des Veys) was assessed using four highly polymorphic microsatellite loci which have revealed strong intra-locality genetic diversity. In the eastern Baie de Seine, both temporal (1994–1996) and spatial (1994) genetic differentiation were relatively low but significantly different from zero despite a 15-day dispersing larval stage. Such structures may be explained by the settlement of larvae from different gene pools and differing recruitment histories among sites within the eastern Baie de Seine. At a larger scale, similar levels of spatial differentiation were observed in 1999 between the eastern Baie de Seine and the Baie des Veys. The lack of any significant differences in gene diversity and allelic richness rules out a source–sink functioning at the scale of our study. The present paper provides further knowledge on the population dynamics of a univoltine species and its persistence in a highly dispersive environment via a shifting spatial mosaic.

Keywords *Pectinaria koreni* · Microsatellites · Metapopulation · Genetic heterogeneity · Effective dispersal

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Introduction

Species are typically divided into a number of populations more or less interconnected by migration. Such a dynamic feature has many evolutionary consequences on populations, especially in terms of genetic diversity and population structuring. Due to a finite population size, genetic drift would tend to decrease the total amount of genetic diversity in small populations unless it is counterbalanced by migration. Besides, populations have finite life expectancies, making local extinction/recolonisation processes likely to be a key factor for the persistence of the species at the level of a set of local populations. Although the implication of subdivision and persistence of local populations on the evolutionary dynamics of species has long been emphasised (Wright 1940; Andrewartha and Birch 1954; MacArthur and Wilson 1967), the term "metapopulation" was first introduced by Levins (1969) and originally referred to a spatially structured population which persists through re-colonisation events despite localised extinctions.

Pannell and Charlesworth (2000) have reviewed the implications of extinction/recolonisation dynamics on the amount of genetic differentiation between local populations. They consider two founding models (the <migrant pool and the propagule pool), each having different genetic consequences depending on the theoretical population model used, the rate of extinction, and the migration rate (Slatkin 1977; Whitlock and McCauley 1990). Under the *n*-island-model with migrant pool colonisation, extinction and recolonisation tend to reduce genetic differentiation and increase the genetic diversity of the newly established populations only when the number of founders colonising new vacant areas is twice the number of migrants exchanged between extant populations (Wade and McCauley 1988; McCauley 1991). Under a propagule pool model, extinction is expected to (1) increase the level of differentiation relative to the case of no local extinction (Wade and McCauley 1988; Whitlock and McCauley 1990), and (2) reduce the amount of genetic diversity in newly established popula-

tions (Pannell and Charlesworth 2000). However, these predictions are also affected by the metapopulation structure itself (Dias 1996; Harrison and Hastings 1996; Hanski and Gilpin 1997). The extinction/recolonisation process establishes an age structure among local populations, which may either enhance or reduce the amount of genetic differentiation among subpopulations, as a function of both the migration model and the relative importance of parameters such as the number and the origin of founders (Slatkin 1977; Whitlock and McCauley 1990; Pannell and Charlesworth 2000).

Given those theoretical studies, the marine environment offers considerable potential for metapopulation studies. First, it is a highly dispersive environment characterised by strong substrate heterogeneity. Secondly, most benthic invertebrates are characterised by a complex life-cycle involving a benthic adult breeding stage followed by a dispersive larval stage (Thorson 1946), the duration of which may vary from only a few hours up to several months. As a consequence, the persistence and stability of fragmented benthic populations is governed by two processes: (1) the rate of migration, which depends on hydrodynamic processes (i.e. advective currents, turbulent mixing and eddy diffusion), intrinsic biological properties of the larvae, the reproductive effort of the adults and larval interactions in the plankton, and (2) the rate of population extinction, which also relies on hydrodynamics (i.e. disturbance at the benthic boundary layer), larval recruitment processes (i.e. substrate choice, intra-/inter-specific competition) and predation on adults. According to the supply-side theory (Underwood and Fairweather 1989; Grosberg and Levitan 1992), the spatio-temporal dynamics of bentho-pelagic populations, and thus the extinction rate, is initially dependent on larval input which is, in turn, influenced either by larval dispersal and larval retention in the vicinity of adult populations or by larval homing towards the adult population after a short dispersal phase.

In this context, the tubicolous polychaete *Pectinaria koreni* (Malmgren) is characterised by interesting biological features which can be used to analyse the metapopulation processes governing the evolution of natural populations in the marine environment. Based on recent findings, populations of this univoltine species living for 15–18 months may be described as an assemblage of geographically isolated populations potentially linked together by a planktonic larval phase of approximately 15 days (Lagadeuc and Retière 1993; Ellien et al. 2000). Thus, populations of *P. koreni* might constitute a metapopulation along the French coast of the eastern English Channel. Indeed, at the scale of the English Channel, both a strong tidal regime and the action of wind-induced currents govern patterns and rates of larval dispersal during the reproductive season (Ellien et al. 2000). In addition, demographic monitoring of French populations has revealed that some are transient whereas others are not (Ellien et al. 2000; F. Gentil and E. Thiébaut, personal observation), which supports the adequacy of a source–sink metapopulation model (as defined by Pulliam 1988, with growth rate (r) being >0 in sources and <0 in sinks in the absence of migration) at least at the regional scale of the Baie de Seine (eastern Baie de Seine; Baie des Veys). In this study, we use molecular tools to investigate the likelihood of a source–sink functioning between the eastern Baie de Seine (BS) and the Baie des Veys (BV).

The eastern Baie de Seine may correspond to a potential source because it is considered to be the most important population in the English Channel both in density and surface area (300–650 individuals per square metre on a 400 km^{-2} area), and it exhibits a relatively stable inter-annual recruitment (Thiébaut et al. 1997). On the other hand, the Baie des Veys population might be regarded as a sink: suitable habitats are significantly smaller (in the order of 100 km^{-2}) and the population is demographically unstable, fluctuating from extremely low densities which may eventually lead to extinction, up to relatively high abundances (Ellien et al. 2000). In addition, hydrodynamic modelling of larval dispersal reveals that potential exchanges between both bays take place mainly from the eastern Baie de Seine towards the Baie des Veys (Ellien et al. 2000). Although there is no direct evidence of extinction events in the latter site between 1973 and 2000, the lack of annual demographic data does not allow definite conclusions (i.e. demographic data were collected at irregular time intervals, thereby disregarding the univoltine character of the species). Molecular ecology may provide powerful tools to describe and determine the relationships between populations in terms of recruitment, dispersal and origin of founders.

The present study aims to address the following questions: (1) Considering that *P. koreni* is univoltine, is there any temporal genetic variation in a population at one particular patch that could indicate an interannual settlement of genetically different larval cohorts? (2) Is the potential source population a mosaic of several genetic entities or are the patches relatively well interconnected? (3) Does the Baie des Veys represent a sink population? (i.e. can extinction events take place without larval supply from other geographic origins?), in which case lower levels of genetic diversity are expected relative to the source because of genetic drift associated with founding (Gaggiotti and Smouse 1996). Finally, are the results provided by hydrodynamic modelling (Ellien et al. 2000) representative of the effective larval dispersal?

Methods

Biological model and sampling procedure

Populations of *P. koreni* (Malmgren) exhibit a highly fragmented distribution throughout the English Channel, associated with the distribution of muddy fine sands confined to bays and estuaries. Similarly, at the scale of a population, individuals form aggregates of highly variable densities (Lambert 1991). *P. koreni* is characterised by a bentho-pelagic life cycle with a main breeding period occurring between March and July, with the release of 20,000–430,000 oocytes per female (Elkaim and Irlinger 1987;

Table 1 Population names, location, sampling year and numbers of *Pectinaria koreni* (*N*) screened for microsatellite analysis for each sampling year

	Latitude	Longitude	Date	*N*
Eastern Baie de Seine				
BS15	49°27.50′ N	0°02.30′ E	March 1999	33
BS29	49°18.50′ N	0°91.98′ W	March 1999	54
BS191	49°28.30′ N	0°02.28′ E	May 1994/95/96	24/24/24
BS123	49°36.10′ N	0°04.50′ E	May 1994	23
BS186	49°23.76′ N	0°03.45′ E	May 1994/96	25/19
Baie des Veys				
BV21	49°32.00′ N	1°15.50′ W	March 1999	40

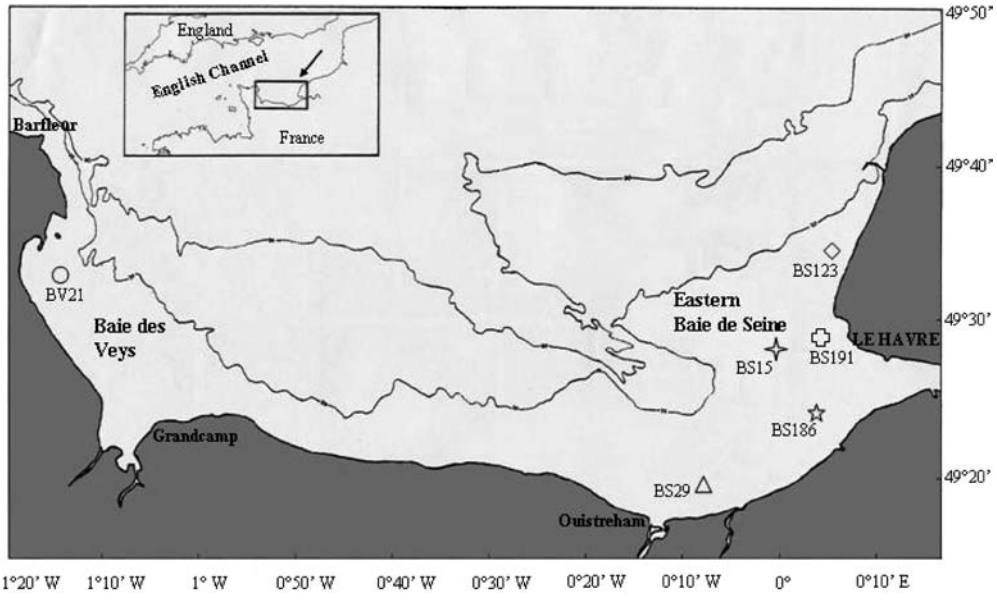

Fig. 1 Study area and location of sampling stations in the Baie de Seine [eastern Baie de Seine (*BS*); Baie des Veys (*BV*)] for 1994 and 1999 (details are given in Table 1). *Labels* represent sampling locations

Irlinger et al. 1991). Larvae may be transported up to 30–40 nautical miles from their site of release, depending on the hydrodynamic residual circulation and on the intensity of environmental forcing (Lagadeuc 1992; Thiébaut et al. 1998). In addition, Lambert (1991) showed that post-larval re-settlement may take place if the substrate where larvae first settle is not appropriate. While the density of settling post-larvae may reach 28,409 ind. m^{-2}, intraspecific competition may eliminate 40%–100% of young settlers (Lambert 1991).

Sampling was undertaken from May 1994 to March 1999 within the Baie de Seine. A preliminary survey of five sites was performed within the eastern Baie de Seine (1994–1999) and one additional site was sampled in the Baie des Veys (1999) (see Table 1 for details). Individuals of *P. koreni* were obtained by sampling the top 10 cm of the sediment with a 0.25 m^{-2} Hamon grab. Samples were sorted directly on board, flash-frozen and kept in liquid nitrogen until DNA extraction. The study area is represented by Fig. 1.

DNA extraction and microsatellite genotyping

Gills were removed while individuals were still frozen and grinded directly in 600 µl of a 60°C preheated 2% CTAB (cetyltrimethylammonium bromide) buffer solution, which differentially precipitates DNA from polysaccharides. The tissues were digested at 60°C with 0.1 mg ml^{-1} proteinase K and the extraction was performed overnight. Proteins, lipids and carbohydrates were removed using a standard phenol/chloroform procedure and DNA was precipitated with isopropanol after 1 h incubation at 37°C in the presence of 2 µl RNase (500 µg/ml) for RNA removal. The tubes were finally centrifuged at 15,000 rpm for 10 min. DNA pellets were washed with 70% ethanol and resuspended in 20–50 µl 1×TE (10 mM Tris- 20 mM EDTA pH 8) buffer prior to electrophoresis on 0.8% agarose gels to check the quality of the extracted DNA.

A total of 218 individuals were screened over four highly polymorphic microsatellite loci (PKGT1; PKAT/GT1; PKAT/GT2 and PKAT/GT4) isolated by Weinmayr et al. (1999).

DNA amplifications were performed on an MJResearch thermal cycler (PTC-200) with the following conditions: first an initial denaturation step at 94°C for 5 min, followed by 40 cycles of denaturation at 94°C for 1 min, annealing for 40 s (PKAT/GT1–2-4 at 58°C and PKGT1 at 51°C) and elongation at 72°C for 35 s and a final elongation at 72°C for 10 min. PCR reactions were performed into a 20 µl reaction volume consisting of 1×PCR buffer (supplied with polymerase enzyme); $MgCl_2$ at a concentration of 1.5 mM (PKGT1) or 2 mM (PKAT/GT1–2-4); 0.12 mM dNTP; 0.8 µM of forward and reverse primers; 0.01 µg $µl^{-1}$ T4gene32 protein; 0.5 U of high fidelity *Taq* polymerase (ABgene) (PKAT/GT1–2-4) or 0.5 U Thermoprime plus *Taq* polymerase (ABgene) (PKGT1); 25–100 ng CTAB-extracted genomic DNA. For each locus, one of the primers was labelled with Texas Red fluorochrome for genotyping. PCR products were run on a 6% polyacrylamide/8 M urea sequencing gel, using a Vistra DNA sequencer (Amersham Pharmacia Biotech).

Statistical analysis

Genetic diversity as summarised by (1) the observed heterozygosity (H_o), (2) the expected heterozygosity (H_{NB}; Nei 1987), (3) the number of alleles per locus per population (N_O) and the allelic richness (R_S), was estimated using FSTAT 2.9 software (Goudet 1995). Standard errors of gene diversity values were estimated using GENETIX 4.02 (Belkhir et al. 1996) and tested using a simple Student's *t*-test. Tests of genotypic disequilibrium between each pair of loci in each population and over all populations were performed by using Fstat 2.9 (Goudet 1995). Deviations from Hardy-Weinberg equilibrium were examined for each population at each locus by calculating Wright's fixation index F_{IS} as estimated by Weir and Cockerham's (1984) *f* and using Fisher's exact test on GENEPOP 3.3 software (Raymond and Rousset 1995).

The genetic structure over all the samples was first analysed by calculating Wright's F_{ST} statistics for each locus, estimated with GENEPOP 3.3 (Raymond and Rousset 1995) following Weir and Cockerham's (1984) θ value. Exact tests for the null hypothesis of identity of allelic distribution across populations were performed with GENEPOP 3.3. Multiloci θ values were also estimated and tested between pairwise combinations of populations by using the same software. To illustrate the extent of both spatial and temporal genetic variation among samples, a principal components analysis (PCA) was performed on allelic frequency data using PCAGEN 1.2 software (Goudet; http://www.unil.ch/izea/research.html#softs).

Results

Genetic diversity

Testing for linkage disequilibrium did not reveal any association of alleles between any of the four diploid loci examined, demonstrating the independence of the information provided by the four loci used.

Overall, the fixation index (F_{IS}) is high across all loci, with significant departure from Hardy-Weinberg proportions (multilocus average F_{IS}=0.341; P<0.001). Such heterozygote deficiencies are not explained by specific reproductive traits, but may result from the presence of genetically differentiated entities (Whalund effect). However, our data also suggests that null alleles may occur (Weinmayr et al. 1999; M.T. Jolly, personal observation). Nevertheless, the lack of correlation between F_{IS} and the number of individuals per locus which were not amplified suggests that null alleles are only partly responsible for the inflated F_{IS} values. Moreover, the F_{IS} values are almost identical whatever populations or sampling dates. All together, this indicates that our conclusions on the genetic structure should not be affected. However, new studies are under progress to determine the origin of these large F_{IS} values.

High and similar levels of genetic diversity (N_O; H_{NB}; R_S) were observed throughout the eastern Baie de Seine in both 1994 and 1999 (Table 2), and for all loci. With the exception of PKAT/GT4, for which a lower number of alleles was sampled in 1994, levels of gene diversity (H_{NB}) were similar for both PKAT/GT1 and PKAT/GT2 ($H_{NB(mean)}$=96%). The number of alleles increases with sample size, indicating that sample sizes are probably too small to reflect the total diversity of *P. koreni* for the four loci. Comparable levels of genetic diversity have been found between the eastern Baie de Seine and the Baie des Veys (1999) (Table 2). This supports the idea that genetic drift is limited due to the potentially huge effective population size of *P. koreni*. However, significant differences in H_{NB} were found between BS29/BV21 at both PKGT1 and PKAT/GT4 (t=2.2108; P<0.05, and t=3.336; P<0.01, respectively) (Fig. 2).

Spatial heterogeneity

Within the eastern Baie de Seine (1994 and 1999)

The locus PKGT1 was excluded from the 1994 analysis as data were missing for two sampling sites. In 1994, low F_{ST} values were observed throughout (0.001<pairwise F_{ST}<0.012) (Table 3), but both pairwise tests of differentiation (0.0001<P value<0.004) and multi-loci F_{ST} (F_{ST}=0.006; P=0.0001) (Table 4) reveal low but significant genetic substructuring within the eastern Baie de Seine.

Pairwise tests for 1999 data (Table 5) reveal a small but significant level of genetic differentiation (F_{ST}= 0.001; P<0.001) between BS15 and BS29, which is consistent with the levels reported for the 1994 samples between BS186 and BS191 (geographically closest to the 1999 sampling stations). Similarly to 1994, differentiation was mainly due to PKAT/GT2 (F_{ST}=0.006; P=0.0001).

Table 2 Genetic diversity and heterozygosity for each sampling site for all loci and for 2 years (total number of alleles) *N* numbers of individuals, N_O average number of allèles, H_{NB} non-biased expected heterozygosity, R_S allelic richness based on a minimum sample size of 10 individuals except for PKGT1; eastern Baie de Seine (*BS*); Baie des Veys (*BV*)

	PKGT1[34][a]				PKAT/GT1 [48]				PKAT/GT2 [59]				PKAT/GT4 [26]			
Sampling sites	*N*	N_O	H_{NB}	R_S	*N*	N_O	H_{NB}	R_S	*N*	N_O	H_{NB}	R_S	*N*	N_O	H_{NB}	R_S
BS191-94	–	–	–	–	22	24	0.946	14.2	23	22	0.963	14.3	23	19	0.908	11.8
BS186-94	–	–	–	–	22	24	0.972	15.2	23	21	0.949	13.2	22	14	0.866	9.0
BS123-94	–	–	–	–	20	21	0.956	13.8	20	19	0.962	14.0	21	18	0.923	11.9
BS15-99	23	17	0.946	15.7	28	24	0.955	13.7	26	28	0.968	15.1	28	20	0.935	12.1
BS29-99	39	23	0.952	17.5	19	18	0.937	12.9	32	27	0.964	14.6	17	17	0.959	13.4
BV21-99	29	18	0.939	15.6	31	22	0.949	13.1	27	28	0.971	15.4	26	17	0.935	11.7

[a] R_S based on a minimum sample size of 17 individuals

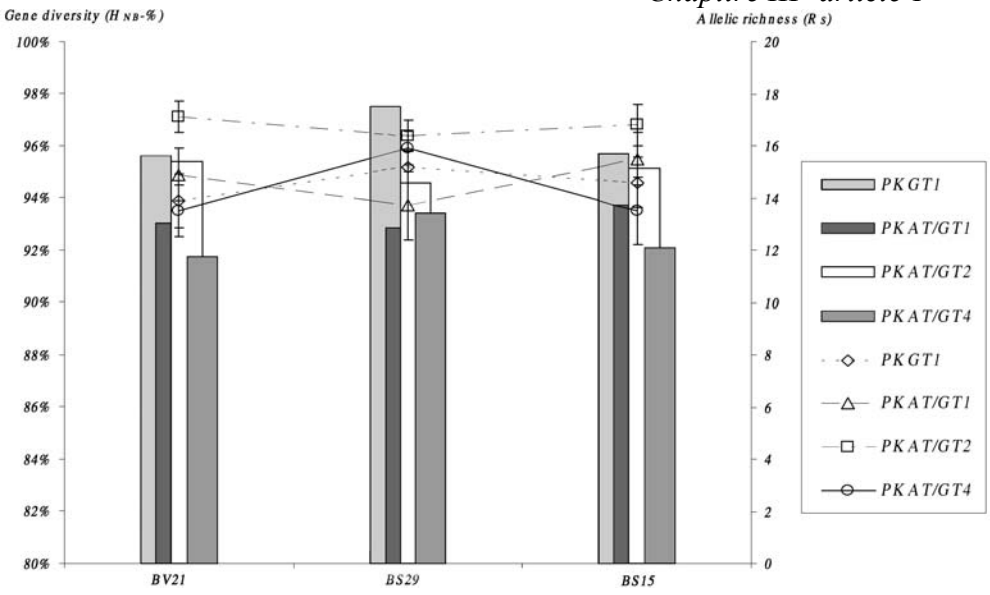

Fig. 2 Graph showing the fluctuations of the gene diversity H_{NB} (Nei 1987) as represented by *lines with standard error bars* (obtained from 1,000 bootstraps), and the allelic richness (R_S) as represented by histograms, for all 1999 samples and for all loci. R_S(PKAT/GT1–2-4) are based on a minimum sample size of 10 diploid individuals whereas R_S(PKGT1) is based on a minimum sample size of 17 diploid individuals

Table 3 Genetic differentiation within eastern Baie de Seine (*BS*) in 1994; Pairwise F_{ST} values at three diploid microsatellite loci (missing data for PKGT1)

	BS191-94	BS186-94	BS123-94
BS191-94	–	0.001**	0.005***
BS186-94		–	0.012***
BS123-94			–

** P<0.01, *** P<0.001

Table 4 Total F_{ST} values for all loci and multilocus average (P value in parentheses) for 1994 and 1999 data [eastern Baie de Seine (*BS*); Baie des Veys (*BV*)]. Tests were performed using Genepop (Raymond and Rousset 1995)

Locus	F_{ST} (BS)-1994	F_{ST} (BS/BV)-1999
PKGT1	–	0.013***
PKAT/GT1	0.005**	–0.001
PKAT/GT2	0.004***	–0.0004
PKAT/GT4	0.008*	0.005**
Multilocus average	0.006***	0.004***
	(0.0001)	(0.001)

* P<0.05, ** P<0.01, *** P<0.001

Table 5 Genetic differentiation within the Baie de Seine in 1999 [eastern Baie de Seine (*BS*); Baie des Veys (*BV*)]. Pairwise F_{ST} values at four diploid microsatellite loci

	BS15-99	BS29-99	BV21-99
BS15-99	–	0.001***	0.008***
BS29-99		–	0.002***
BV21-99			–

*** P<0.001 after sequential Bonferroni correction

Between eastern Baie de Seine and Baie des Veys (1999)

Small but significant genetic differentiation across all 1999 sites (Table 4) (multiloci F_{ST}=0.004; P=0.001) was caused by two loci (PKGT1 and PKAT/GT4). Pairwise F_{ST} values (Table 5) of genetic differentiation are four times higher between BS15 and the Baie des Veys (F_{ST}=0.008; P<0.001) than between the latter site and BS29 (F_{ST}=0.002), suggesting that migration between the two bays would proceed preferentially along the coast via BS29. Elsewhere, the level of differentiation between the eastern Baie de Seine and the Baie des Veys was comparable to that obtained in 1994 (Table 4) and 1999 (Table 5) within the first site.

Overall differentiation

Results from principal components analysis (Fig. 3) using all our genetic data show both a strong temporal and spatial genetic heterogeneity within the eastern Baie de Seine, and between this site and the Baie des Veys. The first axis represents 32.5% of total inertia (i.e. corresponding to a significant F_{ST}=0.01), whereas the second represents only 14.5% of the genetic differentiation.

Temporal variation

Allele frequencies of *P. koreni* were estimated in samples from 1994/1995/1996 and from 1994–1996 at BS191 and BS186, respectively (Fig. 1), in order to trace potential temporal genetic heterogeneity in the eastern Baie de Seine. Table 6 indicates the estimated P values for temporal allelic differentiation for each locus and over the four diploid loci. At both stations a significant differentiation is observed for the most polymorphic locus

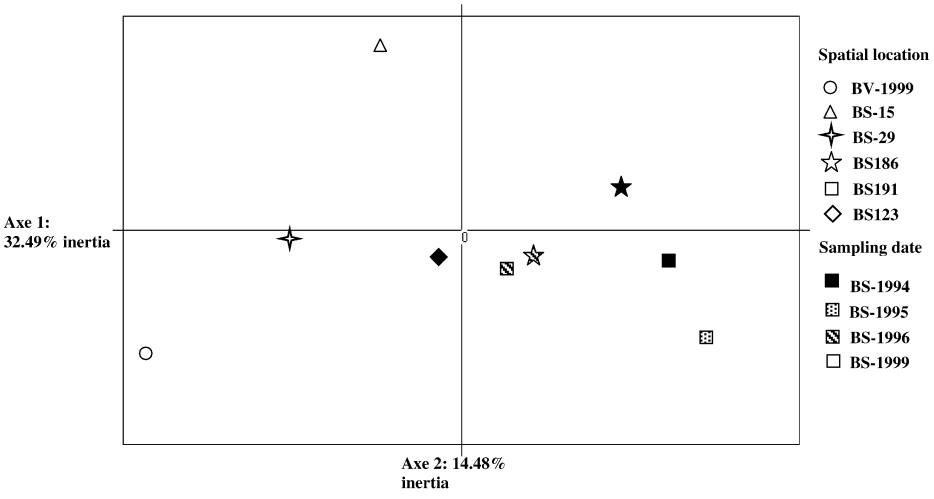

Fig. 3 Principal components analysis (*PCA*) performed using the allele frequency table available for all years and for all sampling stations [eastern Baie de Seine (*BS*); Baie des Veys (*BV*)]. Sampling locations are represented as in Fig. 1

Table 6 Temporal variation *P* values for exact tests of allelic distribution (Weir and Cockerham 1984) for all loci between 1994 and 1996 at two sampling stations within the eastern Baie de Seine (*BS*)

Locus	PKGT1	PKAT/GT1	PKAT/GT2	PKAT/GT4	All loci
BS191-1994/95/96					
P value	0.075	0.611	0.002	0.413	0.008
BS186–1994/96					
P value	–	0.475	0.0001	0.027	0.0002

PKAT/GT2 and to a lesser extent PKAT/GT4 at station BS186. Three loci did not present any differentiation at BS191 (PKGT1, PKAT/GT1–4; $0.075<P<0.611$), and one at BS186 ($P_{(PKAT/GT1)}=0.48$).

Overall, both sampling stations exhibit marked temporal genetic differentiation (multiloci *P* value<0.01 and 0.001 at BS191 and BS186, respectively). Such a structure seems to indicate that pulses of larvae settling between 1994 and 1996 were genetically different.

Discussion

In this study we used four highly polymorphic microsatellite loci to demonstrate (1) a large genetic diversity throughout the Baie de Seine and (2) a small but significant genetic differentiation over time and space. We discuss these results in relation to the relative importance of larval dispersal and retention for the maintenance of both the eastern Baie de Seine and the Baie des Veys populations.

Genetic heterogeneity in the eastern Baie de Seine

Despite the idea that species with long planktonic dispersal are more or less genetically homogeneous over relatively large distances, statistical comparisons of allele frequencies among spatial and temporal samples of *P. koreni* within the eastern Baie de Seine both reveal significant genetic heterogeneity which may be categorised as "chaotic" in a way similar to what Johnson and Black (1982) found for *Siphonaria jeanae*. Indeed, this heterogeneity does not follow a consistent pattern but forms a shifting spatial mosaic (with abundances of *P. koreni* and genetic composition changing on every patch in each generation) without any microspatial directionality in gene flow. It is probable that such a structure is correlated with the variability of wind-induced currents which may, given changes in wind speed and direction, bring about a strong variability in larval dispersal and recruitment patterns (Lagadeuc 1992).

Temporal and spatial genetic change may be explained by (1) a large variance in reproductive success between patches (Hedgecock 1994), and (2) the settlement of differentiated larval cohorts and thus the occurrence of several genetic entities (David et al. 1997). Both hypotheses will be discussed.

