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De la génétique des populations à la gestion durable des résistances : intérêt de l'étude des populations sauvages des pathogènes des cultures. Cas de deux nématodes à kystes et de leur hôte sauvage commun

Cécile Gracianne

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AGRO CAMPUS OUEST

UNIVERSITÉ EUROPÉENNE DE BRETAGNE

Cécile GRACIANNE • 10 avril 2015

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De la génétique des populations à la gestion durable des résistances : intérêt de l'étude des populations sauvages des pathogènes des cultures. Cas de deux nématodes à kystes et de leur hôte sauvage commun.

YANNICK OUTREMAN
Professeur, AGROCAMPUS OUEST, UMR INRA-AO-UR1 IGEPP / *président*

KAREN MCCOY
Chargée de recherche, IRD Montpellier / *rapporteur*

FRANÇOIS DELMOTTE
Chargé de recherche, INRA Bordeaux / *rapporteur*

ANNA-LIISA LAINE
Professeur, Université d'Helsinki / *examinatrice*

ERIC PETIT
Directeur de recherche, UMR INRA-AO-UR1 IGEPP / *directeur de thèse*

JEAN-FRANÇOIS ARNAUD
Professeur, Université de Lille / *co-directeur de thèse*

SYLVAIN FOURNET, Ingénieur de recherche / *invité*
MARC-RICHARD MOLARD, ITB, Paris / *invité*

PhD

Presented by
Cécile GRACIANNE

Prepared within the **Résistance et Adaptation (RA)** team,
UMR 1349 IGEPP

From population genetic to sustainable management of resistances: benefit of the study of wild populations of crops pathogens.
Case of two cysts nematodes and their common wild host

Defended on the 10th of April, 2015 to the committee composed with:

Yannick **OUTREMAN**	Professor, Agrocampus Ouest	President of the committee
Karen **McCOY**	Researcher, IRD Montpellier	Reviewer
François **DELMOTTE**	Researcher, INRA Bordeaux	Reviewer
Anna-Liisa **LAINE**	Professor, University of Helsinki	Reviewer
Marc **RICHARD-MOLARD**	ITB, Paris	Reviewer
Eric **PETIT**	Senior researcher, INRA Rennes	PhD supervisor
Sylvain **FOURNET**	Ingénieur de recherche, INRA Rennes	PhD co- supervisor
Jean-François **ARNAUD**	Professor, University of Lille 1	PhD co- supervisor

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SOMMAIRE

1. INTRODUCTION GENERALE 1

1.1. Potentiel évolutif des agents pathogènes 5
1.1.1. Définition 5
1.1.2. Estimation du potentiel évolutif d'un agent pathogène au travers d'une étude de structure génétique des populations 6
1.1.3. Dynamique évolutive des populations 8
1.1.3.1. Dérive génétique et taille des populations 8
1.1.3.2. Patrons de flux géniques 11
1.1.3.3. Histoire évolutive d'une espèce 13
1.1.4. Autres facteurs pouvant affecter le potentiel évolutif des agents pathogènes 15
1.1.4.1. Mode de reproduction 15
1.1.4.2. Intensité de la sélection et potentiel évolutif 17
1.1.5. Bilan: potentiel évolutif et formation de populations virulentes 17

1.2. Potentiel évolutif, durabilité des résistances et milieu sauvage 18
1.2.1. Potentiel évolutif et durabilité des résistances 18
1.2.2. Les mécanismes de résistances chez les plantes 19
1.2.2.1. La résistance qualitative et les interactions gène-pour-gène 20
1.2.2.2. La résistance quantitative 22
1.2.3. Évaluation du potentiel évolutif d'un pathogène des cultures dans le milieu sauvage 23

1.3. Étude de cas : le complexe *Heterodera schachtii*/*H. betae*/*Beta vulgaris* ssp. *maritima* 25
1.3.1. Les nématodes phytoparasites 25
1.3.2. Biologie des nématodes à kystes (Heteroderinae) 28
1.3.2.1. *Heterodera schachtii* 28
1.3.2.2. *Heterodera betae* 30
1.3.3. Contrôle des nématodes à kystes de la betterave 30
1.3.4. Betteraves sucrière et maritime 32
1.3.4.1. La betterave sucrière 32
1.3.4.2. La betterave maritime 35

1.4. Plan de la thèse 36

2. GEOGRAPHICAL OCCURRENCE AND PATTERNS OF GENETIC DIVERSITY OF TWO CYST BEET NEMATODES AND ITS ASSOCIATED HOST-PLANT, THE SEA BEET 40

2.1. Characterization of the geographical distribution of nematode populations in the wild 42
2.1.1. A comprehensive sampling on the putative species distribution area as a prerequisite 42
2.1.2. Sampling wild nematodes populations is a hard task 43
2.1.3. Preliminary results on geographic structure of genetic diversity of the three species, hypotheses and objectives 45

2.2. Spatial distribution and basic ecology of *Heterodera schachtii* and *H. betae* wild populations developing on sea beet, *Beta vulgaris* ssp. *maritima* 48

2.3. Comparative phylogeography of host/parasite wild populations: wild beets and cyst nematodes as a case study 61

2.4. What is new and what to do next 96
2.4.1. Comparative phylogeography of the three species: a work still in progress 96
2.4.2. *Heterodera betae* is still an open question 97
2.4.3. *Heterodera schachtii*: how explaining large-scale patterns of population genetic structure? 99

3. POPULATION GENETIC STRUCTURE OF *H. SCHACHTII* AT A LOCAL SCALE 102

3.1. Context, objectives and strategy 104
3.1.1. Fine-scale studies to understand patterns of genetic diversity in wild and field nematode populations 104
3.1.2. Sampling in space, but also in time 105
3.1.3. How picturing the population genetic structure in *Heterodera schachtii*? 106

3.2. Heterozygote deficits in cyst plant parasitic nematodes: possible causes and consequences 109

3.3. Temporal sampling and variation in the genetic structure of naturally occurring populations of a plant-parasitic nematode. 1 - Insights from the estimation of effective population sizes 134

3.4. Temporal sampling and variation in the genetic structure of naturally occurring populations of a plant-parasitic nematode. 2 - Separating the relative effects of gene flow and genetic drift 156

3.5. Illuminating the evolutionary biology of *H. schachtii* in the wild and in agro-ecosystems 181
3.5.1. Definition of a nematode population in the wild and consequences in agro-ecosystems 181
3.5.2. Micro-evolutionary processes, host colonization, and large-scale phylogeography 182

4. DISCUSSION AND PERSPECTIVES 185

4.1. What can be learned from