The fact that adults are distributed in patches of highly variable densities offers considerable potential for variance in reproductive success between adult aggregates. External fertilisation success has also been shown to be reduced drastically by the dilution of sperm and gametes when spawning adults occur at low densities (Levitan 1991). This strongly suggests that only disjunct massive aggregates of *P. koreni* are likely to reproduce effectively, possibly leading to the occurrence of different larval pools within the eastern Baie de Seine. However, such a variance in reproductive success is unlikely to be the only explanation for such levels of both temporal and spatial differentiation, although the contribution to a given cohort by a limited number of adults may re-

sult in significant drift effects between cohorts (David et al. 1997). Such a variance linked to the loss of larvae during the pelagic phase and/or the failure to reproduce (variable among patches), would tend to decrease total effective population size (*N*e) and therefore result in demographic instability and increased genetic drift. In contrast, the eastern Baie de Seine population is characterised by a sex ratio close to 1:1 (Irlinger et al. 1991), and is larger (up to 650 ind. m^{-2}; Lambert 1991) and demographically more stable than the Baie des Veys population, presenting a high level of genetic diversity among all patches. Because high genetic diversity may only be explained by either a large effective population size or a high mutation rate, the effective size of the population is expected to be considerable, although probably lower than the census size, as a function of variance in reproductive success. One way to estimate the effective population size is to use the method developed by Waples (1989), based on the analyses of the distribution in allelic frequencies over time. However, this method requires the assumption that the eastern Baie de Seine is a closed population, which does not seem to be true in the light of hydrodynamic modelling of potential larval dispersal (Ellien et al. 2000).

The hypothesis that the temporal heterogeneity of recruits is responsible for spatial genetic discontinuities at a local scale has been proposed from allozyme studies on several species characterised by a long planktonic dispersal phase (e.g. limpets, Johnson and Black 1984; sea urchins, Watts et al. 1990; crabs, Kordos and Burton 1993; McMillen-Jackson et al. 1994; and bivalves, David et al. 1997).

P. koreni is a univoltine species for which two recruitment events (May and July) have previously been observed within the eastern Baie de Seine (Irlinger et al. 1991), the first one being much more important than the second. During the spawning season, several reproductive events may take place at different sites within the eastern Baie de Seine, thereby producing different larval cohorts from different local geographical origins. Depending on the prevailing hydrodynamic conditions at each site of release, dispersal patterns will be affected differently, resulting in variable larval retention rates and geographic shifts in post-larval settlement patterns (Lagadeuc 1992; Ellien et al. 2002).

Given that the retention rates in the eastern Baie de Seine are greater than larval export rates (Ellien et al. 2000), pools of larvae from genetically different local entities within the eastern Baie de Seine may evolve separately according to their respective points of egg release, and subsequently settle at any site of the bay, replacing the old generation and forming a "shifting spatial mosaic" of genetic entities. Temporal variation may thus result from a reproductive asynchrony between genetically different adult aggregates within the eastern Baie de Seine or among nearby populations (Baie des Veys, Penly), depending on local disturbance and habitat quality. Asynchronous spawning events and variance in reproductive success could produce aggregates of differing genetic composition which would show as much genetic heterogeneity within a single patch as between different patches. In contrast, repeated extinction/recolonisation events are the main processes involved in producing similar amounts of both spatial and temporal genetic differentiation in the hydrothermal vent populations of *Alvinella pompejana* (Jollivet et al. 1998).

Spatial differentiation at the scale of the Baie de Seine

A small but highly significant differentiation is observed between the eastern Baie de Seine and the Baie des Veys in 1999. In fact, a similar level of differentiation is detected between sampling sites (i.e. patches or demes) within the eastern Baie de Seine in 1994 as between both distant populations in 1999, but no true extinction events have yet been evidenced. As explained by Balloux and Lugon-Moulin (2002), such small F_{ST} values do not necessarily mean that there is a weak genetic differentiation. Those values are deflated because of the high genetic diversity exhibited by our microsatellite markers.

The assumption of a source–sink metapopulation at such a regional scale (i.e. the Baie de Seine), in which the eastern Baie de Seine would be the source of migrants could explain differentiation by drift as long as the number of colonists arriving from the source is less than the number of migrants exchanged within the source populations. Although both populations are genetically different, there are no overall significant differences in the allelic richness and gene diversity. Therefore, a source–sink functioning at the scale of our study is unlikely.

Elsewhere, under a classical metapopulation model with extinction/recolonisation, we would expect to find greater variation in the genetic diversity between demes than in a subdivided population without recurrent extinction (Pannell and Charlesworth 1999, 2000). Our results therefore tend to indicate that the eastern Baie de Seine and the Baie des Veys belong to one single subdivided population made up of several breeding subpopulations or demes. Alternatively, as genetic differentiation values are close to significance thresholds, both populations may constitute one open population close to subdivision.

Under a migrant pool type of colonisation without extinction and with an unequal recruitment between demes (in both the eastern Baie de Seine and the Baie des Veys), genetic differentiation would be minimised, thereby resulting in no significant differences in genetic diversity. Thus, we may consider all patches as potential sources or sinks, depending on the prevailing hydrodynamic and stochastic environmental processes, where the rate of exchange depends on both the size of the patches and their spatial configuration. Patches are ephemeral and the yearly replenishment of the population is accompanied by a shift in the location of aggregates due to changes in hydrodynamic conditions during the spawning season. The Baie des Veys population probably persists chaotically, as no consistent pattern of colonisa-

tion/migration is observed, but in a shifting balance between reproduction, larval supply and the environmental stochasticity, thereby producing a mosaic of genetic entities throughout the whole Baie de Seine.

Fitting population genetic data to larval dispersal modelling

The Baie des Veys population appears to be genetically distinct from the eastern Baie de Seine. We can therefore assume that migration is substantially reduced between both populations, although both have similar levels of gene diversity and allelic richness. This result agrees relatively well with the 2-D larval dispersal model (Ellien et al. 2000, 2002), in that migration between both bays is very limited, with the Baie des Veys populations persisting at low densities. Migration mainly (if not only) occurs from the eastern Baie de Seine to the Baie des Veys with north eastern winds of 7 m s^{-1} (Ellien et al. 2002) (the reverse is only possible for strong south western winds of 15 m s^{-1}), with migration events occurring preferentially via sites which are at the range limit of both populations (e.g. BS29), which agrees with our genetic data. According to Ellien et al. (2000), larval retention rates in the Baie des Veys are more stable relative to the eastern Baie de Seine area, whatever the wind speed. This is mainly because tidal advection is stronger in the former site than in the latter, where diffusion is the dominant process. Larvae released in the Baie des Veys are therefore trapped in the Barfleur gyre, resulting in a more stable self-recruitment than in the eastern Baie de Seine. As a result, populations in the Baie des Veys may still persist locally in the absence of continuous migration events from the eastern Baie de Seine. Both modelling and genetic data therefore tend to discard the possibility of a source–sink metapopulation at the scale of the Baie de Seine.

Although colonisation of larvae from neighbouring populations into the eastern Baie de Seine is potentially weak (Ellien et al. 2000, 2002), potential larval fluxes may also occur from Penly (situated further north along the French coast) to the eastern Baie de Seine only when north eastern winds of 9 m s^{-1} are blowing (the reverse is possible only for south western winds of 12 m s^{-1}). The eastern Baie de Seine could therefore recruit stochastically from Penly, which in turn may be connected, under certain hydrodynamic conditions, to populations living around the Straits of Dover. Although the relative importance of delayed larval metamorphosis and post-resettlement behaviour on migration rates has not yet been quantified, migration may possibly be enhanced by such processes.

The present paper provides evidence for temporal and spatial genetic heterogeneity in a univoltine marine species with planktonic dispersal. The genetic structure of *P. koreni* populations in the Baie de Seine is that of a shifting mosaic, where genetic patchiness is renewed in each generation. Contrary to previous expectations from demographic monitoring, there is no source–sink process (*sensu* Pulliam 1988; Gaggiotti 1996) between the eastern Baie de Seine and the Baie des Veys. Rather, both populations seem to constitute one single subdivided population, where migration is oriented from the Seine estuary to the Baie des Veys, with the latter site able to sustain itself at least in the short term.

There is an obvious need for a wider study at the scale of the English Channel in order to determine whether populations on both French and English coasts constitute a metapopulation and to what model they conform. Elsewhere, a more thorough sampling programme needs to be applied in both the eastern Baie de Seine and the Baie des Veys, in order to accurately estimate the effective population size and the total genetic diversity. A longer temporal genetic study of adults in these two areas would enable us to monitor patterns of genetic changes over time and to see whether there is synchrony/asynchrony in the genetic composition of the two areas. In addition, the analysis of larval clouds and/or newly settled recruits in the Baie de Seine and along the French coastline would provide information on their respective origins (within the bay or from other populations), in an attempt to determine the source of temporal variation in the genetic composition of recruits.

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Spatial and temporal genetic variation in a local metapopulation of the polychaete tubeworm *Pectinaria koreni* in the Baie de Seine (France): testing the shifting spatial mosaic hypothesis

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En préparation

Abstract

Populations of sessile coastal invertebrates which display a bentho-pelagic life cycle are often composed of several, disjunct local larval sources. Among these, local landscape features and stochastic environmental fluctuations may directly influence biotic characteristics such as growth rate and interaction strength. Depending on patch location and size, food availability and competition, strong physiological or phenotypic adjustments may lead to asynchronous local dynamics and temporal shifts in the genetic composition of local demes. The polychaete *Pectinaria koreni* is characterised by discrete generations whereby the spawning season coincides with population turnover and local extinction-recolonisation events. Adult demes in the Baie de Seine form a mosaic of differentiated entities structured in a chaotic manner both spatially and temporally. While the population dynamics of the species may contribute to a "shifting spatial mosaic" structure at that scale whereby genetic variation is renewed on every patch at each generation, it is unclear whether such a structure is directly associated with the coexistence of differentiated larval cohorts at each generation or is the result of micro-evolutionary forces acting on structuring the adult aggregates after post-larval settlement. To test whether significant levels of micro-scale differentiation could exist throughout the reproductive period between temporal samples collected at different localities in the Baie de Seine, we performed a spatial and temporal sampling of the individuals before, during and throughout population turnover.

Keywords : spatio-temporal genetic structure, reproductive asynchrony, micro-evolutionary forces

Introduction

In essence, large scale patterns of evolution cannot be fully understood without further knowledge of the underlying micro-evolutionary changes taking place within natural populations. At the level of a group of interconnected subpopulations (i.e. a metapopulation), local adaptations may arise from fine-scale population differences (variance in reproductive success, asynchrony in reproductive timing, metapopulation processes) which may be extrapolated over many generations to drive speciation and macro-evolution if the factors responsible are sustained over time (see Hendry & Troy, 2005). Especially in cases where extinction-recolonisation dynamics are apparent, metapopulation properties have wide consequences for both macro and micro-evolutionary processes (McCauley, 1991; Gaggiotti & Smouse, 1996; Pannell & Charlesworth, 1999, 2000; Schlutter, 2000) as allele frequencies will be directly affected over time, either because of the finite size of closed subpopulations, or because of migration in open subpopulations. Since temporal variation in genetic structure provides the background for micro-evolutionary changes to occur, investigating local population dynamics together with a spatial and temporal population genetic approach may reliably capture information on (1) the effects of population turnover and (2) essential parameters of population evolution such as the effective population size (N_e), the ratio of colonists-to-migrants and the patterns of gene exchange. With regards to the above, the empirical study of species with short generation times and high population subdivision is particularly interesting because they have the greatest potential for rapid adaptive changes in relation to the heterogeneity of the habitat.

The polychaete tubeworm *Pectinaria koreni* (Malmgren, 1865) constitutes an ideal and well studied biological model to tackle the implications of metapopulation properties on the micro-evolutionary processes that affect population structure at both local and mesoscales. First, the species has discrete (i.e. non overlapping) generations. Adults live up to 15- 18 months (Elkaïm & Irlinger, 1987), have an equilibrated sex ratio (close to 1:1 according to

Irlinger *et al*., 1991) and die off after spawning. Second, individuals form aggregates of highly variable densities which may exhibit differences in reproductive timing (Lambert, 1991), and differences in fecundity and growth rate depending on local environmental conditions (Brouazin, 1988; Lambert, 1991). This implies a potential for a large variance in reproductive success between adult aggregates which may be strengthened by the effects of a great mortality rate during the early larval and juvenile stages: intraspecific and interspecific competition may eliminate 40% to 100% of the young settlers (Lambert, 1991). This makes the combination of demography and spatio-temporal genetic studies in this species very attractive in terms of studying how the interaction of biotic and abiotic factors such as post-resettlement mechanisms (habitat selection and competition avoidance) affects the distribution of the genitors in the Baie de Seine (France; Lambert, 1991; Olivier *et al*., 1996).

The considerable level of spatial patchiness and abundance heterogeneity caracterising the Baie de Seine population, together with discrete recruitment periods during the spawning season, could suggest aggregate-dependant reproductive events which may produce separate larval cohorts from different local geographic origins. Depending on the prevailing hydrodynamic conditions at each site of release, dispersal patterns may be affected differently, resulting in variable larval retention rates and geographic shifts in post-larval settlement patterns (Lagadeuc, 1992; Ellien *et al*., 2004). Together with a large variance in the recruitment rates and the heterogeneity of the habitat in space and time, both the biological and ecological characteristics of *P. koreni* make the local model of *meta*population structure in the Baie de Seine a very interesting area of research with regards to confronting the theoretical predictions of metapopulation models with empirical data on the population structure over a few discrete, non-overlapping generations.

A previous study only provided a “snapshot” of the spatial and temporal genetic structure of *P. koreni* in the Baie de Seine (Jolly *et al*., 2003), but showed that usual metapopulation

source-sink dynamics were unlikely to describe the relationship between both embayments: among adults, no reduction in genetic diversity was detected between the putative sink (i.e. the Baie des Veys) and the putative source (i.e. the eastern Baie de Seine), and highly significant genetic structuring was observed across all sampling locations of the Baie de Seine. Whether embayments represent two distinct functional entities or one spatially structured population, the whole Baie de Seine nonetheless reflects true metapopulation properties. As stated by Lambert (1991) who described two stable genitor populations in the eastern Baie de Seine, more than one source of larvae exists and at least some aggregates may exist as sources and sinks. All together, Jolly *et al*. (2003) concluded that the strong spatial and temporal genetic patchiness in the Baie de Seine was the result of the coexistence of differentiated larval "cohorts" at each generation thereby creating an original "shifting spatial mosaic" structure corresponding to yearly population turnover. However, the large number of processes structuring adult subpopulations between the recruitment period and the spawning season, together with the fact that too few individuals were collected at each locality and each sampling date (once a year) might have biased estimates of genetic diversity and genetic differentiation.

In this study, we undertook an extensive and finer spatio-temporal survey over one generation to verify or rectify previous assumptions and to discriminate more readily between micro-evolutionary processes shaping the "*meta*"population of the Baie de Seine. We performed a spatial and temporal sampling of the individuals before, during and throughout population turnover. This work was undertaken in several putative subpopulations (demes) located within relatively stable areas of abundance (i.e. the genitor populations) or at the margins of the eastern Baie de Seine where individual growth rates are slower (Lambert, 1991). Using microsatellite markers, our aims were (1) to measure the effects of population dynamics, such as population turnover and habitat recolonisation, on the genetic structure of

subpopulations (i.e. are there any spatial/ temporal restriction on larval dispersal?), (2) to test whether genetic differentiation could exist between juvenile stages and adults at the scale of either one deme or the whole population, and (3) to see whether models of colonisation (i.e. propagule-pool, migrant-pool models) could explain observed "chaotic" structure of *P. koreni*. With respect to these objectives, we tested whether significant levels of micro-scale differentiation could exist throughout the reproductive period between temporal samples of each locality in the Baie de Seine and could explain or contribute to a "shifting spatial mosaic" structure. Particularly, does the genetic composition change on every patch at each generation as Jolly *et al*. (2003) stated, or does it constitute solely a spatial mosaic structure shifting/ revolving around more than one homogeneous and relatively temporally stable aggregate? The effective size of the genitor population was also estimated from temporal samples.

Materials and methods

Sampling strategy

The *P. koreni* population of the Baie de Seine is one of the largest in the English Channel and the dynamics of *P. koreni* in the eastern embayment is well known (Elkaïm & Irlinger, 1987; Irlinger *et al*., 1991; Lambert, 1991; Lagadeuc & Retière, 1993; Thiébaut *et al*., 1997; Ellien *et al*., 2000; Ellien *et al*., 2004). Individuals in the eastern bay are widely distributed over the 400 km^{-2} of suitable habitat, with abundances significantly greater and more stable (650 $ind.m^{-2}$; Lambert, 1991; Ellien *et al*., 2000) than those from the Baie des Veys where suitable habitats only represent 80 km^{-2} (33.9 $ind.m^{-2}$ in March 1997; Dauvin *et al*., 2004). Early in the summer, the breeding population is replaced by the arrival of at least two larval recruitment waves. These settlements correspond to the two main reproductive events taking place in the Baie de Seine in April-Mai and June-July respectively (Lambert, 1991). By

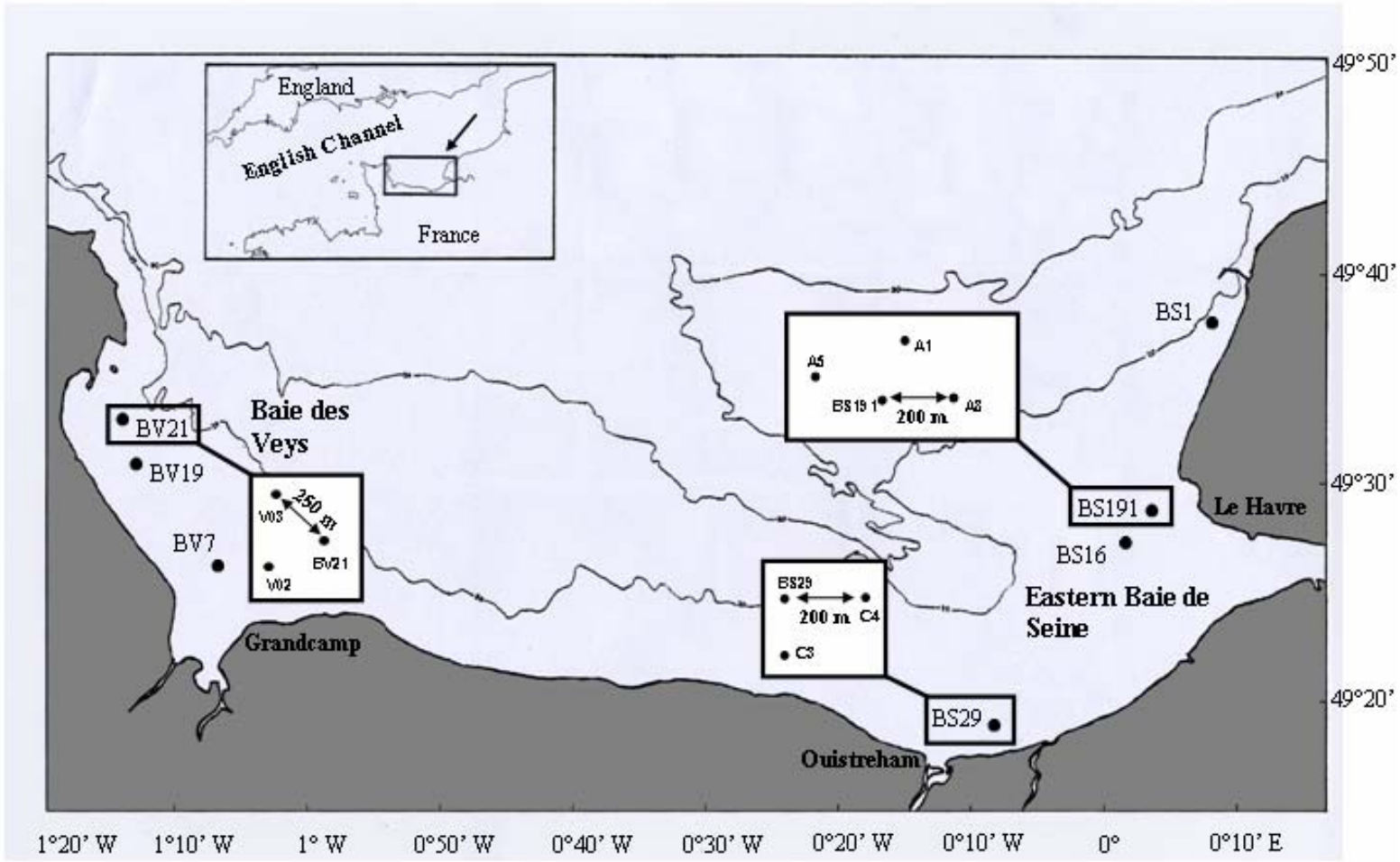

Figure 1. Study area and location of sampling stations in the Baie de Seine [eastern Baie de Seine (BS); Baie des Veys (BV)] for 2003 (details are given in Table 1, see "Results" section).

September, the turnover of the population should be complete although some adults remain and late reproductive events could still take place in Autumn.

Based on these observations, sampling was performed on three consecutive periods in 2003. Sampling sites and dates are presented in figure 1 and table 1 respectively. The mature adult population was sampled at 5 localities in March 2003 (BS1, BS191, BS16, BS29 and BV21). Newly settled juveniles and some mature adults were sampled across 14 localities and sub-localities in July 2003 (BS1, BS191-A1-A5-A8, BS16, BS29-C3-C4, BV21-V2-V3, BV19 and BV7). Juveniles and a few big adults were collected at 7 sites in September 2003 (BS1, A1, BS16, BS29, BV21-V2-V3). The eastern Baie de Seine was subjected to fine scale sampling, the localities of which (BS1, BS16, BS191and BS29) corresponded to those observed by Lambert (1991) as being: (1) the more stable aggregate representing more than 200 ind.m^{-2} (BS16), (2) the locality marginally situated at the immediate vicinity of BS16 (i.e. BS191) and where growth rate was as high, (3) The locality situated at the margins of suitable habitat where densities and growth rate was comparatively lower (BS29 and BS1). Figure 2 shows the distribution and abundance of *P. koreni* in the Eastern Baie de Seine as described by Lambert (1991) for 1987 and 1988, together with the positions of our sampling localities in 2003.

In March 2003, sampling was done using a "Rallier du Batty" dredge (one dredge sampled approx. 1/3 m^{-2} of sediments). However, because of the scarcity of adults in the Baie des Veys, dredging was conducted over a larger area in March 2003 (from BV21 towards BV19, see figure 1), which made impossible the study of temporal genetic fluctuations in the Baie des Veys during the March-July transition. In the two subsequent sampling periods (July and September 2003), a 0.1 m^{-2} Smith McIntyre grab was used to refine the scale of sampling and to explore the "within-locality" genetic patchiness. To test for microspatial genetic heterogeneity, the sampling scheme was refined to a perimeter of approximately 200-250 m

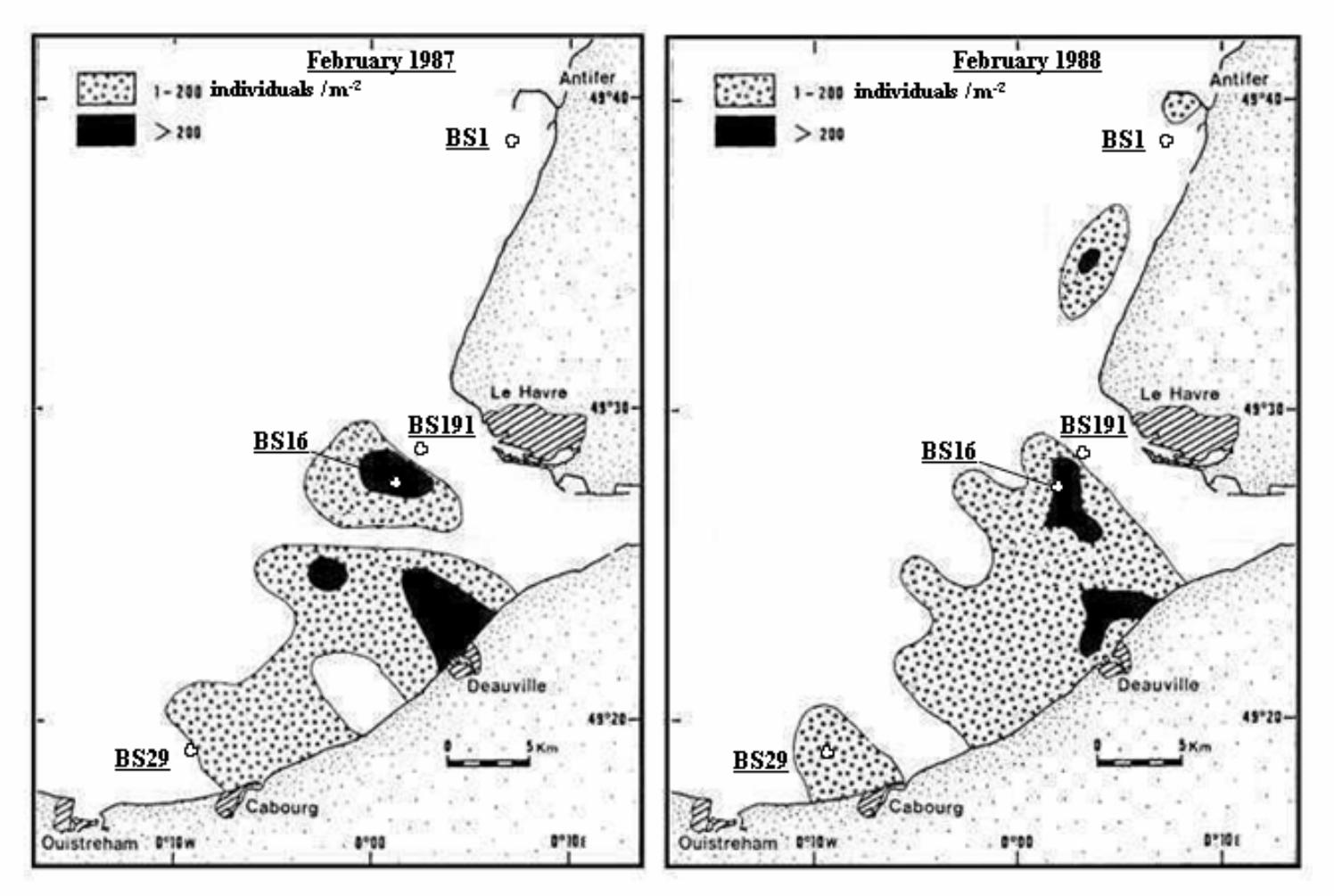

Figure 2. Distribution and abundance of *Pectinaria koreni* in the eastern Baie de Seine between 1987 and 1988 (data from Lambert, 1991), with the relative positions of our main sampling sites in 2003.

around the center of the aggregate (samples A and C around BS191 and BS29 respectively, Figure 1) and at one location in the Baie des Veys (sampling site V around BV21).

Density and age structure

All samples taken for genetic analyses were directly flash-frozen in liquid Nitrogen on board the research vessel. To look at the respective demographic patterns in our sampling locations (i.e. density and age structure), separate samples of taken. Individuals were sieved through a 1-mm circular mesh sieve and preserved in 10% buffered formaldehyde prior to sorting, counting and measuring, with the exception of individuals from March 2003 which came from preserved genetic samples. The diameter of the cephalic disk (CD) of *P. koreni*, defined as the best biometric index of this species (Nicolaidou, 1983; Irlinger *et al*., 1991), was measured individually using a microscope and a micrometer, for both formalin and flash-frozen individuals.

DNA extraction and microsatellite genotyping

DNA extraction was performed according to Jolly *et al.* (2003) and individual genotypes were screened over the four highly polymorphic microsatellite loci (PKGT1, PKAT/GT1, PKAT/GT2 and PKAT/GT4) isolated by Weinmayr *et al*. (1999).

The amplification of microsatellite loci were carried out using a PTC200^{TM} thermocycler (MJ Research): (1) an initial denaturation step at 94°C for 4 min, (2) 38 cycles of denaturation at 94°C for 1 min, annealing for 40 s (PKAT/GT1-2-4 at 58°C and PKGT1 at 53°C) and elongation at 72°C for 50 s, (3) a final elongation at 72°C for 10 min. PCR reactions were performed into a 10 µl reaction volume consisting of 1xPCR buffer (supplied with polymerase enzyme); $MgCl_2$ at a concentration of 1.5 mM (PKGT1) or 2.2 mM (PKAT/GT1-2-4); 0.2mM dNTP; 0.4µM of forward and reverse primers; 0.01 $\mu g.\mu l^{-1}$ T4gene32 protein (Qbiogen); 0.5 U of High Fidelity *Taq* polymerase (ABgene) (PKAT/GT1-2-4) or 0.5 U

Thermoprime Plus *Taq* polymerase (ABgene) (PKGT1); 1µl of a 50ng CTAB-extracted genomic DNA. For each locus, one of the primers was labelled with IR^2-700 or IR^2-800 infra-red fluorescent dye for genotyping. The PCR products were run on a 6% polyacrylamide/ 7 M urea sequencing gel, using an automated DNA sequencer (Li-Cor, model 4200^{TM}).

Statistical analysis

Genetic diversity, as summarised by the non biased expected heterozygosity (H_{NB}- Nei, 1987) and the allelic richness (R_S) based on a weighted sample of diploid individuals, was estimated using GENETIX 4.03 (Belkhir *et al.*, 2002) and FSTAT 2.9 software (Goudet, 1995) respectively, together with associated standard error. The latter software was used to test for differences in genetic diversity between groups of localities and sampling dates. Deviations from Hardy-Weinberg equilibrium were examined for each sample at each locus by calculating Wright's fixation index F_{IS} as estimated by Weir & Cockerham's (1984) *f* and genotypic didequilibria were tested using Fisher's exact tests with GENEPOP 3.4 software (Raymond & Rousset, 1995). To analyse the relative contributions of spatial and temporal processes affecting the genetic structure of *P. koreni*, an F_{ST} based analysis of molecular variation (AMOVA) was performed using ARLEQUIN v. 2 (Schneider *et al.*, 2000) using first the whole March-July-September dataset, and then, only using the March-July dataset.

The spatial genetic structure for each sampling period was analysed using GENEPOP 3.4, by calculating pairwise Wright's F_{ST} statistics (Wright, 1969) estimated using Weir & Cockerham's (1984) θ. Testing for the null hypothesis of identity in allelic distributions across localities and sampling dates were performed using Fisher's exact tests in GENEPOP 3.4. When multiple tests were performed, significance levels were corrected using the sequential Bonferroni procedure (Rice, 1989). The effective population size (N_e) was estimated for the eastern Baie de Seine using samples collected at BS16 in March 1999 (data in Jolly *et al*., in prep.) and 2003. This was done according to the sampling strategy described

Table 1. Locality names, position and numbers (*N*) of *Pectinaria koreni* screened using microsatellites for each sampling date. CD = diameter of the cephalic disk (mm); D = density (ind.m^{-2}); A, C and V correspond to sublocalities sampled within the localities BS191, BS29 and BV21 respectively.

Sampling locations	Geographic coordinates			Sampling date (2003)		
	Latitude	Longitude		March	July	September
Eastern Baie de Seine						
			N	54	46	80
BS1	49°38.86' N	0°07.09' E	CD	[2 - 6]	[0.5 - 2]	[1.5 - 4.5]
			D	27.2	27.5	
			N	81	87	80
BS16	49°27.24' N	0°01.48' E	CD	[4.5 - 8]	[1 - 3]	[1.5 - 5]
			D	269.7	11820	4095
			N	120	80	-
BS191	49°28.27' N	0°02.36' E	CD	[4 - 7.5]	[0.5 - 2]	[1.5 - 5]
			D	133.2	4220	300
A1	49°28.43' N	0°02.35' E		-	80	68
A5	49°28.36' N	0°02.11' E		-	41	-
A8	49°28.31' N	0°02.51' E		-	53	-
			N	58	77	69
BS29	49°18.50' N	0°09.23' W	CD	[2 - 6]	[2 - 4.5]	[1.5 - 2; 2.5 - 5]
			D	27.6	1140	880
C3	49°18.39' N	0°09.22' W		-	75	-
C4	49°18.51' N	0°09.08' W		-	79	-
Baie des Veys						
			N	98*	23	30
BV21	49°31.99' N	1°15.50' W	CD	[2 - 6.5]	[3 - 8]	[1 - 3; 3.5 - 6.5]
			D	21.8		175
V2	49°31.99' N	1°15.72' W		-	26	30
V3	49°32.14' N	1°15.53' W		-	31	35
BV19	49°30.97' N	1°14.91' W		-	56	-
BV7	49°26.99' N	1°06.44' W		-	31	-

*****(sample dredged from BV21 to BV19 for demography)

by Waples and implemented in the "NE estimator" software (Peel *et al*., 2003). This method is based on the temporal changes in allele frequencies of closed populations (i.e. no immigration), assumes genetic drift and mutation as the only acting evolutionary forces, and is best suited for organisms having a high larval/juvenile mortality (Waples, 1989). The precise estimation of the effective population size (N_e) depends on whether the number of individuals and loci samples are adequate. While the standardised variance in allele frequency changes could have been biased by rare alleles and a low number of loci (4 loci), this was counter-balanced by high samples sizes (from 81 to 108 individuals).

Results

Density and age structure

In March, localities sampled near the mouth of the Seine estuary (BS16 and BS191) showed significantly greater abundances (269.7 and 133 ind. m^{-2} respectively) relative to those sampled at the margins of the eastern basin (BS1 and BS29) and in the Baie des Veys (BV21, see Table 1). In addition, the animals collected within sampling stations of high densities were bigger with a greater cephalic disk diameter. Lambert (1991) attributes this to diminished organic matter at the margins of suitable habitat. The turnover of the population during the reproductive season in the eastern Baie de Seine (March-July transition) marks the replacement of the adult population present in March by new settlers (see CD sizes on table 1 and demographic patterns in Appendix 1). While in these populations only a few large individuals may persist until September, no such extinction events occurred in the Baie des Veys where a large number of individuals were maintained in the adult population in September (table 1).

Analyses of the recruitment patterns in July (table 1) show a heterogeneity in size (two peaks of settlers) in the eastern Baie de Seine and no recruitment in the Baie des Veys. The first wave settled at BS29 (average CD = 2 - 4.5 mm) and at BS16 (average CD = 1 - 3 mm).

Table 2. Multilocus genetic diversity and expected heterozygosity per locality and sampling date (*N*: numbers of individuals; R_S= allelic richness based on a minimum sample size of 15 diploid individuals; H_{NB}= non biased expected heterozygosity [Standard Error]).

Sampling location	March 2003			July 2003			September 2003		
	N	R_S	H_{NB} [SE]	*N*	R_S	H_{NB} [SE]	*N*	R_S	H_{NB} [SE]
BS1	54	17.43	0.952 [0.022]	45	17.21	0.949 [0.026]	80	17.88	0.953 [0.027]
BS16	81	17.68	0.955 [0.021]	87	18.02	0.953 [0.025]	80	18.42	0.957 [0.021]
BS191	120	17.71	0.951 [0.026]	64	17.71	0.951 [0.023]	-	-	-
A1	-	-	-	80	17.27	0.951 [0.018]	68	17.06	0.944 [0.031]
BS29	58	16.66	0.944 [0.022]	77	17.82	0.953 [0.024]	69	18.33	0.955 [0.023]
BV21	98*	17.65	0.949 [0.030]	23	15.74	0.944 [0.024]	30	16.52	0.941 [0.032]
V2	-	-	-	26	15.36	0.934 [0.041]	30	16.29	0.938 [0.043]
V3	-	-	-	31	17.74	0.951 [0.027]	35	18.25	0.954 [0.027]

*sample dredged between BV21 and BV19

A second size-group of settlers observed in July (possibly representing the June-July spawning event) was found at BS191 (average CD = 0.5 - 2 mm) and at BS1 (average CD = 0.5 - 2 mm). In contrast, size distributions show no recruitment events in July in the Baie des Veys, but one recruitment in September with a small size-group detected at BV21 (average CD = 1 – 3 mm).

Genetic diversity and heterozygote deficiencies

Despite fine scale sampling, the fixation index (F_{IS}) varied from 0.348 to 0.468 and exhibited significant departures from Hardy-Weinberg proportions at all microsatellite loci (P< 0.001), irrespective of sampling localities and dates (adults and juveniles).

The levels of genetic diversity (H_{NB} and R_S) are presented in Table 2 for each locality and sampling date. They were high and similar across the eastern Baie de Seine localities (BS1, BS16, BS191 and BS29) whatever the sampling date. Note that in BS29 and BS16, allelic richness had increased from March (adults) to September (juveniles). This tendency was also observed between July and September at all other temporal sampling sites, except for sublocality A1. Using the July-September dataset, group comparisons made under FSTAT showed significantly smaller levels of R_S only, in the Baie des Veys (R_S = 16.62) compared to sampling sites within the eastern Baie de Seine (R_S = 17.63; P = 0.009). However, the number of diploid individuals on which these estimates are based is low, because of low sample size at BV21 in July and September. We removed this locality to increase power and determine whether any differences might exist in patterns of genetic diversity between the juveniles collected in July in the eastern Baie de Seine (table 3). Group 1 (BS1, BS16, BS191-A1-A5-A8) had significantly smaller of genetic diversity when compared to group 2 formed by BS29-C3-C4 (P-R_S= 0.006, P-H_{NB}= 0.025 under FSTAT). In addition, levels of R_S were similar between BS16 and BS29-C3-C4 whereas sublocality A8 had the smallest levels. When groups were compared without A8, the expected herozygosity was no longer different

Table 3. Multilocus genetic diversity and heterozygosity for all spatial locations where juveniles were sampled in July 2003 (*N*: numbers of individuals; R_S= allelic richness based on a minimum sample size of 26 diploid individuals; H_{NB}= non biased expected heterozygosity [Standard Error]).

	Spatial locations sampled in July								
	Group 1						Group 2		
	BS1	BS16	BS191	A1	A5	A8	BS29	C3	C4
N	46	87	80	79	41	53	77	75	79
R_S	22.45	24.43	22.94	22.49	22.14	21.17	23.78	25	23.89
H_{NB}	0.949	0.953	0.949	0.951	0.949	0.942	0.954	0.959	0.955
[SE]	[0.026]	[0.024]	[0.024]	[0.018]	[0.015]	[0.028]	[0.024]	[0.019]	[0.022]

Table 4. Pairwise values of genetic differentiation using Weir & Cockerham's (1984) θ, for all adult samples collected in March 2003 in the eastern Baie de Seine, and those from the Baie des Veys collected in July 2003.

	BS1 (54)	BS16 (81)	BS191 (120)	BS29 (58)	BV21 (23)	V2 (26)	V3 (31)	BV19 (56)	BV7 (31)
BS1	-	-0.002	0.001*	0.001***	0.010***	0.007**	-0.001	0.002**	0.009***
BS16		-	0.002***	0.001***	0.003**	-0.001	0.0003	0.002***	0.006***
BS191			-	0.001***	0.009***	0.010***	0.0001	0.001**	0.009***
BS29				-	0.005**	-0.002	0.003*	0.002***	0.011***
BV21					-	0.008***	0.003**	0.008***	0.005**
V2						-	0.007***	0.008***	0.010***
V3							-	0.004***	0.003*
BV19								-	0.009***
BV7									-

* P < 0.05, ** P < 0.01, *** P < 0.001 after sequential Bonferroni correction

(P-R_S= 0.02, P-H_{NB}= 0.20). When comparing the September samples based on 10 diploid juvenile individuals (results not shown), all the sampling sites situated within the eastern Baie de Seine and V3 in (Baie des Veys) showed very similar levels of genetic diversity (R_S and H_S), whereas allelic richness (R_S) only was comparatively diminished in BV21-V2 (P= 0.016, under FSTAT).

Spatial genetic structure between adult populations

All pairwise values of genetic differentiation between adult aggregates of March 2003 are presented in Table 4. With the exception of adults from BS1 and BS16 which were genetically similar, significant genetic structuring was observed between all other adult subpopulations in the eastern Baie de Seine. In addition, adult samples collected in the Baie des Veys in July (the adults of March) were all significantly different from one another. Despite this "chaotic" structuring, adults sampled in sublocalities of the Baie des Veys (V2 and V3) were genetically similar to the dense aggregate of the eastern Baie de Seine (BS16). In particular, samples from V3 were genetically similar to all sites sampled in the eastern Baie de Seine with the exception of BS29.

Genetic differentiation between and within generations

A hierarchical analysis of molecular variance was first conducted by combining all spatial localities, sublocalities, and the 3 sampling dates (March, July and September) and then, using only the March-July samples including the BV21 sample for March (see AMOVA, table 5). The analysis revealed that a significant amount of genetic variation (F_{SC} = 0.0028; P = 0.000) existed among sampling dates within localities rather than between localities. The AMOVA restricted to the March-July samples indicated that all the variation (F_{SC} = 0.003; P =0.000) resided in the transition of adults to juveniles during population turnover between March and July.

Table 5. Hierarchical analysis of molecular variance (AMOVA) of *P. koreni* populations from localities within the Baie de Seine using allele frequencies of four microsatellite loci: (A) among groups of localities for all three sampling dates and (B) among groups of localities for the two first sampling dates.

	Source of variation	Fixation indices	P-value
A) March - July - September	Among groups of localities	F_{CT}= 0.0002	0.153
	Among sampling dates within	F_{SC}= 0.0028	0.000
	Within localities	F_{ST}= 0.0030	0.000
B) March - July	Among groups of localities	F_{CT}= 0.0009	0.147
	Among sampling dates within	F_{SC}= 0.0030	0.000
	Within localities	F_{ST}= 0.0039	0.000

Table 6. Temporal change in gene frequencies both at the inter-generation level (BS: adults of March vs. juveniles of July; BV: adults of July vs. juveniles of September) and at the intra-generation level in the eastern Baie de Seine (juveniles of July vs. juveniles of September). NA: not available; NS: not significant.

Eastern Baie de Seine	**BS1**	**BS16**	**BS191**	**A1**	**BS29**
March - July 2003 (inter-generation)	P < 0.001	NS	NS	NA	P < 0.001
July – September 2003 (intra-generation)	NS	NS	NA	P < 0.001	NS
Baie des Veys	**BV21**	**V2**	**V3**	**BV19**	**BV7**
March - July 2003 (intra-generation)	NA	NA	NA	NA	NA
July – September 2003 (inter-generation)	P < 0.05	P < 0.01	NS	NA	NA

The results of Fisher's exact tests of homogeneity in allele distributions between adults from March and juveniles from July (the inter-generation level, Table 6) indicate significant levels of temporal differentiation mainly for the marginal and less abundant aggregates (BS1 and BS29; $P < 0.001$). However, adults and juveniles of BS16 and BS191 were genetically homogeneous. While the first locality is situated at the center of the densely populated area near the mouth of the Seine estuary (where $N > 200$ ind.m^{-2}), the latter is situated towards its margins. In the Baie des Veys, most genetic differentiation was detected between July and September ($P< 0.05$ at BV21 and $P< 0.05$ at V2, Table 6), which was concomitant with the arrival of newly settled juveniles (see table 1 for age structure/ size distribution).

Within the juvenile recruitment period in the eastern Baie de Seine (intra-generation level from July to September, Table 7) all localities (BS1, BS16 and BS29) showed no differences in allele frequencies, with the exception of sub-locality A1 situated 200 m NE from BS191, at the margins of the main center of abundance (BS191 was not used because of technical difficulties during sampling). Juveniles collected in September at BS16 and BS29 were not different from any of the other localities collected in July (BS1, BS16 and BS29) except between BS16-July and A1-September ($P< 0.05$, see Table 7). The BS1 and A1 samples collected in September were genetically differentiated ($0.01< P < 0.05$) from BS16 and BS29 sampled in July. This latter result may indicate the arrival of a third, differentiated larval cloud from another origin between July and September, but it may also reflect the different degree of spatial overlap between just two separate recruitment events among localities in the eastern Baie de Seine in July.

Micro-spatial changes in the genetic structure of the juveniles in the eastern Baie de Seine between July and September

Levels of genetic differentiation between localities where new settlers were sampled in the eastern Baie de Seine are presented in Table 8, together with the associated levels of

Table 7. Corrected levels of significance for pairwise comparisons of allele frequencies at the intra-generation level in the eastern Baie de Seine (juveniles of July Vs. juveniles of September). NS: not significant.

July sampling sites	September sampling sites (N)			
	BS1 (80)	BS16 (80)	BS29 (69)	A1 (68)
BS1 (46)	NS	NS	NS	NS
BS16 (87)	P < 0.05	NS	NS	P < 0.05
BS29 (77)	P < 0.05	NS	NS	P < 0.05
A1 (79)	P < 0.05	P < 0.05	NS	P < 0.01

Table 8. Pairwise values of genetic differentiation based on Weir & Cockerham's (1984) θ, among newly settled juveniles using all sites from the eastern Baie de Seine in July 2003. The associated significance levels are given after sequential Bonferroni correction for multiple tests (9 populations and 4 loci) (* P< 0.05; ** P< 0.01; *** P< 0.001).

	BS1 (46)	BS16 (87)	BS19 1	A1 (79)	A5 (41)	A8 (53)	BS29 (77)	C3 (75)	C4 (79)
BS1	-	-0.002	-0.002	-0.003	-0.004	0.001**	-0.002	-0.006	-0.004
BS16		-	-0.002	0.003***	-0.001	0.002***	-0.000	-0.001	0.000
BS191			-	0.002***	0.001***	0.002***	0.0002**	0.001***	-0.002
A1				-	-0.002	0.003***	-0.002	-0.001	-0.001
A5					-	0.0001**	-0.002	-0.006	-0.001
A8						-	0.004**	0.000	0.003**
BS29							-	0.000	-0.001
C3								-	0.001
C4									-

significance. Only sampling sites located at the periphery of the main aggregate were very different from nearly all others (i.e. BS191 and to a lesser extent A8).

In July, BS1 was not genetically different from any of the other sampled sites, except with A8 (F_{ST}= 0.001**; P< 0.01, Table 8). Interestingly, while levels of differentiation between localities in March were all significantly different (except between BS16 and BS1, see table 5), all this structure disappeared by July. Note that levels of genetic differentiation between BS16 and A1 in July were highly significant (F_{ST}= 0.003***; P< 0.001) and of similar magnitude than between BS16 and BS191 in March (F_{ST}= 0.003***; P< 0.001, see table 5). In addition, similar levels observed between BS191 and BS29 in March (F_{ST}= 0.001***; P< 0.001) were detected in July between BS191 and C3 (F_{ST}= 0.001***; P< 0.001). By September, no spatial genetic stucture was observed among juveniles of the Baie de Seine except between BS1 and BS16 (F_{ST}= 0.0004**; P< 0.01) and between A1 and BS16 (F_{ST}= 0.002***; P< 0.001). This suggest that over the spawning season, at least two larval recruitments led to the genetic homogenisation of the eastern Baie de Seine populations, with the last wave of larvae reaching the Baie des Veys populations.

Estimation of the effective population size in the eastern Baie de Seine

The BS16 locality showed stable allele frequencies throughout population turnover, which was the result a high self recruitment of juveniles originating from this site. We therefore assumed that the population at this site was closed to migration. All together, the data represented 4 generations of adults (collected in March 1999 and 2003) and an exact test of homogeneity revealed an overall highly significant change in allelic frequencies between the sampling dates (P< 0.001). The Waples test (1989), performed by statistical analysis of a contingency table representing three classes of alleles (first most frequent, second most frequent, others), was highly significant overall. Using the temporal method of Waples (1989) we estimated N_e by processing samples separated by 4 generations [February 1999 (N= 108;

103.5 ind. m^{-2}) and the March 2003 adult genotypes (N= 81; 269 ind. m^{-2})]. This gave an estimate of the effective population size (N_e= 90.8, CI= 54.9 - 169.8) which was close to the average number of individuals used in the analyses (average N $BS16_{99\text{-}03}$ = 94). Note that increasing sample size for 2003 (N= 165) by grouping BS16 for July and September, did not affect the N_e estimate based on 1999 and 2003 data (N_e= 94.4, CI= 56.6 - 180.6).

Discussion

The objective of this study was to combine population demography and fine scale population genetics througout population turnover (1) to test how the genetic structure is affected from adults to newly settled juvenile stages, (2) to confirm or reject the hypothesis of a temporally "shifting" spatial mosaic structure, and (3) to identify the impact of potential micro-evolutionary forces on genetic changes between age-classes which could explain the persistence of "chaotic genetic patchiness" (Johnson & Black, 1982).

The majority of the adult demes sampled in March 2003 were spatially significantly differentiated. However, there was no directionality in gene flow (i.e. chaotic genetic patchiness) nor were there any apparent differences in genetic diversity within the eastern Baie de Seine. In addition, there was highly significant temporal heterogeneity between February 1999 and March 2003 data collected in the centre of abundance at BS16 ($P< 0.001$). These results are fit previous conclusions on the spatio-temporal genetic structure of the adult metapopulation of *P. koreni* in the Baie de Seine (Jolly *et al*., 2003). Furthermore, the lower level of genetic diversity in the Baie des Veys among adults and juveniles, reflects source-sink dynamics at that scale. This latter result was not observed by Jolly *et al*. (2003) because individuals were dredged so that the true genetic diversity of the sampled localities weree masked.

Age structure observations provide evidence for local asynchrony in recruitment events within the eastern Baie de Seine in July and, to a much stronger level, between both

eastern and western embayment in September. From demographic data alone, there is evidence that the new settlers in the Baie des Veys represent outsiders from the eastern Baie de Seine since most of the adult population remained late in the season and thus might not have spawned by the date of sampling in mid-September. This contrasts with what is observed in the eastern Baie de Seine where nearly all the adults had disappeared by July. Asynchrony in reproductive timing therefore appears to be strong, with spawning events in the Baie des Veys occuring at a much later time, either by the end of autumn or maybe the following year. Considering the relationship between size and fecundity ($F = 333.6 \times (CD)^{3.525}$; Ellien *et al*., 2004), there is also a considerable potential for variance in reproductive success associated to differential growth rates between localities situated at the margins of suitable habitats (BS29, BS1; average fecundity = 48 139.66) and those situated in front of the Seine estuary (BS16, BS191; average fecundity = 71 926.39). This fits with what is reported by Lambert (1991) which attributes differential growth to diminished organic matter at the margins of suitable habitat (Lambert et al., 1991). Similarly, the abundance of individuals is always higher (for both mature adults and juveniles) to the East, near the mouth of the Seine estuary (BS16-BS191) than among marginal populations, and is seemingly associated with a higher degree of larval retention over these specific areas (Lambert, 1991; Lagadeuc, 1992).

In the light of demography, our genetic results are particularly interesting in revealing the effects of variance in reproductive success, population turnover and hydrodynamic features controlling larval dispersal, on changes in gene frequencies according to patch size and location. All together, spatially varying biotic/ abiotic conditions, temporal asynchrony in reproductive timing, variance in reproductive success, extinction-recolonisation dynamics and the scale of larval dispersal or retention must create a strong shifting pattern of genetic structure such as that observed in the adult populations (Jolly *et al.*, 2003). Within the eastern Bay de Seine, significant inter-generational genetic change was detected within patches where

adult fecundity and abundances were the lowest (BS29 and BS1), whereas both the highly fecund centre of abundance BS16 and the sampling locality situated at its margins (BS191) showed a stability of gene frequencies. This corresponds to the findings of Lambert (1991) who characterised "two large genitor populations" in the eastern Baie de Seine in 1987 and 1988 which should contribute effectively to the next generation. One of these "genitor populations" correspond to the area around BS16 while the other is situated further south near the entrance of the estuary but was not sampled (see Figure 2). This author also observed that although patch geometry could change yearly, densities within those densely populated aggregates were overall highly stable. The persistence of dense aggregates together with the temporal stability of allele frequencies observed within these BS16 and BS191is most likely related to a high retention rate as proposed by Lewis & Thorpe (1994) to explain the constancy of allele frequencies in the scallop *Aequipecten opercularis*. This higher retention seems to be caused by convergent oceanographic currents at the mouth of the estuary, as observed from *in situ* studies (Irlinger, 1991; Lambert, 1991; Lagadeuc, 1992) and from new fine scale hydrodynamic modellisation of larval dispersal at the scale of the eastern Baie de Seine (P. Guyard and E. Thiébault, unpublished data). During the recruitment period, most of the observed genetic differentiation within the eastern Baie de Seine is found around BS191 (A1, A5, A8). This site is situated very close to the main centre of abundance (BS16) and was itself highly different from any other sites but with the exception of BS1, BS16 and C4. The genetic differences seen at a micro-spatial scale around BS191 may have resulted from a combination of density dependant processes (competition, post-larval re-settlement), immigration and unstable environmental conditions (high salinity variations, dredging of the channel entrance to the port of Le Havre) which might have structured this area. Extinction-recolonisation processes at BS191 and A1 may explain the intra-generation genetic changes observed in this area, whether as a result of habitat selection, competition for food/ space and strengthened small-scale post-resettlement processes because of high abundances in this area

(i.e. habitat selection, Olivier *et al*., 1996), or immigration from a differentiated source of individuals either situated within the Baie de Seine (i.e. the second "genitor population" situated off Deauville, Lambert, 1991) or from an outside population situated further to the North along the Pays de Caux.

The temporal changes observed at less abundant sites cannot be solely be explained by changes in the local pool of reproductive adults and high larval retention since genetic diversity between these sites was similar. It seems that migration was largely oriented from the highly abundant BS16 to the less abundant patches. In cases of inter-generational genetic change (i.e. BS1 and BS29), we also found evidence supporting the hypothesis of a recruitment of juveniles from more than one source in July-September. Indeed, this is obvious at BS29-C3-C4 where genetic diversity was higher than in other areas although not different from BS16 and V3. Our results corroborate both *in situ* and simulated hydrodynamic-based larval dispersal patterns based on 1987 (Ellien *et al*., 2004). The authors showed that strong wind-induced currents during the reproductive season cause advective processes to become stronger, thereby increasing the exportation of larvae outside the boundaries of the main aggregates even if retention rates remain strong within the centre of abundance (i.e. BS16). Simulating an early spawning event in the eastern Baie de Seine on the 15th May 1987, larvae were mainly advected to the south and the west in response to north-eastern winds, with settlement principally occurring off the Seine estuary and along the coasts of the Pays d'Auge (towards BS29). This contrasted with another simulation based on a spawning event on the 26th May 1987 which produced larvae that were mainly advected north and north-eastwards, with a settlement in areas off the Seine estuary and along the Pays de Caux (towards BS1).

Regarding the "shifting" spatial mosaic structure, it is important to note that levels of genetic differentiation within the eastern Baie de Seine in March (0.001* < FST < 0.002***) are maintained throughout population turnover at the margins of the centre of abundance BS16 (0.0001** < F_{ST} < 0.003*** in July; F_{ST} = 0.002*** between BS16 and A1 in

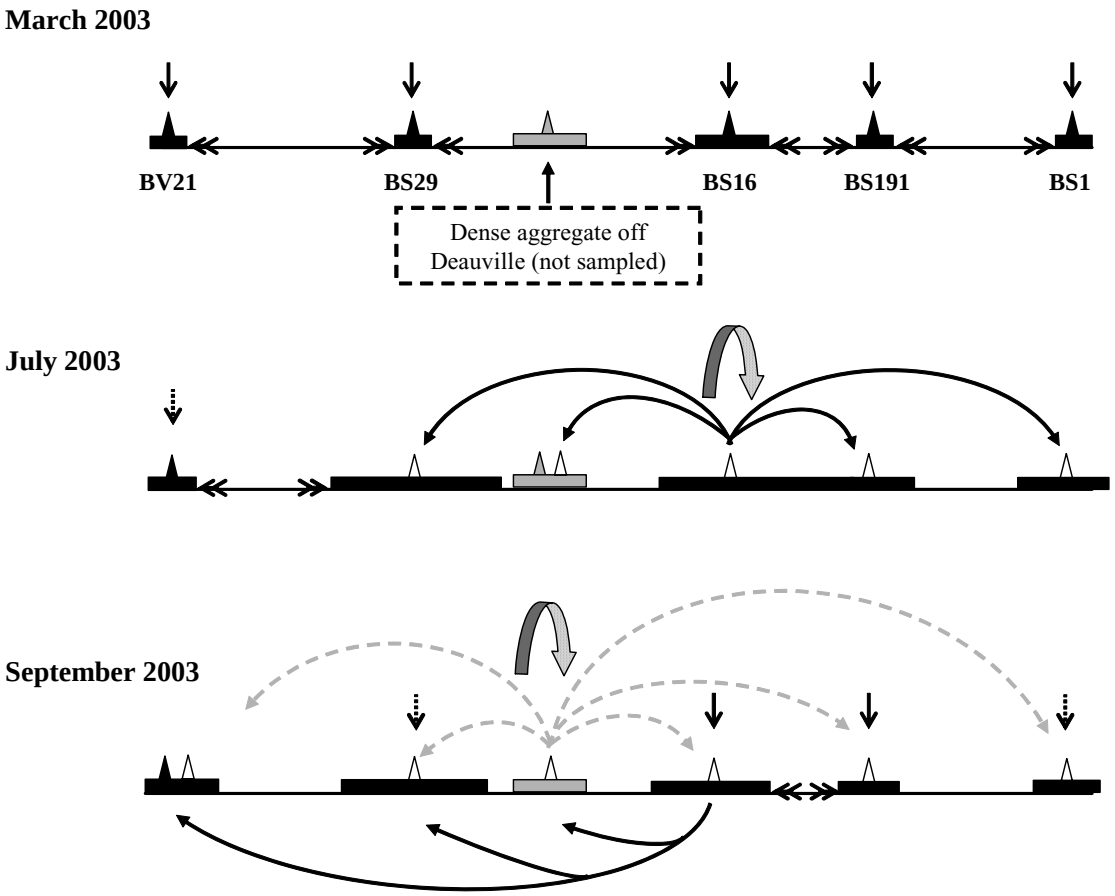

Figure 3. Schematic representation of the dynamics and the evolution of genetic structure during population turnover of *Pectinaria koreni* in the Baie de Seine. Black rectangles : the sampled localities; black and white triangles : adults and newly settled juveniles respectively; << >> : genetic differentiation; full and dotted arrows pointing downwards : respectively greater or weaker mortality; large grey arrows : larval retention; horizontal full black arrows : larval migration as observed with our genetic data; horizontal dotted arrows : hypothetical larval migration from the unsampled locality off Deauville.

September). On the contrary, significantly less genetic differentiation is observed among juveniles at a larger spatial scale. The intricate pattern of "chaotic" genetic structure observed among adults disappeared among juveniles, leading to a genetic homogenisation at the scale of both embayments by September (not considering the remaining adults of March in the Baie des Veys). This indicates that (1) recolonisation at every generation is favoured by extinction of the adult population, at least in the eastern Baie de Seine and that (2) the mechanisms responsible for adjusting the magnitude of the genetic structure of adults is more likely to occur during the Autumn and Winter periods, between the juvenile and the adult stages. Such mechanisms are related to strong reproductive assynchrony between embayments, increased differential mortality associated with competition, post-larval resettlement and small scale juvenile extinction events at the local scale. Figure 3 schematically represents the dynamics of the metapopulation as observed from our data.

To what model of population (spatially-structured population or source-sink metapopulation) the genetic structure of *P. koreni* conforms at the scale of the Baie de Seine is still unclear, although it shows evidence of a spatially structured population with n-islands, mainland-island migration and source-sink dynamics with aspects of both migrant pool and propagule pool models of colonisation. In effect, both latter models seem to be represented among respectively locality BS29 and sub-locality A8 near BS191. Assuming a "migrant-pool" model of colonisation, extinction and recolonisation would tend to reduce genetic differentiation and increase genetic diversity in newly established population, but only when the number of founders colonising new vacant areas is twice the number of migrants exchanged between extant populations (Wade & McCauley, 1990; McCauley, 1991). This model of colonisation fits well with the general patterns observed in the Baie de Seine, especially to that observed at BS29. Nevertheless, the model of colonisation that best characterises A8 in July, situated at the margins of an area of higher abundance relative to BS29, is the "propagule-pool" model whereby extinction is expected to reduce the amount of

genetic diversity in newly established populations (Pannell & Charlesworth, 2000) and to increase the level of differentiation as a function of both the migration model and the relative importance of parameters such as the number and the origin of the founders (Wade & McCauley, 1988; Whitlock & McCauley, 1990). At this locality (BS191-A1-A8), local extinctions as a result of density dependant mortality must have more strongly affected juveniles during the early life stages, relative to areas where abundances are lower (i.e. BS29).

The fine scale spatio-temporal sampling undertaken in this study allows us to re-enforce our original hypothesis of a "shifting spatial mosaic" genetic structure in the Baie de Seine *meta*population of *P. koreni*, but with a slight change to our original understanding since no temporal genetic change was observed within the main centre of abundance (BS16). No variance in reproductive success was found within this locality and estimates of effective population size at BS16 were similar to the average number of individuals sampled in 1999 and 2003 at this locality. We can extrapolate this result to the whole densely populated area around BS16 where N > 200 ind.m^{-2}. According to Lambert (1991), adults are grouped in an area representing roughly 10% of the total area occupied by *P. koreni* (which was approx. 200 km^{-2}). This population roughly occupies an area of approximately 20 km^{-2} populated by 5-6 billion individuals. If we assume local reproductive success to be similarly high over this whole area, we can imagine how effective gene flow originating from this area might be, in nearly completely homogenising the Baie de Seine by September. In effect, juveniles were much less structured than adults at the scale of the whole bay and reflected the role of gene flow originating from BS16 as a homogenising force. However, significant levels of differentiation still exist among peripheral areas around BS191, which shows that very small-scale "mosaic" genetic structuring remains throughout population turnover. While the stability and persistence of genetic variation in the whole spatio-temporal system of the Baie de Seine may be strengthened by mechanisms of asynchronous reproductive dynamics and local extinction-recolonisations, the effects are likely to be different according to patch size

and density. Both physical conditions and behavioural responses to such conditions, habitat quality and competition, interact to structure the adult population between September and March. Because F_{IS} values were identical whatever locality or sampling dates, they should not have affected our conclusions on the spatio-temporal genetic structure of *P. koreni*. While these heterozygote deficiencies may be consistent with a Walhund effect arising from micro-scale genetic heterogeneity within the sampled demes, a more likely explanation is the presence of null alleles, whereby apparent homozygotes are in reality heterozygotes between a visible and a null allele (Chakraborty *et al*., 1994). This study not complete without micro-spatial and temporal sampling of the second main centre of abundance described by Lambert (1991) as situated off Deauville, to test for a differentiated larval source and to see whether similar spatio-temporal patterns emerge among peripheral areas such as that observed around BS191.

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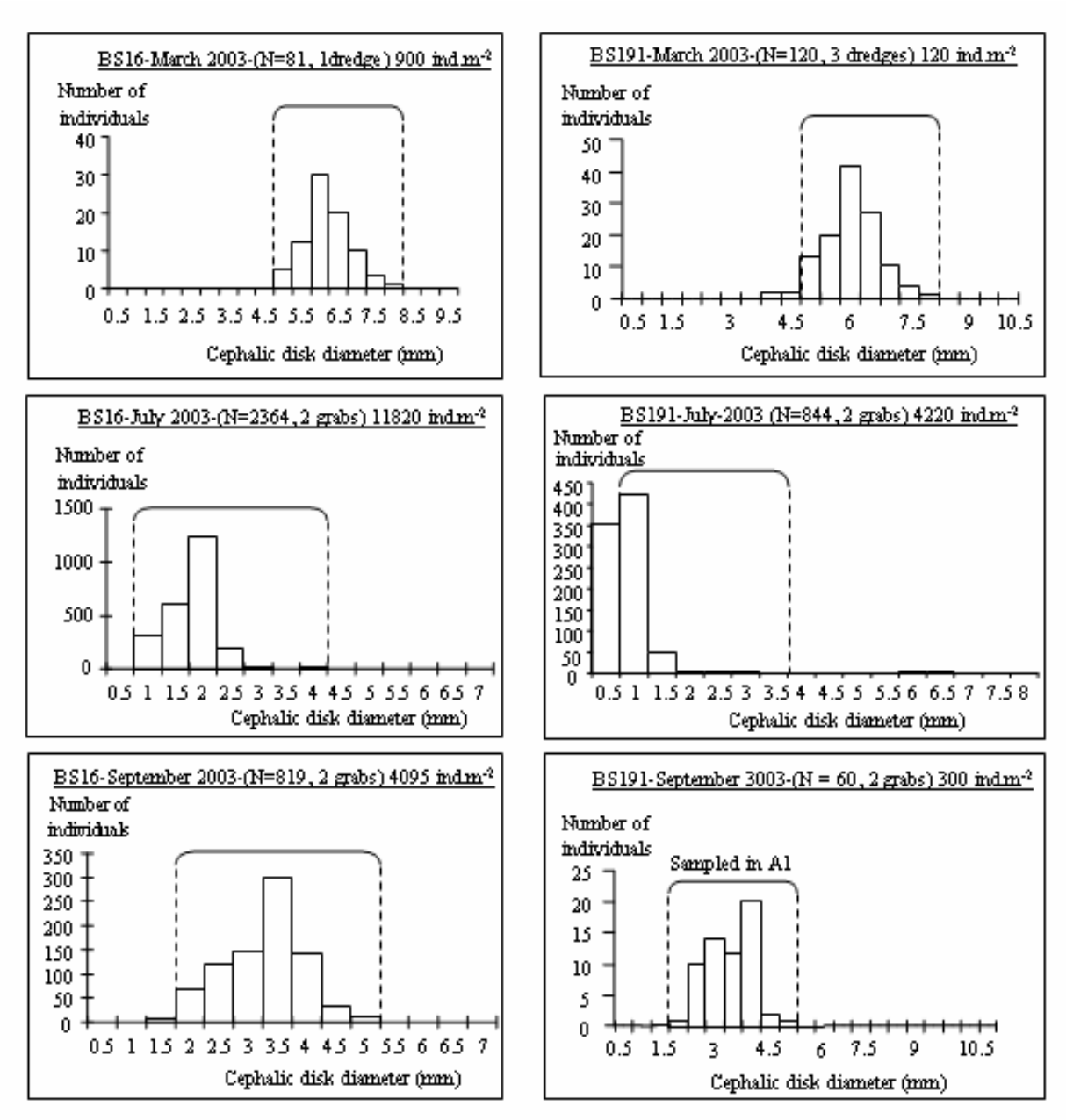
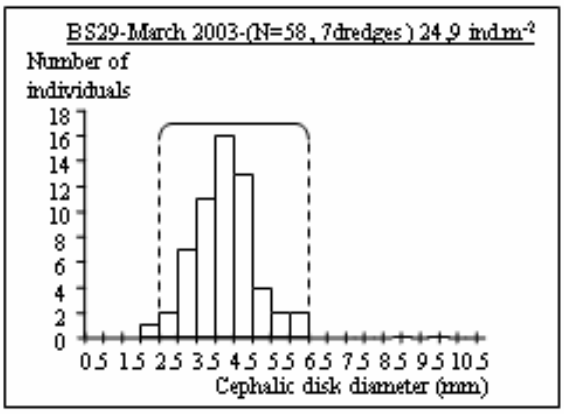
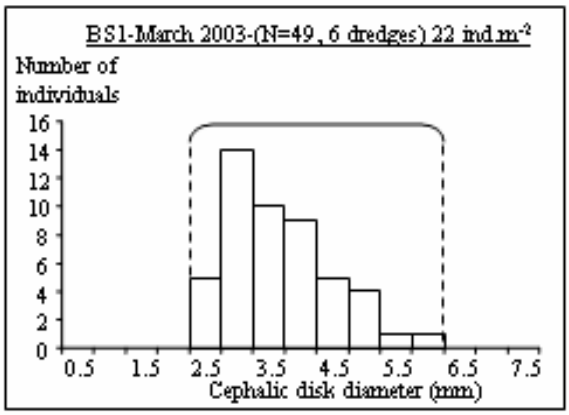
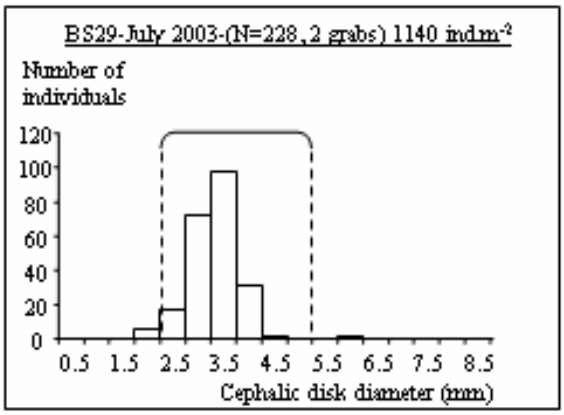
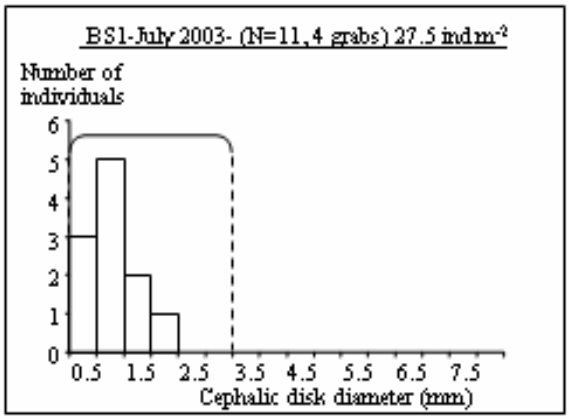
Appendix 1. Demographic patterns recorded from 2003 data, before (March), during (July) and after (September) population turnover of *Pectinaria koreni* in the Baie de Seine. The interval representing the size classes of the individuals taken for genetic analyses are also shown.

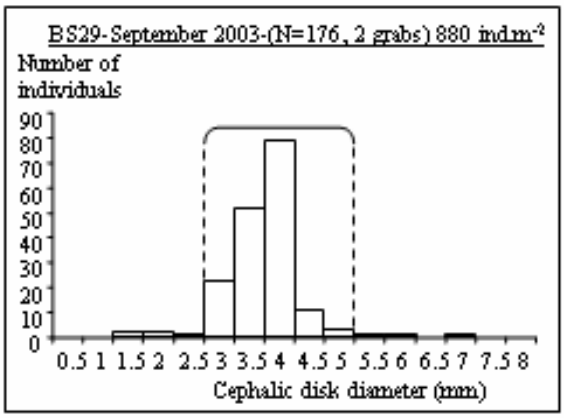


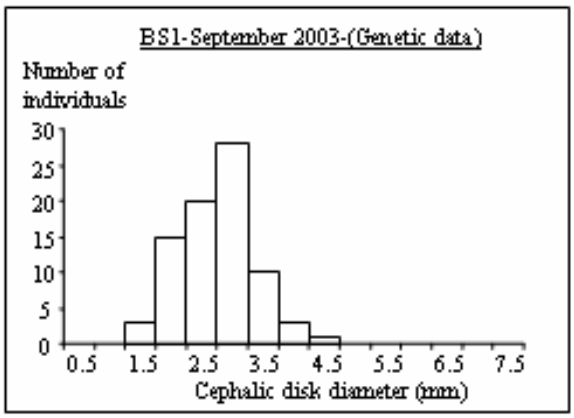

BS29-March 2003-(N=58, 7dredges) 24,9 ind.m-2
Number of individuals
Cephalic disk diameter (mm)

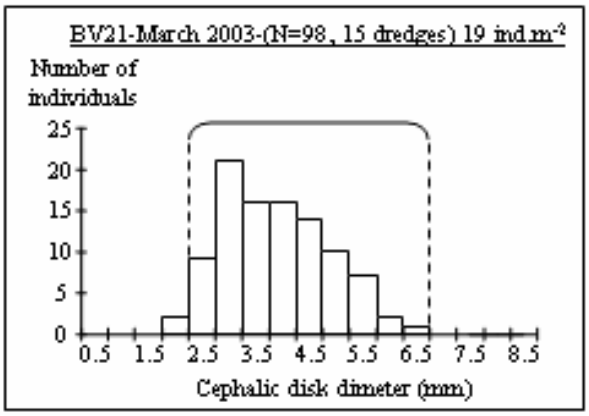

BS1-March 2003-(N=49, 6 dredges) 22 ind.m-2
Number of individuals
Cephalic disk diameter (mm)

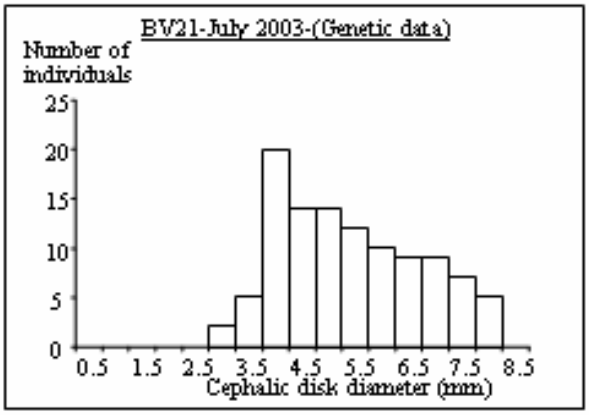

BS29-July 2003-(N=228, 2 grabs) 1140 ind.m-2
Number of individuals
Cephalic disk diameter (mm)


BS1-July 2003- (N=11, 4 grabs) 27.5 ind.m-2
Number of individuals
Cephalic disk diameter (mm)


BS29-September 2003-(N=176, 2 grabs) 880 ind.m-2
Number of individuals
Cephalic disk diameter (mm)


BS1-September 2003-(Genetic data)
Number of individuals
Cephalic disk diameter (mm)


BV21-March 2003-(N=98, 15 dredges) 19 ind.m-2
Number of individuals
25
20
15
10
5
0
0.5 1.5 2.5 3.5 4.5 5.5 6.5 7.5 8.5
Cephalic disk dimeter (mm)


BV21-July 2003-(Genetic data)
Number of individuals
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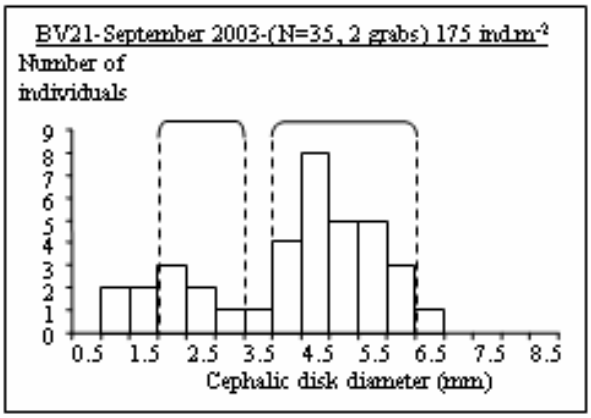
20
15
10
5
0
0.5 1.5 2.5 3.5 4.5 5.5 6.5 7.5 8.5
Cephalic disk diameter (mm)


BV21-September 2003-(N=35, 2 grabs) 175 ind.m-2
Number of individuals
9
8
7
6
5
4
3
2
1
0
0.5 1.5 2.5 3.5 4.5 5.5 6.5 7.5 8.5
Cephalic disk diameter (mm)

CONCLUSION

La métapopulation de *P. koreni* est caractérisée par une nette asynchronie reproductrice entre la Baie de Seine et la Baie des Veys, mais probablement aussi entre les deux noyaux d'abondance de la Baie de Seine (BS16 et Deauville) qui présentent une grande stabilité dans le temps et sont sources de larves pour les populations marginales. Cette asynchronie pourrait refléter des différences au niveau de la qualité de l'habitat (voir la thèse de Lambert, 1991) : les individus des populations marginales de la Baie des Veys (e.g. BV21) et de la Baie de Seine (e.g. BS29, BS1) grandissent plus lentement que ceux recrutant au niveau de l'estuaire de la Seine où l'apport trophique est plus élevée (i.e. au niveau des noyaux d'abondance tel que la station BS16). Les populations marginales présentent une différenciation temporelle significative (entre adultes de Mars et nouvelles recrues de Juillet), indiquant le recrutement d'un « pool » de larves génétiquement différenciées provenant probablement des principaux noyaux d'abondance (la BS16). Au contraire, une stabilité temporelle des fréquences alléliques est observée au niveau de la station BS16 pendant la phase de renouvellement de la population. Ces observations s'accompagnent d'un effet homogénéisant que l'on observe sur l'ensemble de la Baie de Seine entre recrues de Juillet, ce qui suggère, non seulement, un auto-recrutement massif sur le site, mais aussi un export massif de larves à l'échelle de la Baie de Seine. Pourtant, il existe tout de même une légère différenciation génétique entre recrues de Juillet et juvéniles de Septembre dans certaines populations en marge du noyau d'abondance BS16. Ceci peut suggérer (1) le recrutement d'une deuxième cohorte génétiquement différenciée, en provenance soit du noyau d'abondance situé au large de Deauville, soit de populations situées le long des côtes Picardes, (2) une dérive génétique exacerbée par une très forte mortalité estivale résultant soit de phénomènes densité-dépendant (compétition inter- et intra-spécifiques ; mécanismes de re-sédentarisation post-larvaire), soit de phénomènes anthropiques destructeurs tels que les évènements de dragages du chenal à

l'entrée du port du Havre et d'Antifer, et associé à la mise en place du nouveau port en eaux profondes (Havres 2000). Au niveau des populations adultes, nos résultats suggèrent que la plupart de la différenciation génétique observée est majoritairement due à des mécanismes de dérive génétique et de sélection différentielle associée à une forte mortalité pendant la période hivernale, en tous cas à l'échelle de la génération. On ne peut, pourtant, pas négliger la possibilité de plusieurs recrutements successifs provenant de populations spatialement différenciées se traduisant par la mise en place d'une structure génétique en mosaïque spatiale fluctuante au niveau de la métapopulation (Jolly *et al*., 2003). Aux échelles spatiale et temporelle de notre étude, nous observons une structure proche du modèle continent-îles avec des aspects de type source(s)-puit(s) entre (1) la Baie de Seine orientale (la source) et la Baie des Veys (le puit), et (2) le noyau d'abondance BS16 (la source) et les populations marginales BS29, BS19, BS1 (les puits).

Pour résumer, nous avons mis en évidence (1) un effet homogénéisant de la migration pendant la phase de renouvellement des populations (phase de recrutement), (2) une asynchronie reproductrice entre noyaux agissant vraisemblablement sur la différenciation locale des populations, (3) une stabilité des fréquences alléliques au sein du principal noyau d'abondance, lié à un auto-recrutement massif sur ce site, (4) une instabilité des fréquences alléliques dans les populations marginales, lié au recrutement d'un « pool » larvaire génétiquement différencié, (5) un rôle potentiel des processus de densité-dépendance tels que la compétition intra- et inter-spécifiques et les mécanismes de re-suspension postlarvaire sur la micro-structuration de la Baie de Seine orientale, et (6) l'importance des effets de dérive génétique par mortalité des juvéniles, pendant la période hivernale.

ANNEXES
CHAPITRE III

Annexe III. 1 : *Pectinaria koreni.* Fréquences alléliques pour cinq locus microsatellites (PKGT1; PKAT/GT1; PKAT/GT3; PKAT/GT2 et PKAT/GT4). Jolly *et al.* (2003).

	POPULATIONS								
PKGT1	BS29-99	BS15-99	BV21-99	DR191-94	DR191-95	DR191-96	DR186-94	DR186-96	DR123-94
(N)	39	23	29	21	18	0	0	0	0
198			0.0345			------	------	------	------
200			0.0345			------	------	------	------
202	0.0128		0.0172			------	------	------	------
204		0.0435	0.0690			------	------	------	------
206	0.0256		0.0345			------	------	------	------
208			0.0345			------	------	------	------
212	0.0256	0.0217	0.1379			------	------	------	------
214		0.0435	0.0345			------	------	------	------
216			0.0690			------	------	------	------
218			0.1034			------	------	------	------
220	0.0256	0.1087	0.0345			------	------	------	------
222	0.0513		0.0172			------	------	------	------
224	0.0256	0.1087	0.0345			------	------	------	------
226	0.0256	0.0435		0.0476		------	------	------	------
228	0.0897		0.1207	0.0476		------	------	------	------
230	0.0897	0.0435				------	------	------	------
232	0.0641		0.0690	0.0476	0.0556	------	------	------	------
234	0.0641	0.0435	0.0345	0.0238		------	------	------	------
236	0.0128	0.0217	0.0862	0.0238		------	------	------	------
240	0.1026	0.0435			0.0556	------	------	------	------
242	0.0641	0.0870		0.0238		------	------	------	------
244	0.0641	0.0870		0.0238		------	------	------	------
246	0.0385	0.1087		0.0952	0.0556	------	------	------	------
248	0.0256	0.0435		0.2143	0.1389	------	------	------	------
250	0.0385	0.0217		0.0238		------	------	------	------
252				0.0952	0.1111	------	------	------	------
254	0.0256			0.0476	0.1389	------	------	------	------
256		0.0435		0.0476	0.0556	------	------	------	------
258	0.0769	0.0870		0.1429	0.1667	------	------	------	------
260	0.0128				0.0556	------	------	------	------
262	0.0128				0.0556	------	------	------	------
268	0.0256			0.0952		------	------	------	------
282					0.1111	------	------	------	------
316			0.0345			------	------	------	------

	POPULATIONS								
PKAT/GT1	BS29-99	BS15-99	BV21-99	DR191-94	DR191-95	DR191-96	DR186-94	DR186-96	DR123-94
(N)	19	28	31	22	23	20	22	14	20
228									0.0250
328		0.0357							
332		0.0179	0.0323		0.0217				
334	0.0526		0.0161			0.0250			
336		0.0179	0.0161	0.0227			0.0682		
338	0.0263	0.0179		0.0455		0.0500			
340	0.0789		0.0161	0.0227	0.0217	0.0250			
342	0.0263	0.0536	0.0645						0.0250
344	0.2105	0.0357	0.0323	0.0227		0.0250	0.0227		
346	0.0789	0.0357	0.1290		0.0217		0.0227	0.0357	0.1000
348	0.0526	0.0714	0.0645	0.0682	0.0435	0.0500		0.0714	0.0750
350					0.1087		0.0682		0.0250
352	0.0526	0.0357	0.0484	0.0455	0.0217	0.0750			0.0250
354	0.0263	0.0893		0.0227	0.0435	0.0250	0.0455	0.0357	0.0500
356	0.0263	0.0357	0.0806	0.0227	0.0217		0.0682	0.0714	0.0250
358		0.0357	0.0645	0.0455	0.0217	0.0750	0.0455		
360	0.0263	0.0893	0.0484	0.0227	0.0435			0.0357	0.0750
362	0.0526	0.0179	0.0161	0.2045	0.1304	0.2000	0.0909	0.1071	
364	0.0789	0.1250	0.1129	0.0227	0.0435	0.0500			0.1000
366	0.0526	0.0714		0.0682		0.0750	0.0455	0.0357	0.1250
368	0.0526		0.0161	0.0682	0.0435	0.0500	0.0455	0.0714	0.0250
370		0.0179	0.0323		0.0217		0.0227		
372		0.0714	0.0323	0.0455		0.0500	0.0455	0.0357	0.0250
374	0.0526	0.0357		0.0227	0.0217			0.0714	0.0250
376		0.0179	0.0323	0.0455	0.0435	0.0250	0.0682	0.0357	0.0250
378							0.0227		0.0750
380			0.0484				0.0227		
382				0.0227	0.0217		0.0455	0.0357	
384				0.0455	0.0435	0.0250		0.0714	0.0500
386	0.0263					0.0250	0.0455	0.0357	0.0250
388				0.0227	0.0217	0.0250	0.0455		
390		0.0179	0.0161			0.0250	0.0227		0.0250
392		0.0179	0.0645	0.0227		0.0250			0.0500
394					0.0217	0.0250			
396	0.0263			0.0227			0.0455	0.1429	
398								0.0714	
400					0.0652				
402		0.0179			0.0652		0.0227		
404			0.0161						
406					0.0652		0.0227		
410		0.0179		0.0227					
412						0.0250			
414								0.0357	
418				0.0227					
420							0.0227		
422					0.0217	0.0250			
426							0.0227		
430									0.0250

	POPULATIONS								
PKAT/GT2	BS29-99	BS15-99	BV21-99	DR191-94	DR191-95	DR191-96	DR186-94	DR186-96	DR123-94
(N)	32	26	27	23	24	24	23	15	20
232				0.0435		0.0417			
238		0.0192							
242		0.0769							
248						0.0417			
250				0.0217					
258						0.0208			
260				0.0217	0.0208				
266			0.0556						
268		0.0192							
270		0.0192					0.0435	0.0333	
276						0.0417			
278	0.0313		0.0370			0.0417		0.0333	
280	0.0156	0.0385	0.0556	0.0870					
282	0.0313				0.0625	0.1042	0.0217	0.2000	0.0500
284	0.0156	0.0192	0.0370	0.1087	0.0625	0.1042	0.0870		0.0500
286	0.0781	0.0385	0.0185	0.0652	0.0208		0.0435		
288	0.0156	0.0192	0.0370		0.0833	0.0208			0.0500
290	0.0156	0.0577	0.0741	0.0217	0.0208		0.0435		0.0750
292	0.0156		0.0370			0.0417		0.0667	
294	0.0156	0.0192	0.0185	0.0435	0.0208		0.1087	0.0667	
296		0.0577	0.0185	0.0435		0.0417		0.0667	
298	0.0313	0.0385	0.0185						
300				0.0435		0.0417		0.1000	
302	0.0781			0.0435		0.0417	0.1304	0.0333	
304	0.0156	0.0192			0.0833			0.0667	0.0250
306	0.0313			0.0217	0.0833	0.0208	0.0217		0.0750
308	0.0313	0.0192	0.0370	0.0217	0.0208	0.0208	0.0217		0.0500
310	0.0156				0.0208		0.1087		0.0500
312	0.0469	0.0769	0.0741		0.0625		0.0217		
314	0.0313	0.0385	0.0185	0.0435	0.0625	0.0208			
316	0.0781	0.0192							
318							0.0217		
320			0.0556	0.0652	0.0625	0.0208			0.0500
322	0.0313		0.0185			0.0417	0.0217		
324		0.0192	0.0370	0.0435		0.0417	0.0217		0.0250
326		0.0769	0.0370		0.0208			0.0667	
328	0.0625		0.0370				0.0217	0.0667	0.0500
330		0.0192	0.0185		0.0417	0.0625			0.0500
332			0.0185	0.0435					0.0500
334	0.0313	0.0192	0.0185		0.0208	0.0208	0.0217		
336		0.0962	0.0185	0.0435	0.0625	0.0208	0.0435		
338	0.0469			0.0217	0.0208	0.0208	0.0435		0.0250
340		0.0192		0.0435			0.0435	0.0667	
342					0.0417				0.1250
344		0.0192	0.0370		0.0208	0.0417			
346			0.0370	0.0870	0.0208	0.0417			
348	0.0938	0.0577	0.0926						0.0250
350		0.0385							0.0500
352	0.0469								
354	0.0469								
356			0.0185		0.0208	0.0417	0.0217	0.0667	0.0750
360	0.0313								
362							0.0870		0.0500

PKAT/GT2 (suite)	BS29-99	BS15-99	BV21-99	DR191-94	DR191-95	DR191-96	DR186-94	DR186-96	DR123-94
364		0.0192							
368		0.0192							
376					0.0417				
384	0.0156								
392				0.0217				0.0667	
394			0.0185						

	POPULATIONS								
PKAT/GT3	BS29-99	BS15-99	BV21-99	DR191-94	DR191-95	DR191-96	DR186-94	DR186-96	DR123-94
(N)	22	19	22	20	24	19	18	18	16
136			0.0455						
142		0.0526	0.0455			0.0526			
144	0.0455				0.0417				
145			0.0455						
146		0.0526	0.0455	0.0500	0.0417	0.1053			0.1250
148	0.2727	0.2105	0.2273	0.1000	0.1667	0.1053	0.1111	0.0556	0.1250
150	0.1818	0.1053	0.1818	0.0500	0.1667	0.0526	0.0556	0.0556	0.1250
152	0.1364	0.1053	0.0909	0.1000	0.0833		0.1111	0.3889	0.0625
154	0.0909	0.1053	0.0455	0.3500	0.1250	0.4211	0.2222	0.1667	0.1250
156		0.1579	0.0909	0.3000	0.0833	0.1053	0.1667	0.0556	0.3125
158	0.0455	0.1579	0.0455	0.0500	0.0833	0.0526	0.2222	0.1667	0.0625
160		0.0526	0.0455		0.0417			0.0556	0.0625
162					0.0417		0.0556	0.0556	
164			0.0455			0.0526	0.0556		
166	0.0909				0.0417	0.0526			
168	0.0909								
170			0.0455						
172	0.0455				0.0417				
174					0.0417				

	POPULATIONS								
PKAT/GT4	BS29-99	BS15-99	BV21-99	DR191-94	DR191-95	DR191-96	DR186-94	DR186-96	DR123-94
(N)	17	28	26	23	23	21	22	10	21
334							0.0227		0.0238
336		0.0179			0.0217			0.0500	0.0238
338		0.0179		0.0435	0.1739	0.0476	0.1591	0.0500	0.0476
340	0.0588	0.1429		0.2609	0.2609	0.0952	0.2727	0.2000	0.2143
342	0.0588	0.0714	0.1154	0.1087	0.1522	0.1190		0.1000	0.1190
344	0.1176	0.0179	0.1346	0.0435	0.0217	0.0476		0.1000	0.0476
346	0.0588		0.1346	0.0217	0.0217		0.0227	0.0500	0.0238
348			0.0385	0.0217	0.0217	0.0476	0.0455	0.0500	0.0476
350	0.0882		0.0385	0.0217			0.0227		0.0238
352	0.0588	0.0357	0.0577		0.0435	0.0238	0.1136		0.0238
354		0.0357	0.0192	0.0217	0.0435			0.1000	0.0714
356			0.0385	0.0217	0.0217	0.0476	0.0227		
358	0.0294	0.1429	0.0192	0.1087	0.0435	0.1667	0.1818	0.1500	
360	0.0588	0.1071	0.0385	0.0435	0.0217	0.0238	0.0227		0.0714
362	0.0882	0.0179	0.0192	0.0435	0.0217	0.0476	0.0455		0.1190
364	0.0294	0.0536	0.0769	0.0217				0.0500	0.0238
366	0.0882	0.0536	0.0769	0.0652	0.0652	0.0476		0.0500	0.0476
368	0.0588	0.0893	0.0577			0.0238	0.0227		0.0238
370	0.0588	0.0179	0.0769	0.0217	0.0217	0.0952		0.0500	
372		0.0536		0.0435	0.0217	0.0476			0.0238
374	0.0588	0.0179		0.0217		0.0952	0.0227		
376	0.0294	0.0179	0.0192	0.0435					0.0238
378	0.0294	0.0357	0.0385	0.0217	0.0217		0.0227		
380	0.0294	0.0179							
386		0.0357							
390						0.0238			

Annexe III. 2 : *Pectinaria koreni* . Mission "Pectow· Mars 2003" : Fréquences alléliques pour quatre locus microsatellites (PKGT1; PKAT/GT1; PKAT/GT2 et PKAT/GT4). Jolly *et al* . (en préparation).

	POPULATIONS				
PKGT1	BS1	BS16	BS29	BS191	BV21
(N)	38	49	36	84	66
218	0.0132			0.0060	0.0152
220				0.0417	
222	0.0132	0.0102			0.0379
224	0.0526		0.0139	0.0357	0.0076
226	0.0395	0.0408	0.0278	0.0238	0.0076
228	0.0132	0.0306	0.0417	0.0595	0.0152
230	0.0132	0.0408		0.0595	0.0682
232	0.0395	0.0510		0.0536	
234	0.0263	0.0204	0.0556	0.0655	0.0530
236	0.0132	0.1020	0.0556	0.0774	0.0909
238	0.0526	0.0918	0.0972	0.0238	0.0455
240	0.0789	0.0918	0.0972	0.0774	0.1136
242	0.1316	0.1224	0.0972	0.0833	0.1288
244	0.1447	0.1020	0.1944	0.0774	0.0833
246	0.0526	0.0408	0.0694	0.0952	0.0606
248	0.0789	0.0306	0.0278	0.0298	0.0227
250	0.1053	0.1122	0.0417	0.0476	0.0455
252	0.0395	0.0306	0.0139	0.0536	0.0530
254	0.0395	0.0204	0.0694	0.0179	0.0606
256	0.0132		0.0278	0.0298	0.0379
258		0.0102		0.0060	0.0152
260			0.0417	0.0298	
262	0.0263	0.0408		0.0060	0.0076
264		0.0102	0.0278		0.0152
276	0.0132				
278					0.0152

	POPULATIONS				
PKAT/GT1	BS1	BS16	BS29	BS191	BV21
(N)	51	76	57	114	86
226		0.0132			
318				0.0132	
326		0.0132	0.0088		0.0116
328		0.0066		0.0219	
330	0.0098	0.0066		0.0044	
332		0.0066		0.0044	0.0058
334		0.0066	0.0088	0.0219	0.0116
336	0.0392	0.0197	0.0088	0.0219	0.0116
338		0.0395	0.0351	0.0044	0.0116
340	0.0196	0.0592	0.0263	0.0088	0.0407
342	0.0686	0.0461	0.0351	0.0746	0.0291
344	0.0392	0.0395	0.0088	0.0395	0.0465
346	0.0588	0.0132	0.0175	0.0482	0.0465
348	0.0294	0.0329	0.0702	0.0307	0.0407
350	0.0588	0.0329	0.0614	0.0702	0.0291
352	0.0294	0.0461	0.0789	0.0219	0.0349
354	0.0686	0.0329	0.0439	0.0570	0.0465
356	0.0686	0.0263	0.0175	0.0614	0.0465
358	0.0196	0.0329	0.0614	0.0175	0.0291
360	0.0392	0.0461	0.0702	0.0439	0.0640
362	0.0294	0.0592	0.0526	0.0482	0.0523
364	0.0392	0.0395	0.0175	0.0482	0.0291
366	0.0490	0.0461	0.0175	0.0175	0.0349
368	0.0098	0.0197	0.0526	0.0175	0.0640
370	0.0588			0.0219	0.0233
372	0.0294		0.0263	0.0175	0.0233
374	0.0098	0.0197	0.0088	0.0175	0.0174
376	0.0294	0.0263		0.0307	0.0233
378	0.0098	0.0658	0.0439	0.0263	0.0291
380	0.0784	0.0197		0.0044	0.0407
382	0.0098	0.0197	0.0351	0.0044	0.0291
384		0.0329	0.0175		0.0174
386	0.0098	0.0132	0.0526	0.0219	0.0116
388	0.0294	0.0263	0.0175	0.0088	0.0291
390	0.0196	0.0329	0.0439	0.0219	0.0233
392		0.0066	0.0175	0.0132	
394	0.0098	0.0197		0.0175	
396			0.0088	0.0175	0.0058
398	0.0196	0.0132	0.0088		0.0174
400	0.0098	0.0132		0.0088	0.0116
402				0.0175	
404			0.0088	0.0044	
406			0.0175	0.0219	0.0058
408		0.0066			
410				0.0044	
414				0.0044	0.0058
416				0.0088	
420				0.0088	

	POPULATIONS				
PKAT/GT2	BS1	BS16	BS29	BS191	BV21
(N)	52	81	57	116	96
238				0.0043	
256				0.0086	0.0052
258					0.0052
264			0.0088		
266			0.0088		0.0052
268	0.0192				0.0417
270		0.0185		0.0043	0.0104
272		0.0062	0.0088		0.0052
274		0.0247		0.0086	0.0104
276	0.0288	0.0494	0.0088	0.0172	0.0365
278	0.0577	0.0185	0.0439	0.0560	0.0260
280	0.0288	0.0432	0.0263	0.0345	0.0573
282	0.0192	0.0185	0.0439	0.0345	0.0104
284	0.0096	0.0370	0.0088	0.0216	0.0208
286	0.0288	0.0185	0.0088	0.0129	0.0208
288	0.0096		0.0526	0.0086	0.0208
290	0.0096	0.0185		0.0302	0.0052
292	0.0192	0.0185		0.0302	0.0260
294	0.0288	0.0247	0.0088		0.0156
296	0.0096	0.0309	0.0351	0.0388	0.0313
298	0.0385	0.0123	0.0175	0.0388	0.0365
300	0.0481	0.0432	0.0175	0.0216	0.0208
302		0.0556	0.0439	0.0345	0.0260
304		0.0123	0.0439	0.0517	0.0156
306	0.0192	0.0494		0.0216	0.0208
308	0.0096	0.0185	0.0175	0.0388	0.0104
310		0.0185	0.1053	0.0259	0.0313
312	0.0288	0.0062	0.0263	0.0172	0.0156
314	0.0288	0.0370	0.0175	0.0129	0.0156
316		0.0370	0.0351	0.0259	
318	0.0288	0.0185	0.0175	0.0345	
320	0.0288	0.0370	0.0088	0.0302	0.0260
322	0.0192	0.0123	0.0614	0.0172	0.0104
324	0.0481	0.0123	0.0088	0.0302	0.0313
326	0.0192	0.0123	0.0789	0.0216	0.0313
328	0.0288	0.0123	0.0088	0.0259	0.0156
330	0.0288	0.0494		0.0345	0.0469
332	0.0481	0.0309	0.0526	0.0302	0.0208
334	0.0481	0.0185	0.0351	0.0086	0.0313
336	0.0385	0.0185	0.0526	0.0129	0.0469
338	0.0385	0.0247	0.0088	0.0086	0.0156
340	0.0288	0.0123		0.0172	0.0208
342	0.0096	0.0370	0.0175	0.0216	0.0104
344	0.0096		0.0088	0.0129	0.0260
346	0.0192	0.0123	0.0175	0.0086	0.0052
348	0.0096		0.0088	0.0129	0.0052
350		0.0123		0.0129	0.0052
352	0.0192			0.0129	0.0052
354			0.0175		0.0260
356				0.0086	0.0052
358	0.0096		0.0088	0.0086	0.0208
360	0.0192			0.0043	
362	0.0096	0.0185			0.0052
364		0.0062			0.0104
366	0.0192	0.0185			
370					0.0052

PKAT/GT2 (suite)	BS1	BS16	BS29	BS191	BV21
372	0.0192			0.0043	
374				0.0043	0.0156
376		0.0123		0.0086	
378	0.0096				0.0104
380		0.0062			
382				0.0043	
384				0.0043	

	POPULATIONS				
PKAT/GT4	BS1	BS16	BS29	BS191	BV21
(N)	54	81	54	119	93
264	0.0093				
278					0.0054
338		0.0432	0.0093	0.0042	0.0430
340	0.0648	0.0741	0.0741	0.0672	0.0430
342	0.1111	0.0988	0.2037	0.2059	0.2258
344	0.1481	0.0926	0.0926	0.0882	0.1075
346	0.0833	0.0802	0.0463	0.0252	0.0591
348	0.0556		0.0185	0.0294	0.0215
350	0.0093	0.0247	0.0370	0.0462	0.0215
352	0.0370	0.0370	0.0370	0.0168	0.0054
354		0.0247	0.0185	0.0042	0.0161
356		0.0370	0.0370	0.0252	0.0215
358	0.0370	0.0309	0.0278	0.0336	0.0215
360	0.0648	0.0802	0.0741	0.0966	0.0538
362	0.0741	0.0741	0.0370	0.0672	0.0591
364	0.0370	0.0679	0.0556	0.0714	0.0430
366	0.0370	0.0309	0.0556	0.0420	0.0323
368	0.0463	0.0432	0.0185	0.0546	0.0806
370	0.0648	0.0370	0.0370	0.0210	0.0323
372	0.0556	0.0370	0.0370	0.0168	0.0054
374	0.0093	0.0062	0.0185	0.0126	0.0108
376	0.0093	0.0185	0.0185	0.0084	0.0161
378	0.0185	0.0370	0.0185	0.0252	0.0591
380		0.0185		0.0126	
382	0.0093		0.0093	0.0084	0.0161
384	0.0185		0.0185	0.0126	
388				0.0042	
392		0.0062			

Annexe III. 3 : *Pectinaria koreni* . Mission "Pectow 2003-Juillet" : Fréquences alléliques pour quatre locus microsatellites (PKGT1; PKAT/GT1; PKAT/GT2 et PKAT/GT4). Jolly *et al.* (en préparation).

	POPULATIONS													
PKGT1	BS1	BS16	DR191	A1	A5	A8	BS29	C3	C4	BV21	V2	V3	BV19	BV7
(N)	26	56	42	53	26	33	47	49	55	15	17	23	37	22
206				0.0094										
208		0.0089												
210												0.0435		
216	0.0192	0.0089						0.0204						
218		0.0179	0.0119		0.0385			0.0306	0.0182			0.0217		
220		0.0089		0.0283		0.0303	0.0319	0.0306			0.0588	0.1304		
222		0.0179	0.0238	0.0377	0.0769		0.0106		0.0182			0.0217	0.0405	0.0909
224		0.0179	0.0238	0.0566			0.0532	0.0204	0.0182			0.0870	0.0135	0.0909
226	0.0192	0.0268	0.0476	0.0189	0.0385		0.0213	0.0408	0.0091		0.1176		0.0405	
228	0.0577	0.0357	0.0714	0.0377		0.0303	0.0106	0.0510	0.0182	0.1000		0.0217		
230	0.0192	0.0089	0.0119	0.0377	0.0192	0.1364	0.0319	0.0306	0.0364	0.2000		0.0652		0.0227
232	0.0962	0.0446	0.0238	0.0472	0.1154	0.0606	0.1277	0.0918	0.0545		0.0588	0.0217	0.1081	0.0682
234	0.0192	0.0357	0.0238	0.0472		0.0303		0.0612	0.0455				0.0541	0.0909
236	0.0769	0.0625	0.0238	0.0943	0.0769	0.0758	0.0745	0.0816	0.0364	0.1667			0.0541	0.1591
238	0.0962	0.1518	0.1548	0.1038	0.0577	0.0758	0.1170	0.0306	0.1455	0.0667	0.2647	0.0435	0.1622	
240	0.0769	0.0714	0.1190	0.0472	0.0577		0.0957	0.0408	0.0818	0.0667	0.0588	0.2174	0.0541	0.0455
242	0.1346	0.0536	0.1310	0.1038	0.0385	0.1061	0.0851	0.0714	0.0636	0.1333	0.1176	0.0435	0.0405	0.1364
244	0.0385	0.0714	0.0238	0.0566	0.0385	0.1061	0.1064	0.0306	0.1091	0.0667	0.1765	0.0435	0.0811	0.0455
246	0.1346	0.0268	0.0714	0.1038	0.0769	0.0455	0.0426	0.0816	0.1364			0.0652	0.0676	0.0682
248		0.0446	0.0476	0.0283	0.0769	0.0909	0.0319	0.0408	0.0455		0.0882		0.0811	0.0682
250	0.0385	0.0536	0.0238	0.0755	0.0385	0.0758	0.0319	0.0204	0.0364		0.0294	0.1087	0.0135	
252	0.0577	0.0268	0.0357	0.0189	0.0769	0.0606	0.0319	0.0714	0.0273				0.0270	
254	0.0769	0.0625	0.0357	0.0094	0.0577	0.0303		0.0408	0.0182	0.0667				
256		0.0089	0.0238	0.0094		0.0455	0.0426						0.0270	
258	0.0192	0.0268					0.0213	0.0306	0.0273		0.0294		0.0405	0.0909
260		0.0179			0.0385			0.0204	0.0091	0.0667			0.0541	
262		0.0179	0.0238		0.0385			0.0204	0.0182	0.0667		0.0217	0.0270	
264							0.0106					0.0435		
266		0.0179	0.0238											
268					0.0385		0.0106							
270	0.0192							0.0204					0.0135	
272				0.0189										
274								0.0102						
276		0.0179							0.0273					0.0227
278			0.0238	0.0094										
282							0.0106							
290								0.0102						
296		0.0179												
300		0.0089												
304		0.0089												

	POPULATIONS												
PKAT/GT1	BS1	BS16	DR191	A1	A5	A8	BS29	C3	C4	BV21	V2	V3	BV19	BV7
(N)	45	86	74	68	34	47	70	73	77	21	26	31	51	32
316							0.0143							
322				0.0147		0.0213	0.0143							
324									0.0065					
326				0.0074	0.0294			0.0205	0.0390			0.0161	0.0392	0.0156
328		0.0058	0.0068	0.0074	0.0147		0.0143	0.0137	0.0130		0.0577		0.0196	0.0313
330			0.0135			0.0106	0.0071		0.0130			0.0161		
332			0.0068	0.0147		0.0213	0.0071	0.0068	0.0065				0.0196	
334	0.0222	0.0116		0.0441	0.0147	0.0319	0.0286	0.0137					0.0196	
336		0.0233	0.0203	0.0147			0.0071	0.0137	0.0130			0.0323		
338	0.0444	0.0233	0.0203	0.0221			0.0071	0.0342			0.0385		0.0196	0.0156
340	0.0222	0.0116	0.0135	0.0809	0.0147	0.0213	0.0357	0.0205	0.0455	0.0476	0.0192	0.0161		0.0313
342	0.0444	0.0349	0.0473	0.0735	0.0882		0.0714	0.0274	0.0195	0.0238		0.0323	0.0098	0.0781
344	0.0222	0.0291	0.0676	0.0221	0.0441	0.0319	0.0571	0.0205	0.0584	0.0476	0.0769	0.0484	0.0098	0.0313
346	0.0333	0.0465	0.0135	0.0515	0.0882	0.0638	0.0429	0.0342	0.0325	0.0238	0.0577	0.0323	0.0980	0.0781
348	0.0333	0.0640	0.0473	0.0441	0.1029	0.0426	0.0143	0.0479	0.0260	0.0714	0.0385	0.0323	0.0392	0.0313
350	0.0333	0.0407	0.0676	0.0662	0.0147	0.0213	0.0429	0.0274	0.0519	0.0238	0.0769	0.0484	0.0588	0.0313
352	0.0333	0.0640	0.0135	0.0221	0.0441	0.0426	0.0143	0.0479	0.0325	0.0476	0.0192	0.0161	0.0588	0.0469
354	0.0111	0.0349	0.0338	0.0147	0.0441	0.0106	0.0429	0.0274	0.0065		0.0192	0.0968		0.0469
356	0.0333	0.0233	0.0608	0.0809	0.1029	0.0851	0.0571	0.0342	0.0455	0.1190	0.0577	0.0645	0.0392	0.0781
358	0.0556	0.0523	0.0473	0.0147	0.0588	0.0745	0.0357	0.0685	0.0519	0.0238	0.0192	0.0484	0.0392	0.0156
360	0.0778	0.0291	0.0203	0.0147	0.0441	0.0851	0.0214	0.0616	0.0325		0.0385	0.0161	0.0882	0.0313
362	0.0667	0.0465	0.0473	0.0662		0.0638	0.0429	0.0342	0.0584	0.0714	0.0192	0.0806	0.0294	
364	0.0556	0.0756	0.0608	0.0368	0.0441	0.0106	0.0214	0.0616	0.0130	0.0476	0.0385	0.0161	0.0588	0.0156
366	0.0333	0.0174	0.0541		0.0294	0.0851	0.0357	0.0137	0.0390	0.0238	0.0385	0.0161	0.0098	0.0938
368	0.0444	0.0058	0.0338	0.0147	0.0294		0.0214	0.0342	0.0390		0.0385	0.0484		0.0625
370	0.0444	0.0349	0.0135	0.0441	0.0147	0.0106	0.0214	0.0137	0.0390	0.0952		0.0323	0.0196	0.0156
372		0.0407	0.0135	0.0441	0.0294	0.0106	0.0571	0.0205	0.0325	0.0714	0.0769	0.0484	0.0490	
374	0.0222	0.0174	0.0405	0.0147	0.0147	0.0213	0.0357	0.0479	0.0325			0.0161	0.0196	0.0156
376	0.0222	0.0233	0.0338	0.0441		0.0319	0.0214	0.0342	0.0065	0.0238	0.0192	0.0645	0.0294	
378	0.0333	0.0581	0.0608	0.0221		0.0319	0.0071	0.0137	0.0260	0.0476	0.0962		0.0098	
380	0.0222	0.0349	0.0338	0.0074	0.0441		0.0357	0.0342	0.0065			0.0161	0.0490	0.0313
382	0.0111	0.0116	0.0270	0.0221	0.0147	0.0213	0.0286	0.0411	0.0195			0.0645	0.0392	0.0156
384	0.0556	0.0291	0.0135	0.0221	0.0147	0.0213	0.0143	0.0068	0.0260				0.0490	0.0156
386		0.0233					0.0071	0.0137	0.0325	0.0238	0.0192		0.0098	
388	0.0111	0.0174	0.0068	0.0147		0.0213	0.0071	0.0274	0.0130	0.0952	0.0192	0.0484		
390	0.0111	0.0116	0.0135			0.0319			0.0390	0.0238	0.0769			0.0469
392	0.0111	0.0058	0.0068	0.0074		0.0106	0.0143	0.0205	0.0065				0.0098	
394	0.0111	0.0116	0.0068	0.0074			0.0286	0.0068					0.0294	
396		0.0058	0.0203				0.0143						0.0098	0.0469
398	0.0111	0.0174			0.0147	0.0106		0.0068		0.0476			0.0098	0.0156
400	0.0111	0.0058				0.0213	0.0071		0.0195					0.0313
402				0.0221	0.0147	0.0106		0.0137	0.0065			0.0323		
404	0.0111	0.0058			0.0147				0.0195					
406	0.0111					0.0213		0.0068						0.0156
408	0.0222		0.0068		0.0147		0.0071	0.0068					0.0098	
410	0.0111	0.0058						0.0068	0.0130					0.0156
412							0.0071							
414								0.0068	0.0065					
416			0.0068					0.0068	0.0065					
420											0.0385			
422									0.0065					
424							0.0071							
426							0.0214							

	POPULATIONS													
PKAT/GT2	BS1	BS16	DR191	A1	A5	A8	BS29	C3	C4	BV21	V2	V3	BV19	BV7
(N)	44	83	78	78	40	50	72	71	74	20	25	29	51	31
252													0.0098	
256				0.0128										
258			0.0064							0.0250				
260					0.0125									
262									0.0068	0.0500				
264					0.0125		0.0069							
266				0.0128	0.0125	0.0100	0.0139							
268			0.0128	0.0064		0.0200	0.0139	0.0141	0.0068				0.0196	
270		0.0060	0.0192				0.0347		0.0068					
272		0.0120	0.0064				0.0208	0.0141		0.0250	0.0200	0.0172		0.0161
274	0.0227	0.0301	0.0321	0.0513	0.0125	0.0500		0.0282	0.0270	0.0250				
276		0.0301	0.0385		0.0250	0.0600	0.0139	0.0070			0.0200		0.0294	0.0161
278		0.0181	0.0192	0.0577	0.1000	0.0400	0.0417	0.0704	0.0405			0.0690	0.0196	
280	0.0341	0.0241	0.0321	0.0128	0.0250	0.0100	0.0278	0.0211	0.0338	0.0250	0.0400	0.0172	0.0294	0.0645
282	0.0341	0.0241	0.0192	0.0064		0.0100	0.0347	0.0141	0.0068					
284	0.0114	0.0181		0.0321	0.0250			0.0282			0.1000		0.0098	
286		0.0120	0.0256	0.0385			0.0069		0.0203	0.0750	0.0200		0.0098	
288	0.0227	0.0181			0.0125	0.0100		0.0352	0.0203	0.1500		0.0172	0.0588	0.0161
290	0.0114	0.0241	0.0385	0.0641		0.0300	0.0347	0.0211	0.0068		0.0200	0.0517	0.0686	0.0323
292	0.0568	0.0181		0.0321	0.0375	0.0100	0.0208	0.0352	0.0135	0.0500	0.0600	0.0345	0.0490	0.0645
294	0.0341	0.0361		0.0192	0.0250	0.0400	0.0417	0.0352	0.0135	0.0500	0.0600	0.0172	0.0392	0.0161
296	0.0341	0.0602	0.0321	0.0256	0.0250	0.0600	0.0208	0.0211	0.0135		0.1000	0.0690	0.0098	0.0484
298	0.0114	0.0422	0.0192	0.0513	0.1125	0.0500	0.0417	0.0282	0.0405	0.0500	0.0400	0.0172		0.0323
300	0.0455	0.0301	0.0641	0.0128	0.0375	0.0100	0.0278	0.0141	0.0473	0.0500		0.0172	0.0294	
302	0.0227	0.0361	0.0256	0.0128	0.0125	0.0200	0.0417		0.0405	0.0250		0.0345	0.0588	0.0968
304	0.0341	0.0181	0.0385	0.0385	0.0250	0.0600	0.0625	0.0352	0.0135		0.0200	0.0517	0.0588	0.0645
306	0.0455	0.0120	0.0256		0.0625	0.0200	0.0208	0.0141	0.0135			0.0690	0.0294	0.0161
308	0.0568	0.0422	0.0385	0.0256			0.0278	0.0141	0.0270		0.0400	0.0172		0.0161
310	0.0114	0.0301	0.0256	0.0192	0.0375	0.0200	0.0069	0.0352	0.0541	0.1000	0.0800	0.0345	0.0392	0.0323
312	0.0455	0.0301	0.0064	0.0256	0.0500	0.0300	0.0139	0.0423	0.0541			0.0517	0.0098	0.0484
314	0.0114	0.0120	0.0064	0.0513	0.0250	0.0300	0.0347	0.0141	0.0338	0.0250		0.0345	0.0098	0.0161
316	0.0227	0.0301	0.0256		0.0250	0.0100	0.0139	0.0211	0.0068	0.0250		0.0345	0.0196	0.0161
318	0.0114	0.0241	0.0256	0.0128	0.0125	0.0200	0.0139	0.0493	0.0473	0.0250		0.0172	0.0294	0.0161
320	0.0341		0.0449	0.0064	0.0125	0.0200	0.0208	0.0211	0.0405	0.0500	0.0400	0.0345	0.0098	
322	0.0455	0.0060	0.0385	0.0321		0.0100	0.0208	0.0141	0.0338		0.0200			0.0161
324	0.0568	0.0422	0.0128	0.0256	0.0375		0.0417	0.0352	0.0135		0.0200	0.0345	0.0196	0.0323
326	0.0227	0.0241	0.0192	0.0128	0.0125	0.0200	0.0347	0.0423	0.0405		0.0600		0.0490	0.0323
328	0.0341	0.0361	0.0321		0.0625		0.0278	0.0352	0.0203	0.0250		0.0345	0.0980	0.0484
330	0.0341	0.0181	0.0449	0.0513	0.0625	0.0300	0.0208	0.0282	0.0270			0.0172		0.0161
332	0.0455	0.0120		0.0513	0.0250	0.0600	0.0278	0.0493	0.0405		0.0200	0.0345	0.0196	
334			0.0385	0.0128		0.0300		0.0211	0.0203	0.0500	0.0400	0.0172	0.0196	0.0323
336	0.0568	0.0241	0.0385	0.0321		0.0800	0.0139	0.0141	0.0068		0.0200	0.0172	0.0196	0.0484
338		0.0422	0.0064	0.0192	0.0125		0.0347	0.0211	0.0135			0.0172	0.0196	0.0484
340		0.0060	0.0192				0.0139		0.0203					
342	0.0114	0.0241	0.0385	0.0064			0.0069		0.0068		0.0200	0.0172	0.0098	
344	0.0114	0.0120	0.0064	0.0192	0.0125		0.0069		0.0405			0.0172	0.0098	0.0161
346		0.0120	0.0192					0.0070			0.0400			0.0323
348			0.0064	0.0064	0.0125	0.0400	0.0069	0.0070	0.0068		0.0200		0.0098	
350		0.0301		0.0321	0.0125	0.0400	0.0069	0.0070		0.0250				
352	0.0114	0.0120	0.0256	0.0064			0.0139	0.0070		0.0500			0.0196	
354		0.0241		0.0128		0.0200	0.0139		0.0068			0.0172		
356	0.0227	0.0060		0.0064			0.0069	0.0070						0.0161
358			0.0064			0.0200			0.0068					
360				0.0128				0.0141	0.0068					
362			0.0128	0.0064		0.0100	0.0069	0.0070						

PKAT/GT2 (suite)	BS1	BS16	DR191	A1	A5	A8	BS29	C3	C4	BV21	V2	V3	BV19	BV7
364				0.0064					0.0068			0.0172	0.0098	
366					0.0125		0.0069	0.0141			0.0400			
368			0.0064					0.0141	0.0135	0.0250			0.0196	
370		0.0120					0.0139				0.0200			
372		0.0060		0.0064			0.0069	0.0070	0.0068					
374							0.0069		0.0068			0.0345	0.0196	
376	0.0114			0.0128										
378	0.0227													0.0161
380		0.0120												0.0161
382									0.0068			0.0172	0.0098	
384								0.0070						
390											0.0200			
396									0.0068					
398								0.0070						
400									0.0068					

	POPULATIONS													
PKAT/GT4	BS1	BS16	DR191	A1	A5	A8	BS29	C3	C4	BV21	V2	V3	BV19	BV7
(N)	44	83	78	68	34	47	64	72	75	18	24	29	48	29
264										0.0278				
332									0.0067					
336	0.0114				0.0294			0.0069	0.0133				0.0104	0.0172
338	0.0568	0.0181	0.0192	0.0515	0.0735	0.0638	0.0313	0.0625	0.0400	0.1111	0.0417		0.0313	0.0690
340	0.1705	0.0361	0.0769	0.1618	0.1324	0.0638	0.1172	0.1111	0.1000	0.0833	0.1458	0.1379	0.0833	0.2586
342	0.1591	0.2048	0.1987	0.1324	0.1912	0.2553	0.1016	0.1736	0.1333	0.1111	0.1250	0.1207	0.1979	0.0862
344	0.0795	0.1145	0.0833	0.0588	0.0882	0.0319	0.1328	0.0764	0.0733	0.0278	0.1042	0.0690	0.0104	0.0172
346	0.0455	0.0301	0.0128	0.0441	0.0147	0.0319	0.0391	0.0139	0.0400	0.0278	0.0625	0.0172	0.0208	0.0345
348		0.0422	0.0321	0.0441	0.0147	0.0213	0.0156	0.0417	0.0200	0.0278	0.0208	0.0172	0.0208	0.0172
350		0.0241	0.0577			0.0851	0.0469	0.0139	0.0267	0.0278	0.0625		0.0417	0.0172
352		0.0361	0.0321	0.0515	0.0147	0.0213	0.0234	0.0208	0.0267	0.0278	0.0208	0.0517	0.0104	
354	0.0114	0.0181	0.0321	0.0074	0.0294	0.0213	0.0156	0.0208	0.0200	0.0278	0.0208	0.0345	0.0313	0.0517
356	0.0227	0.0542	0.0449	0.0294	0.0294	0.0638	0.0547	0.0556	0.0333	0.0278	0.0417	0.0172	0.0625	
358	0.0455	0.0301	0.0513	0.0515	0.0441	0.0745	0.0781	0.0278	0.0400	0.0833		0.0345	0.0729	0.1207
360	0.0455	0.0663	0.0641	0.0735	0.0588	0.0213	0.0781	0.0486	0.0333		0.0625	0.0345	0.0417	0.0172
362	0.0455	0.0482	0.0577	0.0294	0.0294	0.0426	0.0391	0.0764	0.0467	0.0278	0.0625	0.0517	0.0521	0.0345
364	0.0568	0.0482	0.0513	0.0515	0.0588	0.0532	0.0625	0.0278	0.0867	0.0833	0.1250	0.0862	0.0833	0.0690
366	0.0909	0.0663	0.0192	0.0368	0.0441	0.0426	0.0469	0.0347	0.0533	0.1111	0.0208	0.0345	0.0625	0.0172
368	0.0455	0.0542	0.0321	0.0368		0.0319	0.0469	0.0417	0.0733	0.0278	0.0208	0.0345	0.0938	0.0172
370	0.0341	0.0181	0.0128	0.0074	0.0294	0.0106		0.0139	0.0333	0.0833	0.0208	0.0690	0.0208	0.0345
372	0.0227	0.0301	0.0256	0.0221	0.0147	0.0106		0.0208	0.0400			0.0345	0.0208	0.0690
374		0.0181	0.0128	0.0221	0.0147		0.0234	0.0347	0.0067			0.0345		0.0172
376		0.0060	0.0064	0.0368	0.0147	0.0106	0.0078	0.0208	0.0333	0.0278	0.0417	0.0517	0.0208	0.0172
378	0.0114	0.0241	0.0192	0.0221	0.0441			0.0208	0.0200	0.0278		0.0517	0.0104	0.0172
380	0.0114	0.0120	0.0577	0.0221	0.0147	0.0213		0.0069						
382	0.0227			0.0074			0.0078	0.0208						
384						0.0213	0.0156	0.0069						
386					0.0147		0.0078							
388	0.0114						0.0078					0.0172		

Annexe III. 4 : *Pectinaria koreni* . Mission "Pectow 2003-Juillet" : Fréquences alléliques pour quatre locus microsatellites (PKGT1; PKAT/GT1; PKAT/GT2 et PKAT/GT4). Jolly *et al.* (en préparation).

	POPULATIONS						
PKGT1	BS16	A1	BS29	BS1	BV21	V2	V3
(N)	53	46	42	54	20	16	27
210		0.0109					
216			0.0238				
218	0.0283	0.0435					0.0185
220	0.0566		0.0476	0.0093			0.0370
222		0.0109	0.0119	0.0648			
224		0.0109	0.0238	0.0278			0.0556
226	0.0377	0.0109	0.0357	0.0463	0.0250		
228	0.0660	0.0652	0.0357	0.0278	0.0500	0.1250	0.0185
230	0.0377	0.0761	0.0476	0.0556			0.0926
232	0.0849	0.0761	0.0476	0.0926	0.1250		0.0556
234	0.0377		0.0119	0.0833	0.0750		0.0185
236	0.0472	0.1087	0.0357	0.0648	0.1750	0.1250	0.1296
238	0.0472	0.0870	0.1310	0.0648			0.1481
240	0.1038	0.0761	0.0595	0.0648	0.1500	0.1875	0.0556
242	0.0943	0.1196	0.0476	0.0648	0.1000	0.2188	0.0926
244	0.0566	0.0435	0.0238	0.0648	0.0500	0.1563	0.0741
246	0.0189	0.0978	0.1310	0.1111		0.0625	0.0556
248	0.0377	0.0217	0.0595	0.0093	0.0500	0.0313	0.0370
250	0.0189	0.0543	0.0833	0.0370	0.1000	0.0625	
252	0.0377	0.0109	0.0595				0.0370
254	0.0283	0.0217	0.0476	0.0463	0.0500		0.0370
256	0.0660		0.0357	0.0185		0.0313	0.0370
258	0.0472						
260	0.0094	0.0326		0.0093			
262	0.0094			0.0093	0.0250		
264	0.0189	0.0217			0.0250		
274				0.0278			
278	0.0094						

	POPULATIONS						
PKAT/GT1	BS16	A1	BS29	BS1	BV21	V2	V3
(N)	78	64	65	80	28	30	35
242	0.0064						
250	0.0064						
318	0.0128						
322		0.0156					
324				0.0125		0.0167	
326		0.0156				0.0333	0.0286
328		0.0234	0.0231	0.0125			0.0143
330	0.0064	0.0078	0.0231	0.0188			
332	0.0321	0.0078	0.0231	0.0125			0.0286
334			0.0077	0.0313	0.0536	0.0167	
336	0.0192		0.0154	0.0188	0.0179	0.0333	0.0286
338	0.0192	0.0078	0.0154	0.0250	0.0357	0.0500	0.0286
340	0.0064	0.0625	0.0308	0.0313	0.0179	0.1000	
342	0.0192	0.0469	0.0385	0.0562		0.0167	0.0143
344	0.0385	0.0391	0.0231	0.0500		0.0167	0.0714
346	0.0769	0.0234	0.0154	0.0313	0.1250	0.0500	0.0143
348	0.0385	0.0625	0.0308	0.0562	0.0179	0.0500	0.0286
350	0.0449	0.0078	0.0154	0.0437		0.0333	0.0571
352	0.0321	0.0547	0.0231	0.0063	0.0536	0.0167	
354	0.0064	0.0625	0.0154	0.0313	0.0714	0.0167	0.0429
356	0.0385	0.0391	0.0615	0.0375	0.0357	0.0333	0.0286
358	0.0321	0.0547	0.0538	0.0313	0.0179	0.1000	0.0571
360	0.0577	0.0078	0.0154	0.0500	0.0357	0.0167	0.0143
362	0.0577	0.0625	0.0923	0.0375	0.0179	0.0833	0.0714
364	0.0449	0.0156	0.0692	0.0562	0.0179		0.0714
366	0.0321	0.0625	0.0154	0.0437	0.0893	0.0500	0.0429
368	0.0256	0.0391	0.0538	0.0188	0.0536		0.0429
370	0.0192	0.0391	0.0154	0.0313	0.0179		0.0143
372	0.0256	0.0078	0.0615	0.0188	0.0357	0.0333	0.0286
374	0.0192	0.0078	0.0385	0.0313	0.0714		0.0143
376	0.0256	0.0313	0.0154	0.0125		0.0167	0.0286
378	0.0192	0.0156	0.0154	0.0125	0.0179	0.0167	0.0143
380	0.0192		0.0077	0.0188		0.0167	0.0143
382	0.0192	0.0078	0.0538	0.0250	0.0536	0.0333	0.0571
384	0.0385	0.0313	0.0154	0.0063	0.0179		
386	0.0385	0.0234	0.0077	0.0313	0.0893		
388	0.0192	0.0156	0.0077	0.0125		0.0500	0.0143
390	0.0064	0.0078	0.0077	0.0188			0.0143
392	0.0064		0.0308				
394	0.0321	0.0234	0.0154	0.0125	0.0179	0.0167	0.0143
396	0.0128	0.0078	0.0308	0.0125			
398		0.0078		0.0125	0.0179	0.0333	
400	0.0128					0.0167	0.0143
402	0.0064	0.0078	0.0077				0.0429
404	0.0192			0.0063		0.0333	0.0143
406		0.0234		0.0063			
408		0.0078		0.0125			
410							0.0143
414		0.0156					
418			0.0077				
424	0.0064						
426							0.0143
436				0.0063			

	POPULATIONS						
PKAT/GT2	BS16	A1	BS29	BS1	BV21	V2	V3
(N)	79	67	68	79	30	29	35
248			0.0074				
252					0.0167		
258	0.0127				0.0167		
260			0.0074	0.0063			
264						0.0172	
266			0.0074			0.0172	
268		0.0149	0.0147	0.0063			
270	0.0063	0.0075	0.0147				
272	0.0127	0.0075	0.0147		0.0167	0.0345	0.0143
274	0.0127	0.0075	0.0074	0.0190		0.0172	
276	0.0316	0.0224	0.0221	0.0316		0.0172	0.0143
278	0.0316	0.0373	0.0368	0.0190	0.0833	0.0345	
280	0.0380	0.0373	0.0294	0.0190		0.0172	0.0429
282	0.0063	0.0149	0.0147	0.0127	0.0167	0.0172	
284	0.0190		0.0074	0.0253		0.0172	0.0143
286	0.0127	0.0373	0.0074	0.0190	0.0333	0.0172	0.0143
288	0.0127	0.0299	0.0221	0.0190	0.0167	0.0172	
290	0.0253	0.0075	0.0588		0.0167		
292	0.0253	0.0224	0.0368	0.0063	0.0167	0.0517	0.0286
294	0.0127	0.0672	0.0147	0.0063	0.0333	0.0345	0.0286
296	0.0063	0.0299	0.0294		0.0500		0.0143
298	0.0380	0.0522	0.0221	0.0443	0.0167	0.0172	
300	0.0380	0.0075	0.0294	0.0380	0.0500	0.1034	0.0286
302	0.0127	0.0373	0.0221	0.0316	0.0500		
304	0.0190	0.0075	0.0221	0.0443		0.0862	0.0429
306	0.0443	0.0299	0.0221	0.0253	0.0167		0.0143
308	0.0443	0.0224	0.0147	0.0127			0.0143
310	0.0127	0.0149	0.0221	0.0316	0.0333	0.0345	
312	0.0190	0.0224	0.0221	0.0316		0.0345	0.0143
314	0.0380	0.0075	0.0221	0.0063	0.0333	0.0345	
316	0.0253	0.0299	0.0294	0.0443	0.0167	0.0172	0.0143
318	0.0190	0.0149	0.0074	0.0253	0.0167		0.0286
320	0.0443	0.0149		0.0696	0.0333	0.0172	0.0286
322	0.0190	0.0149	0.0147	0.0127		0.0172	0.0143
324	0.0127		0.0147	0.0253	0.0500	0.0345	0.0143
326	0.0063	0.0448	0.0368	0.0380	0.0167	0.0172	0.0286
328	0.0127	0.0373	0.0368	0.0316		0.0172	0.0286
330	0.0443	0.0821	0.0074	0.0380	0.0167	0.0172	0.0143
332	0.0633	0.0149	0.0221	0.0316	0.0333	0.0345	0.0143
334	0.0063	0.0448	0.0441	0.0127	0.0333	0.0517	0.0429
336	0.0316		0.0221	0.0063	0.0333	0.0517	0.0714
338	0.0380	0.0373	0.0294	0.0190	0.0333		0.0286
340	0.0127	0.0224	0.0294	0.0190			0.0429
342	0.0190	0.0149	0.0074	0.0253	0.0500		0.0286
344	0.0127	0.0299	0.0074	0.0253		0.0172	0.0143
346	0.0063	0.0075	0.0074		0.0500		
348	0.0127		0.0074	0.0190			
350	0.0127		0.0074	0.0063			0.0429
352	0.0063				0.0167		
354			0.0147	0.0253			0.0571
356			0.0147	0.0190		0.0345	0.0143
358			0.0074				0.0286
360	0.0127			0.0063			
362	0.0063	0.0075	0.0074	0.0063			0.0143
364	0.0127	0.0075	0.0074			0.0172	

PKAT/GT2 (suite)	BS16	A1	BS29	BS1	BV21	V2	V3
366	0.0063		0.0074		0.0167	0.0172	0.0429
368			0.0074	0.0127			0.0143
370				0.0063			0.0143
372	0.0127	0.0075		0.0063	0.0167		0.0143
374		0.0075	0.0074	0.0063			
376	0.0063					0.0172	0.0286
378					0.0167		
380			0.0074		0.0167		
382					0.0167		
384							0.0143
386			0.0074				
388		0.0149	0.0074				
390	0.0063		0.0147	0.0063			
394			0.0074				
396							0.0143

	POPULATIONS						
PKAT/GT4	BS16	A1	BS29	BS1	BV21	V2	V3
(N)	77	68	68	80	28	28	33
336		0.0147	0.0074			0.0179	
338	0.0130	0.0441	0.0221	0.0250	0.0179	0.0536	0.0152
340	0.0844	0.1324	0.1103	0.1125	0.1607	0.1786	0.1061
342	0.1623	0.2426	0.1691	0.2000	0.2143	0.0893	0.1970
344	0.1169	0.0735	0.0515	0.0500	0.0893	0.0893	0.0455
346	0.0260	0.0147	0.0221	0.0063	0.0179	0.0357	0.0303
348	0.0390	0.0368	0.0368	0.0437	0.0357	0.0357	0.0303
350	0.0325	0.0221	0.0368	0.0375	0.0179		0.1061
352	0.0325	0.0221	0.0294	0.0125	0.0179	0.0179	0.0303
354	0.0390	0.0147	0.0221	0.0188	0.0357	0.0536	
356	0.0260	0.0441	0.0294	0.0375	0.0357	0.0179	0.0455
358	0.0195	0.0294	0.0515	0.0313	0.0536	0.0893	0.0303
360	0.0974	0.0588	0.1029	0.0500	0.0357	0.0714	0.0606
362	0.0260	0.0588	0.0735	0.1000	0.0357	0.0714	0.0606
364	0.0584	0.0221	0.0441	0.0500	0.0893	0.0357	0.0303
366	0.0390	0.0221	0.0441	0.0688	0.0357	0.0536	0.0455
368	0.0455	0.0515	0.0515	0.0250	0.0536	0.0179	0.0758
370	0.0260	0.0221	0.0221	0.0125		0.0179	0.0152
372	0.0455	0.0294	0.0221	0.0250			0.0152
374	0.0130	0.0074		0.0313	0.0357		
376	0.0065	0.0368	0.0074	0.0500			0.0303
378	0.0195				0.0179	0.0357	
380	0.0195						
382	0.0065		0.0221	0.0125		0.0179	0.0152
384	0.0065						
386			0.0074				
388			0.0074				
390							0.0152
392			0.0074				

Annexe III. 5 : *Pectinaria koreni* . Missions "Pectgene 1999" et "Pectow-Mars 2003 (P1) en Baie de Seine: Fréquences alléliques en BS16 pour quatre locus microsatellites (PKGT1; PKAT/GT1; PKAT/GT2 et PKAT/GT4). Jolly*et al.* (en préparation).

PKGT1			**PKAT/GT2**			**PKAT/GT1**			**PKAT/GT4**		
	BS16-99	BS16-P1		BS16-99	BS16-P1		BS16-99	BS16-P1		BS16-99	BS16-P1
(N)	70	49	(N)	100	81	(N)	99	76	(N)	101	81
191	0.0071		236	0.0100		226		0.0132	336	0.0050	
204	0.0143		242	0.0150		326	0.0051	0.0132	338	0.0050	0.0432
212	0.0071		258	0.0050		328	0.0152	0.0066	340	0.0446	0.0741
214	0.0143		268	0.0050		330	0.0152	0.0066	342	0.0594	0.0988
218	0.0071		270	0.0050	0.0185	332	0.0051	0.0066	344	0.0792	0.0926
220	0.0643		272	0.0100	0.0062	334	0.0101	0.0066	346	0.1139	0.0802
222		0.0102	274		0.0247	336	0.0101	0.0197	348	0.0347	
224	0.0500		276	0.0250	0.0494	338	0.0202	0.0395	350	0.0347	0.0247
226	0.0143	0.0408	278	0.0400	0.0185	340	0.0202	0.0592	352	0.0446	0.0370
228	0.0286	0.0306	280	0.0150	0.0432	342	0.0303	0.0461	354	0.0297	0.0247
230	0.0286	0.0408	282	0.0100	0.0185	344	0.0556	0.0395	356	0.0149	0.0370
232	0.0214	0.0510	284	0.0050	0.0370	346	0.0404	0.0132	358	0.0743	0.0309
234	0.0429	0.0204	286	0.0350	0.0185	348	0.0303	0.0329	360	0.0446	0.0802
236	0.0357	0.1020	288	0.0200		350	0.0202	0.0329	362	0.0396	0.0741
238	0.0643	0.0918	290	0.0450	0.0185	352	0.0707	0.0461	364	0.0545	0.0679
240	0.0500	0.0918	292	0.0200	0.0185	353	0.0051		366	0.0594	0.0309
242	0.0929	0.1224	294	0.0250	0.0247	354	0.0455	0.0329	368	0.0693	0.0432
244	0.0643	0.1020	296	0.0250	0.0309	356	0.0354	0.0263	370	0.0446	0.0370
246	0.0929	0.0408	298	0.0350	0.0123	358	0.0354	0.0329	372	0.0396	0.0370
248	0.0429	0.0306	300	0.0150	0.0432	360	0.0707	0.0461	374	0.0149	0.0062
250	0.0429	0.1122	302	0.0350	0.0556	362	0.0404	0.0592	376	0.0198	0.0185
252	0.0286	0.0306	304	0.0200	0.0123	364	0.0657	0.0395	378	0.0149	0.0370
254	0.0214	0.0204	306	0.0100	0.0494	366	0.0253	0.0461	380	0.0347	0.0185
256	0.0214		308	0.0100	0.0185	368	0.0303	0.0197	382	0.0149	
258	0.0286	0.0102	310	0.0150	0.0185	370	0.0253		386	0.0099	
260	0.0286		312	0.0650	0.0062	372	0.0303		392		0.0062
262		0.0408	314	0.0150	0.0370	374	0.0253	0.0197			
264	0.0143	0.0102	316	0.0250	0.0370	376	0.0556	0.0263			
266	0.0214		318	0.0200	0.0185	378	0.0303	0.0658			
276	0.0143		320	0.0100	0.0370	380	0.0101	0.0197			
278	0.0143		322	0.0250	0.0123	382	0.0202	0.0197			
280	0.0214		324	0.0150	0.0123	384	0.0152	0.0329			
			326	0.0400	0.0123	386	0.0152	0.0132			
			328		0.0123	388	0.0101	0.0263			
			330	0.0450	0.0494	390	0.0051	0.0329			
			332	0.0100	0.0309	392	0.0152	0.0066			
			334	0.0100	0.0185	394	0.0051	0.0197			
			336	0.0450	0.0185	398	0.0051	0.0132			
			338	0.0100	0.0247	400		0.0132			
			340	0.0100	0.0123	402	0.0101				
			342		0.0370	408	0.0051	0.0066			
			344	0.0100		410	0.0101				
			346	0.0100	0.0123	426	0.0051				
			348	0.0400							
			350	0.0100	0.0123						
			352	0.0100							
			354	0.0200							
			356	0.0150							
			358	0.0050							
			360	0.0100							
			362	0.0100	0.0185						
			364	0.0050	0.0062						
			366		0.0185						
			368	0.0100							
			370	0.0050							
			372	0.0100							
			376		0.0123						
			380		0.0062						
			396	0.0100							
			398	0.0100							
			400	0.0100							

CONCLUSIONS GENERALES ET PERSPECTIVES

Ce travail de thèse avais pour objectif de mettre en évidence l'impact des processus historiques et contemporains à différentes échelles spatio-temporelles sur la structure des populations côtières des polychètes tubicoles inféodés aux sédiments fins envasés de l'Atlantique Nord Est (*Owenia sp.* et *Pectinaria sp.*). Nos résultats étant à l'origine de nouvelles questions, différentes réponses ont été opposés, dont la plupart à l'origine de nouvelles hypothèses de travail. Dans cette conclusion, nous essayerons de dégager les résultats principaux de ce travail et de discuter les principales interprétations proposées en reprenant les 3 grands axes de cette thèse.

(i) Histoire évolutive des lignées *Pectinaria et Owenia* dans l'Atlantique Nord Est

Non seulement l'hypothèse d'une co-évolution entre les genres *Pectinaria* et *Owenia* semble se confirmer, mais un phénomène vicariant de grande ampleur semble avoir affecté la faune des sédiments meubles pendant la colonisation de l'Atlantique Nord Est et abouti à l'apparition d'espèces cryptiques possédant des distributions largement allopatriques (figure 19). La topologie des arbres phylogénétiques, la distribution géographique des lignées et les réseaux d'haplotypes montrent qu'à l'origine cette colonisation s'est produite selon un axe Nord-Sud. Celle-ci a été accompagnée d'un scindement en deux clades chez les deux espèces il y a au moins 3.7 millions d'années (tenant compte d'un taux de mutation de 2.2×10^{-8} / nucléotide), qui a été suivi, suite au dernier maximum glaciaire (entre -23 000 et -18 000 ans), par une recolonisation de l'Atlantique Nord Est à partir de zones refuges situées au sud (méditerranée/ région ibérique) et au nord (sud-ouest de l'Angleterre et de l'Irlande/ nord-est de l'Ecosse ; figure 20). Dans le cas d'*O. fusiformis*, le clade 3 est le plus divergent. Or, à partir des données préliminaires comparant les populations intertidales et subtidales, il semble que ce clade occupe une distribution au niveau de l'intertidal, ce qui pourrait suggérer une

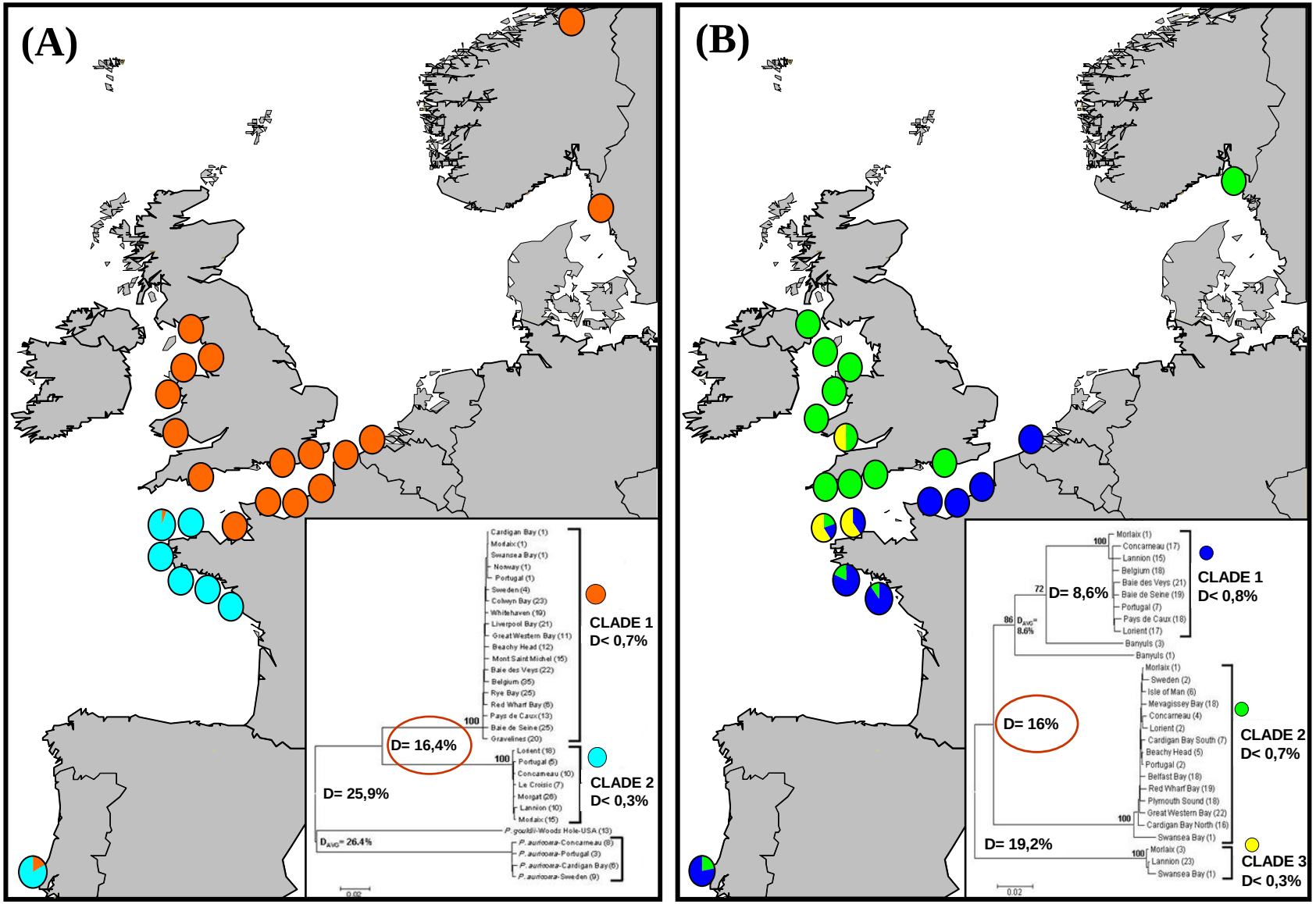
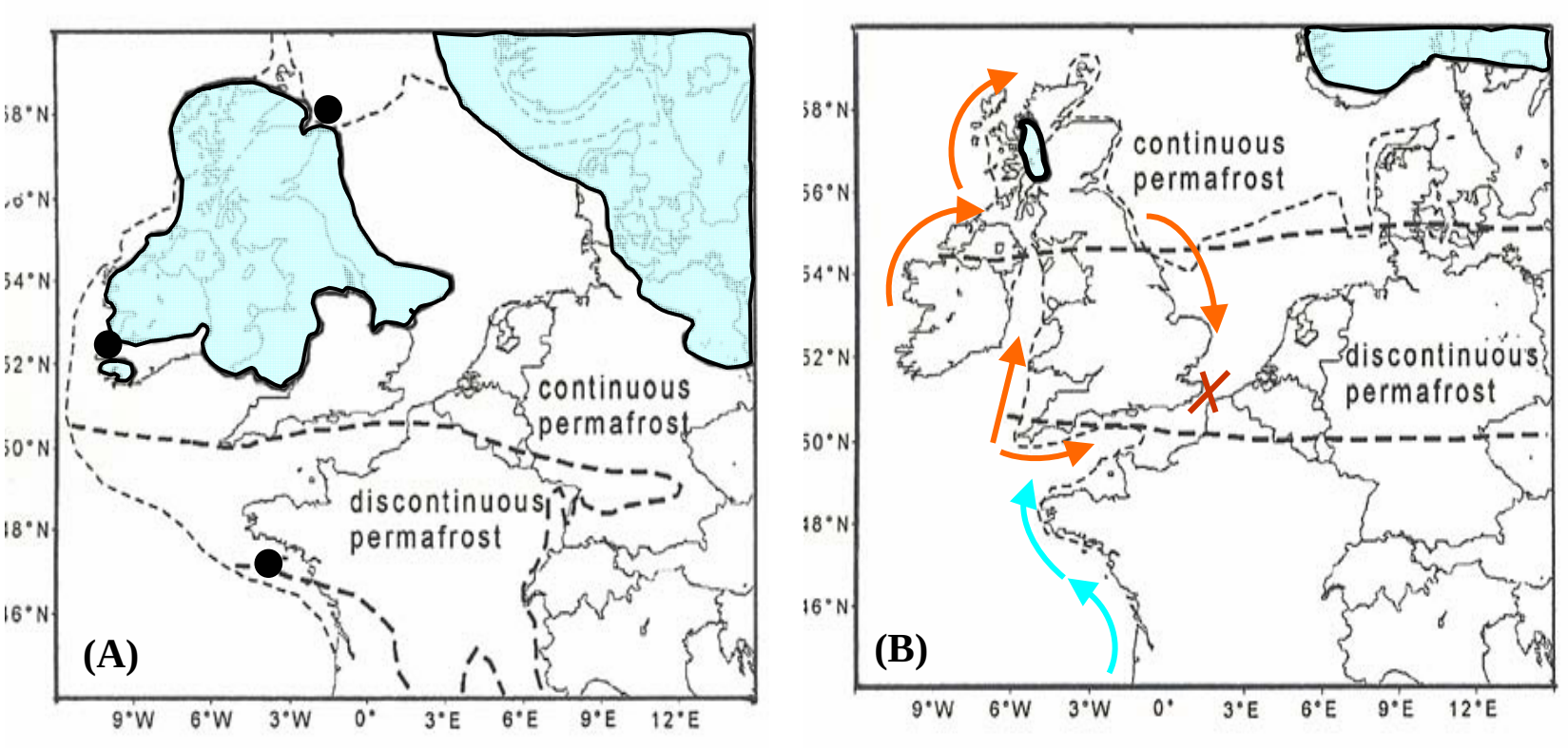


Figure 19. Distributions géographiques des lignées dans l'Atlantique Nord Est et arbres phylogénétiques pour (A) *Pectinaria koreni* et (B) *Owenia fusiformis*.



Le Dernier Maximum Glaciaire
(entre -23 000 et -18 000 ans)

Le Dryas inférieur
(entre -14 et -12 000 ans)

Figure 20. (A) distribution des zones de refuges potentiels pendant le Dernier Maximum Glaciaire (d'après Stewart & Lister, 2001; Luttikhuizen *et al*., 2003); (B) scénario de colonisation de l'Atlantique Nord Est par les clades 1 (rouge) et 2 (bleue) de *Pectinaria koreni* après le Dernier Maximum Glaciaire (Cartes tirées de Renssen & Vandenberghe, 2003); la croix rouge représente l'ouverture du détroit du Pas de Calais il y a environ 8000 ans (Smith, 1989).

spécialisation écologique antérieure, concomitante à la radiation évolutive ayant donné lieu à la formation des espèces *P. auricoma*, *P. belgica* et *P. koreni* chez le genre *Pectinaria sp.*. Cette spécialisation aurait pu conduire à un renforcement de l'isolement allopatrique et/ou sympatrique, se traduisant par des différences aux niveaux phénotypique et comportemental (valeur sélective des larves ou modification du comportement reproducteur des adultes).

Bien que la distribution géographique des clades et les arbres phylogénétiques supportent l'hypothèse d'une histoire de vicariance au niveau des peuplements des sables fins envasés ainsi qu'une co-évolution des genres *Pectinaria* et *Owenia*, les signatures démographiques des lignées évolutives indiquent quand à elles des histoires démographiques différentes entre lignées et espèces : (1) expansion démographique ancienne après la grande glaciation de l'ère Saalienne (*P. auricoma*, *O. fusiformis* clade 3), (2) expansion récente des populations suite à la re-colonisation de l'Atlantique jusqu'au nord des côtes bretonnes pendant la mise en place des courants contemporains (*P. koreni*, clade 2), (3) signature d'une persistance des populations dans au moins un, voire deux refuges glaciaires situés au nord, probablement sur la côte ouest de l'Irlande et au sud-est de l'Angleterre (*P. koreni*, clade 1 ; *O. fusiformis*, clade 2), (4) rétraction et expansion géographiques probablement liées au dernier maximum glaciaire (-23 000 ans) et au Dryas inférieur (-13 000 ans) des populations originelles à partir d'au moins un des deux refuges glaciaires (*O. fusiformis* clade 1).

Les données paléoclimatiques et génétiques indiquent que des zones refuges, non recouvertes de glace, auraient existé au sud de la Bretagne, sud-ouest de l'Irlande, nord-Est de l'Ecosse et même en Norvège (Stewart & Lister, 2001 ; Luttikhuizen *et al*., 2003 ; figure 20). Pendant le Dryas inférieur, la Manche et la Mer d'Irlande étaient ouvertes sur l'Atlantique ce qui aurait permis la colonisation de la Manche par *P. koreni* clade 1 à partir de certaines de ces zones refuges (figure 20). Cette colonisation semble s'être déroulée, d'abord par l'Atlantique et la Mer d'Irlande aux approches occidentales de la Manche, puis par la Mer du Nord après l'ouverture catastrophique de l'îsthme du détroit du Pas-de-Calais il y a environ 8

000 ans (Smith, 1989). Un contact secondaire récent se serait établi en Manche entre des populations préalablement différenciées du clade 1. Au contraire, la recolonisation de l'Atlantique Nord-Est par *P. koreni* clade 2 s'est produite à partir d'une zone refuge située beaucoup plus au sud vers la région méditerranéenne. Vraisemblablement, l'expansion de l'aire géographique vers le nord a été stoppée aux environs de la pointe de Bretagne, pendant la mise en place des courants contemporains de l'Holocène. Si l'action du front d'Ouessant pendant la période de reproduction de la pectinaire agit probablement comme barrière ayant pour rôle de filtrer la dispersion des larves de part et d'autre de la Mer d'Iroise, l'effet sélectif des conditions écologiques propres aux différentes zones biogéographiques (i.e. Lusitanienne et Boréale) pourrait aussi avoir son rôle dans les patrons de distribution observés chez les lignées de *P. koreni* (e.g. différentiel de température dû au brassage des eaux en Manche). Quant à *O. fusiformis*, les patrons de colonisation de la Manche sont beaucoup moins évidents que pour les lignées de *P. koreni*.

De nouvelles questions se posent quant à l'effet vicariant associé aux peuplements des sables fins envasés :

- Cet effet est-il généralisable à l'ensemble des espèces occupant ce type d'habitat?
- Est-il fonction du mode de développement larvaire ?
- Ce dernier a-t-il affecté la répartition ancestrale de ces lignées dans l'Atlantique Nord Est et la Méditerranée ?

Concernant la première question, d'autres études menées ces dernières années ont démontré l'existence d'espèces cryptiques chez d'autres taxons inféodés aux sédiments sablo-vaseux : notamment chez les échinodermes *Echinocardium cordatum* (Chenuil & Féral, 2003) et *Amphiura brachiata* (Muths *et al*., soumis). Dans le cas de l'ophiure *A. brachiata*, la phase pélagique ne dure que quelques jours et les lignées sont distribuées selon leur position sur l'estran (intertidal vs. subtidal) le long de la zone de transition biogéographique Iroise-Manche. Ce taxon présente un niveau de divergence (D_{AVG}= 19.6% selon la distance K2P) qui

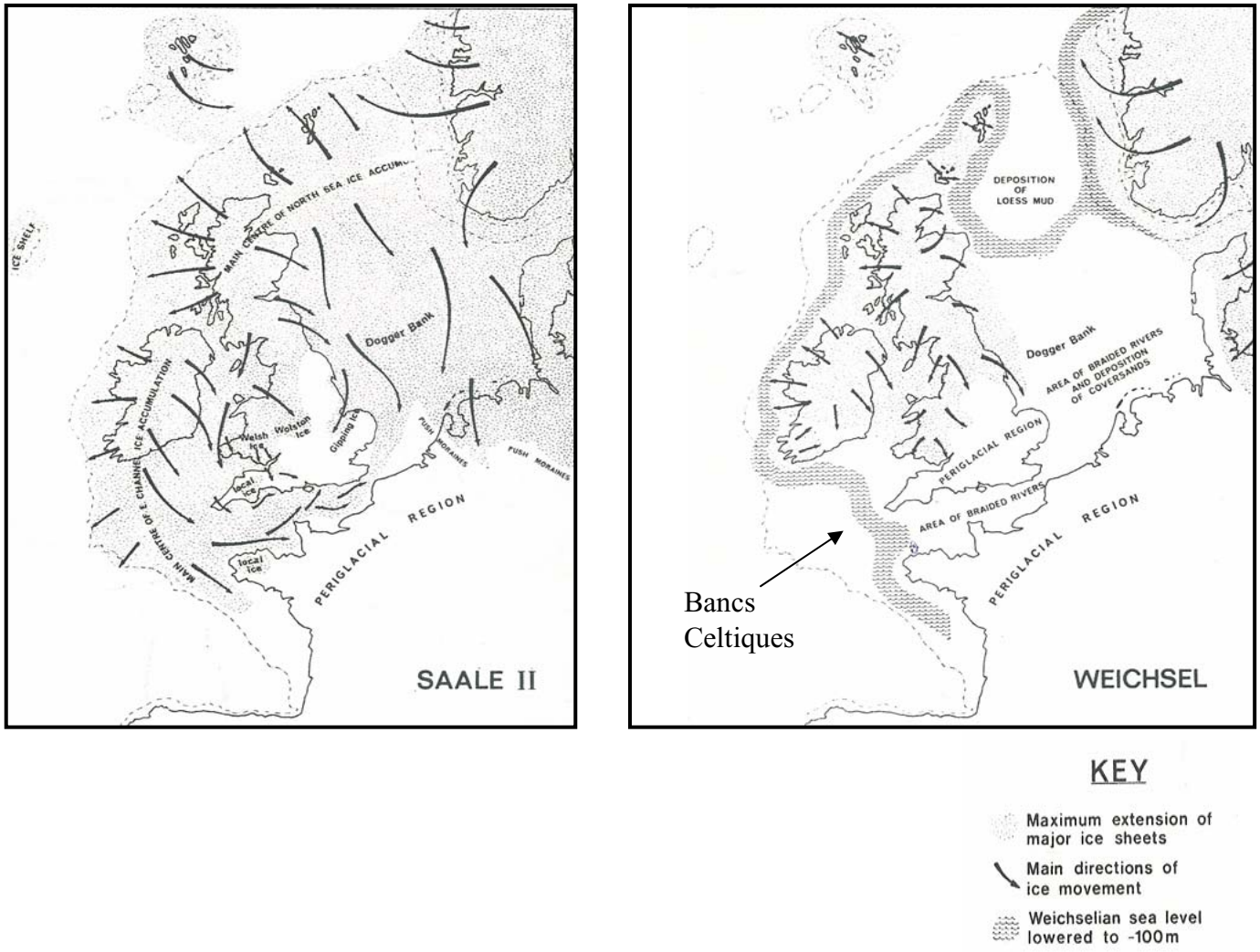


Figure 21. Couverture glaciaire pendant (A) l'ère de glaciation Saalienne (Saale II, il y a environ 128 000 ans) et (B) le dernier maximum glaciaire (Weichsel, entre -23 000 et -18 000 ans) (d'après Kellaway *et al*., 1975).

est du même ordre de grandeur (D_{AVG}= 19.2% selon K2P) que celui observé entre les clades intertidal (clade 3) et subtidal (clades 1 et 2) d'*O. fusiformis* en Baie de Lannion. Dans d'autres types d'habitat, un niveau similaire de divergence est également retrouvé chez les espèces d'hermelles *Sabellaria spinulosa* (subtidal) et *Sabellaria alveolata* (intertidal) présentes en Atlantique Nord Est (Rigal, 2005). De plus, il est à noter que la répartition des deux espèces de moules *Mytilus edulis* et *M. galloprovincialis* est assez proche de celle observée entre les deux lignées de *P. koreni*, bien que la zone d'hybridation soit beaucoup plus étendue (peut être dû à l'action anthropique). Ces résultats convergent et laissent penser que des processus historiques forts ont influencé une large partie des taxons marins européens à cycle de vie bentho-pélagique en Atlantique Nord Est. De nouvelles études sont nécessaires et notamment chez les bivalves inféodés au même type d'habitat (i.e. *Abra alba* et *Phaxas pelucidus*) que *P. koreni* et *O. fusiformis*.

Concernant la répartition historique de ces lignées dans l'Atlantique Nord, deux formations géologiques d'origine pré-Weichsélienne, les bancs celtiques et les vallées incisées de la plate-forme continentale profonde (Reynaud *et al*., 1999 ; figure 21) peuvent nous aider à mieux comprendre les mécanismes d'isolement des différentes lignées. La littérature suggère que les bancs celtiques prédatent le dernier maximum glaciaire, et pourraient correspondre aux reliques d'un système deltaïque ou estuarien mis en place à l'aval du « Fleuve Manche » lors des bas niveaux marins glacio-eustatiques du Quaternaire supérieur (les derniers 3 millions d'années, Gibbard, 1988). Ces dépôts auraient été remaniés sous l'action de courants tidaux durant la dernière transgression postglaciaire (vers -15 000-11 000 ans, Reynaud *et al*., 1999). Des conditions estuariennes existaient aussi au Pliocène (Brault, 2004). A noter que des cours d'eau (e.g. le Rhin) s'écoulaient en Mer du Nord et auraient pu entraîner le dépôt de sédiments fins et vaseux dans la partie nord (Kellaway *et al*., 1975). Selon ces auteurs, le dernier maximum glaciaire du Weichsélien (LGM) est un événement de relativement faible amplitude qui n'aurait que faiblement affecté le plateau

continental en Europe de l'Ouest, comparé à la dernière grande glaciation Saalienne, il y a environ 128 000 ans (Saale II, voir figure 21). De plus, la présence de nos modèles biologiques en Méditerranée et les données fournies sur *O. fusiformis* indiquent que plusieurs épisodes de spéciation ont eu lieu entre la Méditerranée et l'Atlantique après l'ère Messinienne (i.e. après -5.3 million d'années ; McKenzie, 1999).

A côté de nos données mitochondriales, nous avons aussi d'autres évidences d'une divergence importante au sein de *P. koreni*. En effet, la plupart des locus microsatellites ne s'amplifient pas d'un clade sur l'autre. Pour expliquer le maintien de polymorphismes nucléaires pour certains locus dans la zone de transition, il serait nécessaire (1) de caractériser les différences morphologiques tant dans les populations « pures » (présence d'haplotypes d'un seul clade) que dans la zone de recouvrement (i.e. Morlaix- Morgat) pour détecter d'éventuels renforcements phénotypiques par l'action de la sélection, et (2) d'entreprendre des croisements expérimentaux entre mâles et femelles des deux clades, pour étudier le degré d'isolement reproducteur entre ces deux clades et interpréter ainsi nos résultats concernant la ségrégation des allèles sur les marqueurs microsatellites.

(ii) Flux passés et contemporains à l'échelle de la Manche.

L'utilisation de marqueurs microsatellites a permis d'étudier des processus plus récents que ceux mis en évidence avec les marqueurs mitochondriaux. Bien que la différenciation génétique entre populations de pectinaires soit faible à l'échelle de la Manche, il est certain que des échanges se sont établis entre la Mer du Nord et la Manche, notamment via la population de Rye Bay (RB). Existe-t'il un transport de larves de pectinaire entre la mer d'Irlande, le Bristol Channel et la Manche occidentale, et ce, au travers des fronts océaniques tels que le front celtique ou le front d'Ouessant ? Le fait que seulement deux individus aient été récoltés à Cardigan Bay (côte Ouest du Pays de Galles) et dans le Bristol Channel (Swansea Bay) ainsi que quatorze individus à Great Western Bay (côte Sud Ouest de

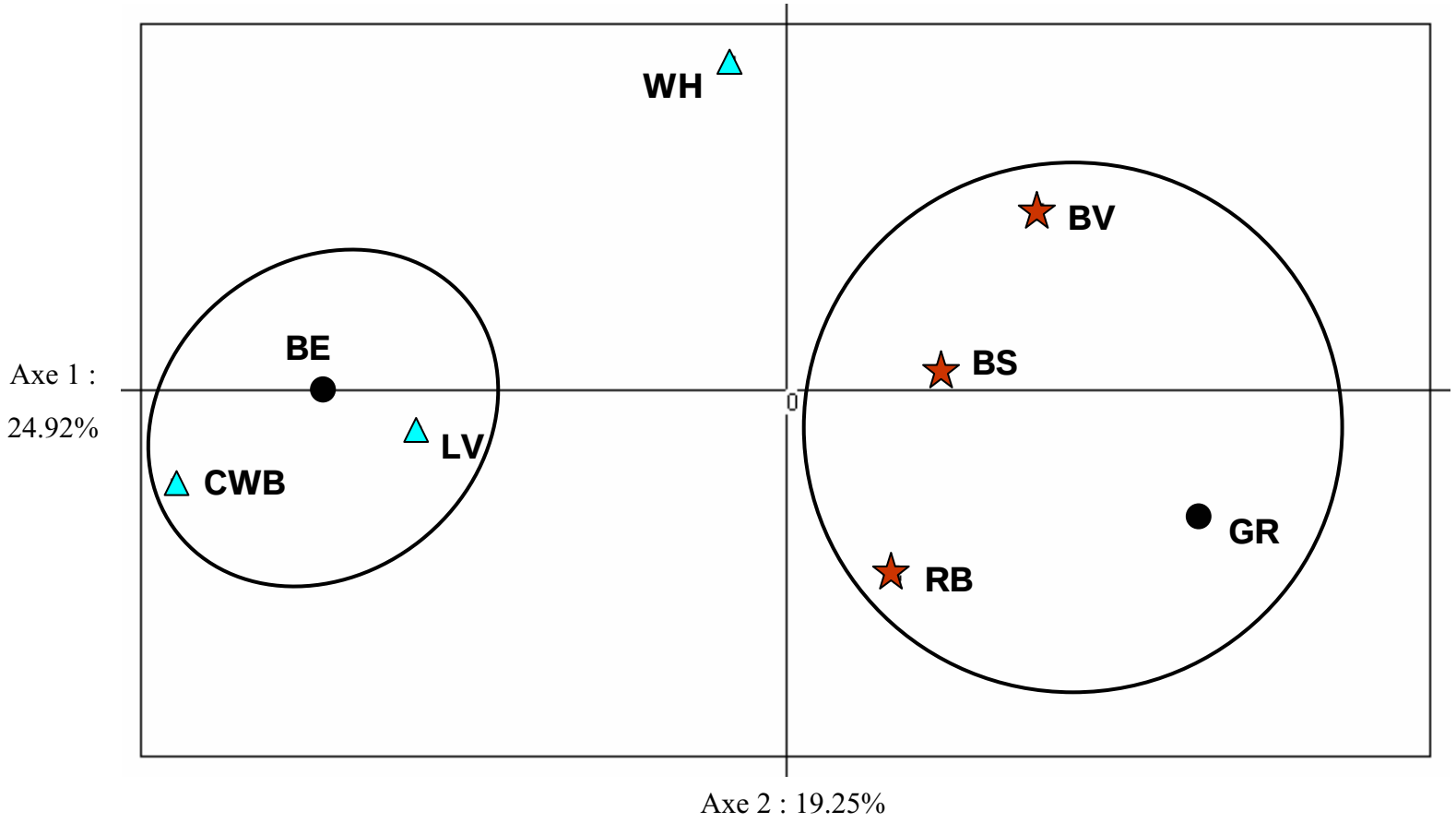


Figure 22. *Pectinaria koreni* clade 1: Analyse en Composante Principale (ACP) obtenue à partir des fréquences alléliques de 4 locus microsatellites, en utilisant le programme PCA-GEN 1.2 (Goudet; http://www.unil.ch/izea/research.html#softs)

l'Angleterre), laisse supposer le passage épisodique de larves au travers de la mer d'Iroise. A ce titre, il faut noter que les prélèvements de méroplancton effectués pour des analyses génétiques lors de la mission PECTIRL (Juin 2004), en des points traversant le front d'Ouessant et le front Celtique présentent un nombre de larves non négligeable (Comtet, comm. pers.). La présence de larves de pectinaire étant avérée, l'analyse moléculaire (assignation d'un génotype multilocus à des populations de référence) devrait être une piste d'étude intéressante pour connaître l'origine de ces larves, bien que nécessitant un très bon échantillonnage des populations adultes et un grand nombre de locus polymorphes.

L'analyse en composante principale basée sur les fréquences alléliques de nos marqueurs microsatellites (figure 22) met en évidence l'existence d'un flux génique entre la population de Belgique (BE) et celles de la Mer d'Irlande (LV, CWB), la population de Gravelines (GR) se regroupant quant à elle, avec celles de la Manche (BS, BV, RB). Ce résultat confirme l'hypothèse établie par les méthodes de coalescence basées sur le gène mtCOI, notamment celle de deux voies de colonisation de la Manche, la première par les approches occidentales de la Manche il y a environ 14 000 ans, la deuxième il y a environ 9 à 8 000 ans à partir de la Mer de Nord. La Manche est donc une zone de contact secondaire entre deux « pools » génétiquement différenciés ayant suivi des voies de colonisation différentes. Ceci expliquerait (1) la faible différenciation génétique entre la Mer d'Irlande et la Manche, (2) la plus faible diversité génétique en Mer d'Irlande comparée à la Manche et à la Mer du Nord, (3) les forts déficits en hétérozygotes observés sur nos marqueurs microsatellites (ie. effet Wahlund).

Nous avons également caractérisé plus finement la structure génétique des populations de *P. koreni* en Manche orientale. Bien qu'il n'y ait aucune différenciation génétique significative à cette échelle, exception faite de la comparaison Gravelines (GR)/ Baie des Veys (BV), les tests d'assignation soulignent l'existence d'un flux trans-Manche entre BV et Rye Bay (RB) sur les côtes anglaises. Ainsi, plus de 20% des individus de BV sont assignés à RB et entre 10-20% des individus de RB sont assignés à BV (figure 23). Bien qu'un flux

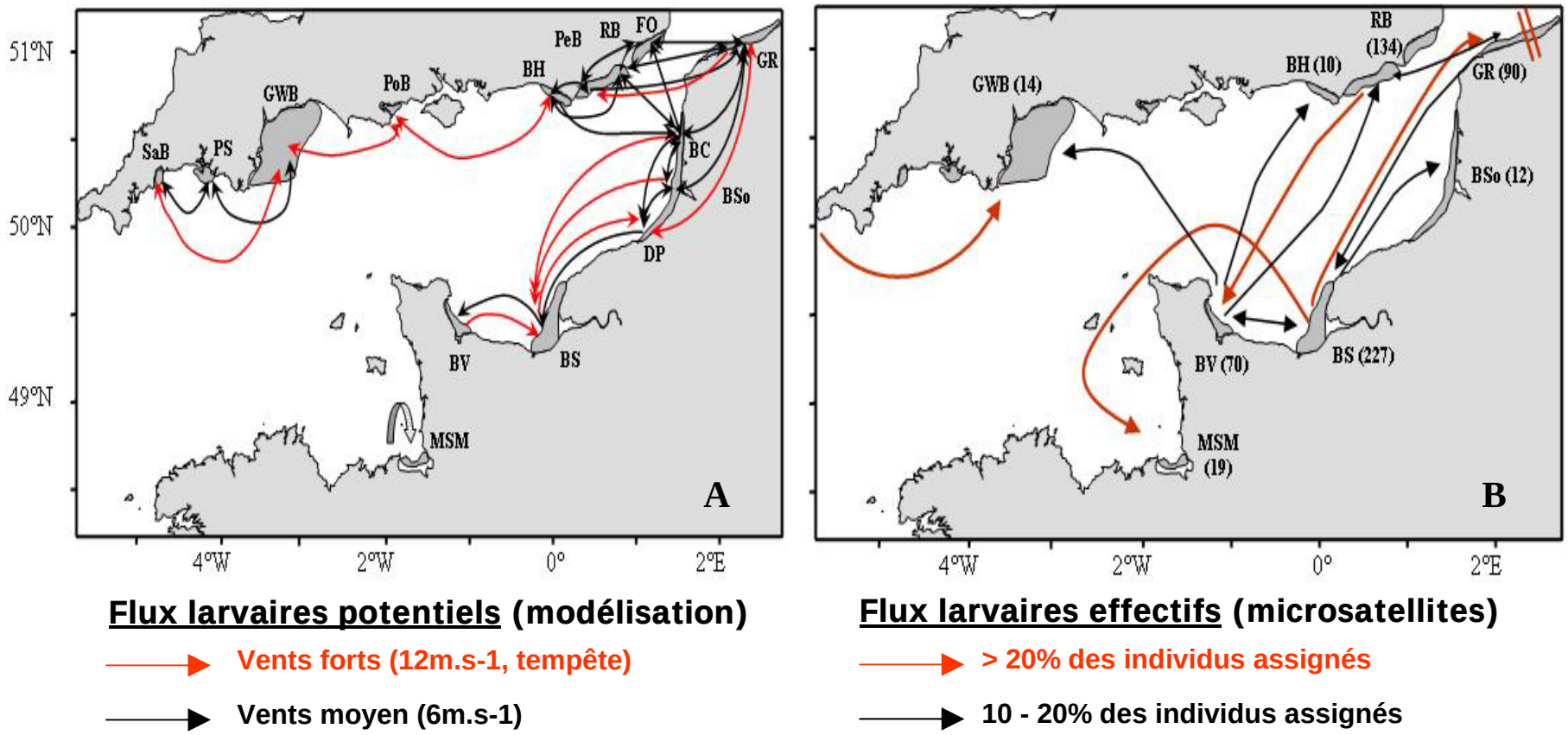


Figure 23. *Pectinaria koreni* clade 1. (A) Synthèse des résultats de simulation hydrodynamique de la dispersion larvaire montrant leséchanges potentiels entre populations de la Manche; (B) Synthèse des tests d'assignation basés sur les génotypes multilocus, représentant les échanges larvaires effectifs; la double barre rouge représente une barrière au flux génique entre la population de Gravelines (GR) et celle de Belgique (BE).

génique trans-Manche ait également été mis en évidence grâce aux allozymes et au gène mtCOI, ces observations sont en contradiction avec les données de simulations hydrodynamiques de la dispersion larvaire (basé sur le modèle 2-D de Salomon & Breton, 1993 ; figure 23). Ce résultat pourrait aussi bien refléter les flux historiques associés à la colonisation récente de la Manche que des flux actuels associés à la dispersion larvaire. Pourtant, des problèmes inhérents au modèle ou même, la considération d'un forçage à long terme et non de perturbations stochastiques, pourraient avoir eu une influence sur les patrons de dispersion larvaire retenus. De plus, l'existence de retard à la métamorphose larvaire ou d'une asynchronie de la reproduction en Baie de Seine pourrait favoriser certaines exportations larvaires vers les côtes anglaises via le tourbillon de Barfleur, pendant la période automnale lorsque les perturbations environnementales sont plus fortes. Il est intéressant de noter que les matrices d'échanges larvaires obtenues grâce au nouveau modèle physique incorporé dans PECTIFLUX (P. Guyard, D. Jollivet, E. Thiébault et F Viard ; voir les caractéristiques du modèle sur la figure 24, et les explications dans l'encadré 3), en fonction de conditions météorologiques réelles couvrant une période de 36 années, a permis de révéler l'existence d'un flux trans-Manche de faible ampleur à partir du tourbillon de Barfleur. Il est maintenant nécessaire de valider les estimations des flux géniques (et non juste des flux potentiels de larves) obtenues par simulation sous PECTIFLUX, en effectuant une analyse d'auto-corrélation spatiale avec nos données microsatellites.

(iii) Etude d'une métapopulation à micro-échelle spatio-temporelle : la Baie de Seine

Ce travail a mis en évidence d'une part, l'impact des phénomènes micro-évolutifs sur la structure locale des populations de la Baie de Seine, et d'autre part, la très nette asynchronie reproductrice des différents noyaux de pectinaires entre la Baie des Veys et la Baie de Seine orientale. Sur la base de nos données, il est difficile de caractériser avec précision le type de métapopulation auquel nous avons à faire car, en fonction du point d'observation dans le

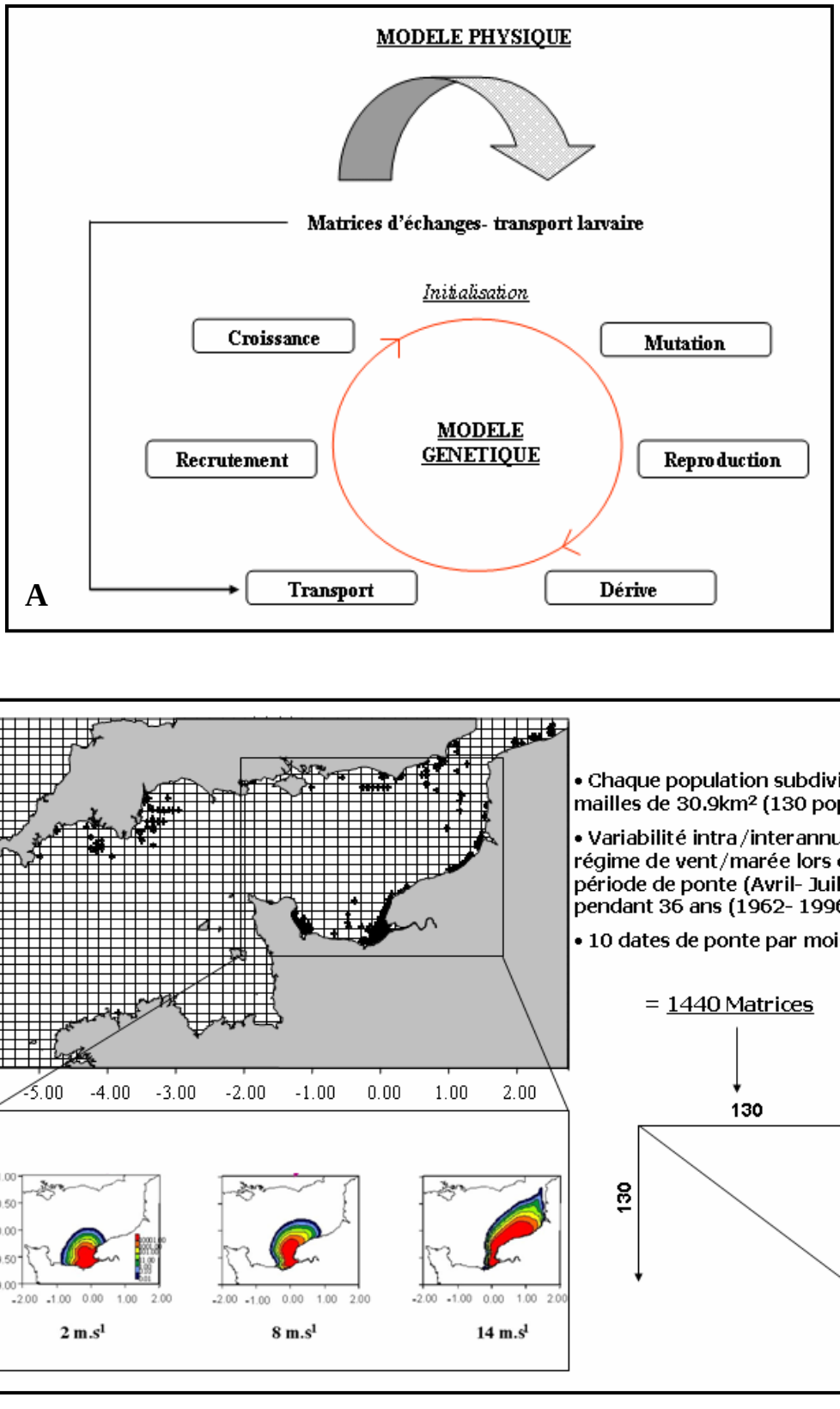


Figure 24. Le modèle PECTIFLUX. (A) le modèle itératif combine un modèle physique (qui tourne sur 1 génération) à un modèle théorique génétique (tournant sur 20 000 générations). (B) Le modèle physique génère des matrices d'échanges larvaires. Ces même matrices sont tirées aléatoirement à chaque génération dans le modèle génétique et ce sur 20 000 générations, pour estimer les flux de gènes entre mailles et calculer les fréquences alléliques de chaque population

Encadré 3. PECTIFLUX : un modèle de flux génique à l'échelle de la Manche

Le modèle 2-D Lagrangien de circulation des eaux de la Manche (Salomon & Breton, 1991, 1993) a été modifié et automatisé pour les simulations de transport larvaire par C. Ellien (Université Paris VI) et P. Guyard (Université de Portsmouth, RU). Ce modèle utilise une base de données de courants résiduels, et interpole les données climatiques variables ou fixées entre 8 situations pré-simulées différentes. La mortalité larvaire est incluse dans cette partie du modèle. Ce modèle permet de calculer une abondance de larves après dispersion à partir d'un seul point d'émission (maille i) et d'une seule date de ponte (date j) sur l'ensemble des mailles découpant la Manche. L'interface entre ce modèle et le modèle de flux génique global (ensemble des populations de la Manche) se fait par le calcul d'un nombre final de larves au niveau de chaque maille après une simulation sur 15 jours, durée moyenne de vie larvaire pour *Pectinaria koreni*. Pour construire une matrice d'échanges de larves entre les différentes mailles de la Manche, il nécessaire d'effectuer une simulation du nuage larvaire à partir de chaque maille et pour toutes les dates de ponte possibles entre Avril et Août. Cent trente populations ont été définies en regroupant (sauf quelques exceptions) 9 mailles du modèle physique (une supermaille), dans lesquelles sont calculées le pourcentage de survie des larves en provenance des 130 populations. A chaque date de ponte (e.g. premier avril 1987) correspond 130 simulations de 15 jours permettant d'obtenir la matrice complète d'échanges larvaires entre toutes les populations. La durée de ponte de la pectinaire étant d'environ 4 mois par an et les données climatiques (marée et vent) obtenues par Météo-France couvrant une période comprise entre1964 et 1999, il a été décidé d'obtenir un nombre de 10 (dates par mois) X4 (mois) X36 (années) de matrices d'échanges 130X130, et ce afin de pouvoir couvrir l'ensemble des possibilités de déplacement des larves en Manche et donc d'obtenir le modèle d'échanges le plus réaliste possible en Manche orientale (1 440 matrices d'échanges).

• Diversité génétique

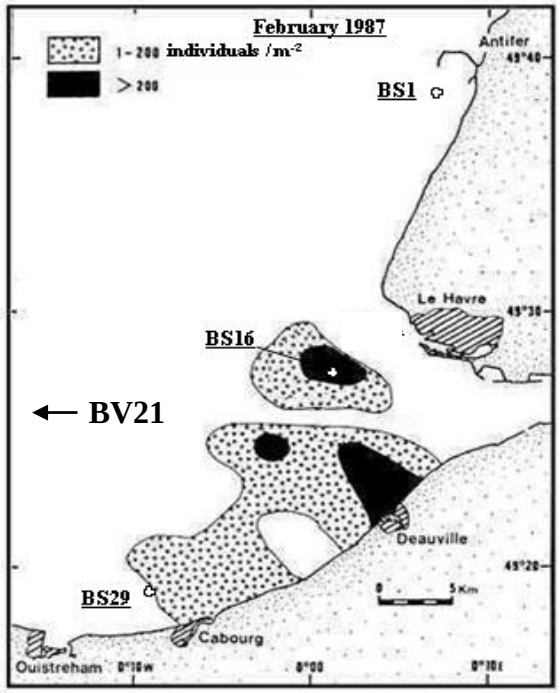
Sampling location	March 2003			July 2003			September 2003		
	N	R_S	H_{NB} [SE]	*N*	R_S	H_{NB} [SE]	*N*	R_S	H_{NB} [SE]
BS1	54	17.43	0.952 [0.022]	45	17.21	0.949 [0.026]	80	17.88	0.953 [0.027]
BS16	81	17.68	0.955 [0.021]	87	18.02	0.953 [0.025]	80	18.42	0.957 [0.021]
BS29	58	16.66	0.944 [0.022]	77	17.82	0.953 [0.024]	69	18.33	0.955 [0.023]
BV21	-	-	-	23	15.74	0.944 [0.024]	30	16.52	0.941 [0.032]

R_S = richesse allèlique basée sur 15 individus diploïdes

• Différenciation génétique temporelle

Baie de Seine	BS1	BS16	BS29	BV21
March - July 2003	P < 0.001	NS	P < 0.001	NA
July – September 2003	NS	NS	NS	P < 0.05

Inter-génération



• Différenciation génétique spatiale

F_{ST}- Adultes (Mars 2003)

	BS1 (54)	BS16 (81)	BS29 (58)	BV21 (23)
BS1	-	-0.002	0.001***	0.010***
BS16		-	0.001***	0.003**
BS29			-	0.005**
BV21				-

* P < 0.05, ** P < 0.01, *** P < 0.001

F_{ST}- Juvéniles (Juillet 2003)

	BS1 (46)	BS16 (87)	BS29 (77)
BS1	-	-0.002	-0.002
BS16		-	-0.000
BS29			-

F_{ST}- Juvéniles (Septembre 2003)

	BS1 (80)	BS16 (80)	BS29 (69)	BV21 (16)
BS1	-	0.0004**	-0.0008	0.0001
BS16		-	0.0000	-0.002
BS29			-	0.0002
BV21				-

Figure 25. *Pectinaria koreni* clade 1. Différenciation génétique temporelle (P value) et spatiale (F_{ST}) des populations dans la Baie de Seine entre Mars et Septembre 2003.

temps et l'espace, les caractéristiques d'échanges se rapprochent d'abord d'un modèle en source-puits (ou continent-îles) lors de la phase de renouvellement de la population, puis correspondent à un modèle en mosaïque spatiale fluctuante où la nature génétique d'un noyau change selon l'origine des recrues. De façon empirique, cette étude nous a permis néanmoins d'analyser le fonctionnement particulier d'une métapopulation en milieu marin, chez une espèce dont le cycle de vie correspond exactement aux hypothèses sous-jacents aux modèles théoriques de métapopulation (i.e. générations non chevauchantes, extinction et recolonisation des parcelles à chaque génération).

A partir des données génétiques obtenues, le scénario proposé est le suivant (figure 25): (1) en Mars, les populations de géniteurs diffèrent presque toutes significativement les unes des autres avec une diversité génétique significativement plus faible en Baie des Veys (BV21), suggérant l'existence d'une population-puit dans cette zone, (2) en Juillet, un auto-recrutement massif est observé au niveau du noyau d'abondance de la BS16 (stabilité des fréquences alléliques inter-génération) de même qu'un recrutement de larves provenant de la BS16 est observé dans les stations marginales BS1 et BS29 de la Baie de Seine orientale (différenciation temporelle inter-génération et effet homogénéisant de la migration au niveau spatial), (3) en Septembre, tandis qu'une stabilité des fréquences alléliques est observée en BS1, BS16 et BS29 entre recrues de Juillet et Septembre, les différences alléliques temporelles observées en BV21 indiquent que le recrutement de larves provient de BS16 (aucune différenciation génétique entre recrues de BV21 et BS16).

Le fait que l'on retrouve, en Septembre, un faible niveau de différenciation entre BS16 et BS1 suggère qu'une deuxième cohorte génétiquement différenciée en provenance soit d'une ponte tardive du noyau d'abondance échantillonné au large de Deauville (non analysé), soit de larves issues des populations Picardes ait été recruté en BS1. Nos résultats indiquent néanmoins que la plupart de la différenciation génétique observée au niveau des populations

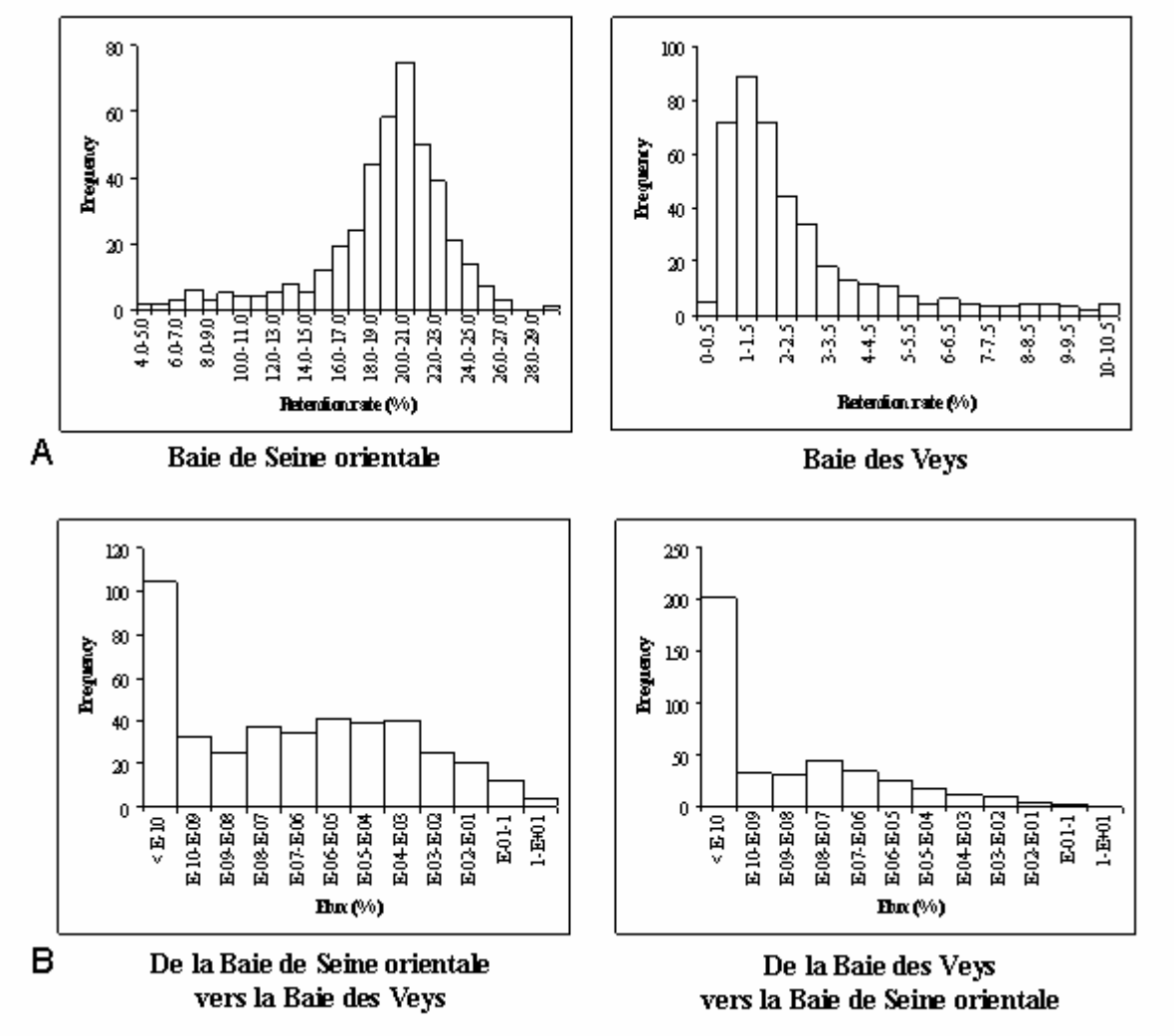


Figure 26. Analyse des matrices d'échanges générées par le nouveau modèle hydrodynamique (Guyard & Thiébaut, comm. pers.), à l'échelle de la Baie de Seine, pour la période Avril-Juillet de 1964 à 1999 (Baie de Seine orientale = 22 mailles ; Baie des Veys = 5 mailles). (A) Taux de rétention moyen par population en Baie de Seine et en Baie des Veys; (B) variabilité spatio-temporelle des flux larvaires d'une baie à l'autre.

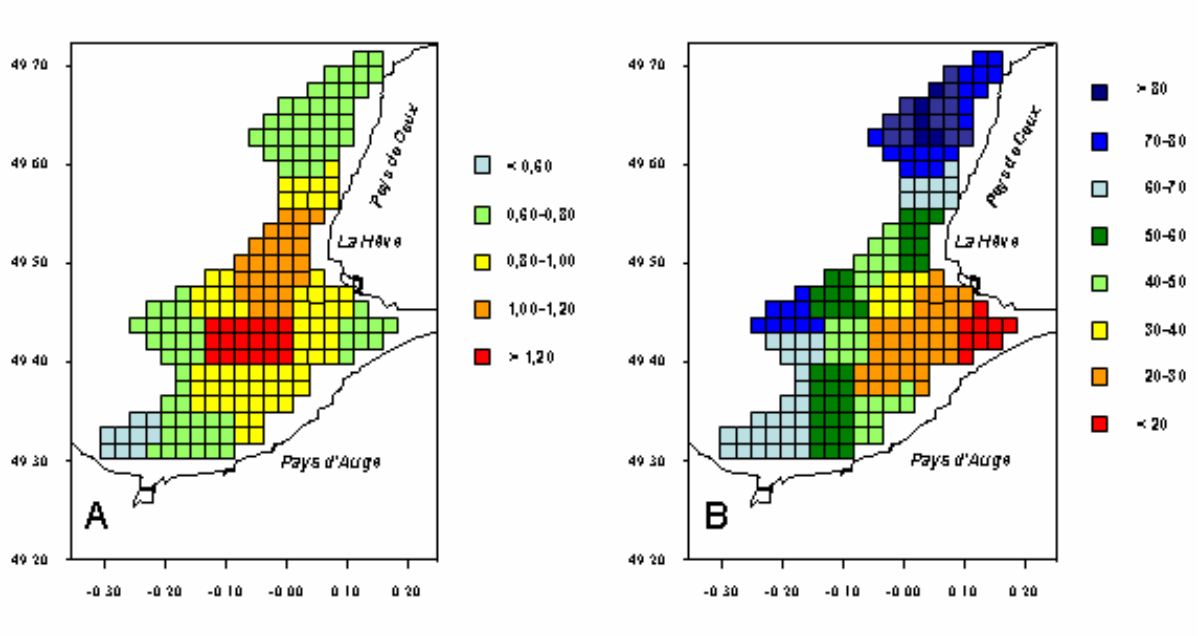


Figure 27. Variabilité spatio-temporelle des apports larvaires à l'échelle locale de la Baie de Seine orientale, à partir des données fournies par un nouveau modèle physique (Guyard & Thiébaut, comm. pers.). (A) Apport larvaire moyen de pectinaires (ind.m^{-2}), (B) coefficient de variation des apports larvaires.

adultes est principalement due à une dérive génétique associée à des fortes mortalités des premiers stades de vie pendant la période hivernale.

Concernant l'asynchronie reproductrice observée entre la Baie des Veys et la Baie de Seine, il semble maintenant évident que ces baies fonctionnent selon un modèle en source-puits. Ceci est confirmé par le nouveau modèle hydrodynamique incorporé à PECTIFLUX 3-D (Ellien, 2001) car les larves situées en Baie des Veys sont perdues au large pour la majorité (figure 26). D'autre part, les échanges inter-baies sont des évènements épisodiques sous le contrôle du forçage climatique et les flux larvaires sont principalement orientés de la Baie de Seine orientale vers la Baie des Veys. Concernant la stabilité des fréquences alléliques dans le noyau de plus forte densité (face à l'embouchure de la Seine), le nouveau modèle hydrodynamique indique que celle-ci est une zone privilégiée pour le recrutement des larves de pectinaires au sein de la baie, et où la variabilité des apports est la moins importante (figure 27).

Ce travail à micro-échelle n'a pu être totalement terminé, faute de temps. Il est en effet nécessaire de génotyper également les individus du deuxième noyau d'abondance situé près de Deauville en Baie de Seine orientale à partir des échantillons récoltés au cours des mêmes campagnes en 2003. Ce complément d'étude devrait permettre de comparer les microstructures génétiques des 2 noyaux, d'identifier les sources potentielles de larves qui peuvent constituer des cohortes génétiquement différentes dans un contexte de mosaïque spatiale fluctuante, et de mesurer la variation inter-génération des fréquences alléliques dans ce deuxième noyau. Cette dernière analyse devrait permettre d'affiner nos estimations de la taille efficace des populations au sein de la Baie de Seine, tout en sachant qu'il est nécessaire d'utiliser un nombre plus élevé de locus microsatellites pour obtenir une meilleure précision dans les calculs. Le rôle de l'asynchronie sur la structure en métapopulation Baie des Veys/ Baie de Seine orientale est importante car, théoriquement, cette asynchronie pourrait permettre de minimiser les risques d'extinction globale de la métapopulation lorsque les

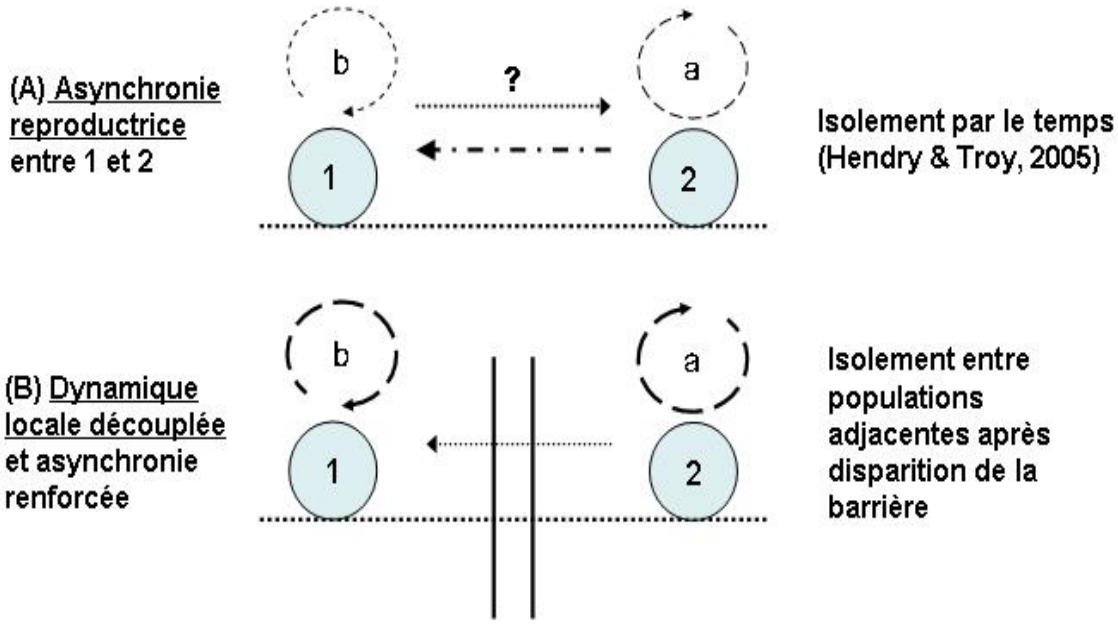


Figure 28. Scénario évolutif proposé pour la séparation des clade 1 et 2 de *Pectinaria koreni*. (A) L'asynchronie reproductrice entre populations adjacentes évoluant vers des pics adaptatifs différents peut mener à leur isolement dans le temps en dépit d'un flux larvaire, ici essentiellement dirigé vers la population 1 (modèle de Hendry & Troy, 2005); (B) l'apparition d'une barrière à la dispersion entre les populations a pour effet de renforcer l'asynchronie de reproduction et donc de rapidement découpler la dynamique locale des populations, même après disparition de cette barrière.

conditions hydrodynamiques locales ne conduisent pas localement (dans l'une ou dans l'autre baie) à assurer un recrutement conséquent, nécessaire au maintien d'un noyau d'abondance. Selon Hendry & Troy (2005), une telle asynchronie reproductrice maintenue entre populations adjacentes pourrait aboutir à un isolement temporel des populations même en présence d'un faible flux génique (figure 28). En effet, ce sont les paramètres environnementaux, biologiques et écologiques propres à chaque localité ainsi que la valeur sélective des migrants qui déterminent l'évolution des populations vers des pics adaptatifs différents (Schlutter, 2000). Cet isolement temporel est d'autant plus rapide si l'apparition d'une barrière, par exemple par effet vicariant, affaiblit l'effet homogénéisant de la migration, renforçant la dynamique locale des populations et donc l'asynchronie reproductrice, même après disparition de la barrière. Dans ce cas, le renforcement de l'asynchronie reproductrice et la rapidité des directions évolutives prises sont fonction du maintien de cette barrière physique dans le temps. En conséquence, l'évolution de lignées distinctes épousant des distributions largement allopatriques chez *P. koreni*, pourrait provenir d'une dynamique locale découplée liée à une asynchronie reproductrice renforcée et maintenue dans le temps.

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Résumé : Au regard des patrons actuels de distribution géographique des espèces marines à cycle bentho-pélagique et de leur conservation, prédire le devenir des systèmes biologiques passe par la compréhension des phénomènes passés, liés à l'histoire évolutive des espèces, c'est-à-dire, à leurs traits d'histoire de vie et au fonctionnement de leurs populations au cours des temps géologiques. En effet, l'évolution d'une espèce ne peut être comprise totalement qu'en intégrant les processus évolutifs s'exerçant à toutes les échelles d'espace (de la parcelle à l'aire de distribution de l'espèce) et de temps (de la génération au temps de résilience de l'espèce). Ce travail de thèse s'inscrit dans cette optique et aborde par l'utilisation du polymorphisme enzymatique, nucléotidique (sous-unité I du gène mitochondrial de la Cytochrome Oxidase, mtCOI), et de taille (microsatellites), (1) l'étude des processus de colonisation à macro-échelle évolutive chez les polychètes des genres *Owenia* et *Pectinaria*, eu égard à l'histoire géologique de l'Atlantique Nord Est, (2) l'étude des flux géniques contemporains entre les populations de *Pectinaria koreni* à l'échelle de la Manche/ Mer d'Irlande, en confrontant plus particulièrement les données génétiques avec celles issues de simulations hydrodynamiques de la dispersion larvaire en Manche et, (3) l'étude des processus jouant sur le fonctionnement spatio-temporel d'une métapopulation locale (échelle micro-évolutive de la Baie de Seine sur 3 ans).
Mots Clés : métapopulation ; histoire évolutive ; *Pectinaria koreni* ; *Owenia fusiformis*.

Abstract : With respect to the large scale patterns of geographic distribution of bentho-pelagic marine species and their conservation, predicting the fate of biological systems passes by the understanding of past processes linked to the evolutionary history of species, associated with life history traits and the functioning of their populations over geological time scales. The evolution of any species cannot be fully understood without integrating evolutionary processes working at all spatial (from a patch to the species' geographical range) and temporal scales (from one generation to the resilience of the species). Considering such a framework, we used enzymatic markers, mitochondrial gene sequences of the Cytochrome Oxidase subunit I (mtCOI), and microsatellite loci to (1) investigate past colonisation pathways on an evolutionary timescale in *Pectinaria sp.* and *Owenia sp.*, with respect to the geological history of the North East Atlantic, (2) identify contemporary gene flow in the English Channel/ Irish Sea between populations of *Pectinaria koreni*, by comparing genetic patterns with those estimated from hydrodynamic modelling of larval dispersal in the English Channel, and (3) assess the micro-evolutionary processes shaping the spatio-temporal structure of the local metapopulation of *P. koreni* in the Baie de Seine.
Key words : metapopulation ; evolutionary history ; *Pectinaria koreni* ; *Owenia fusiformis*.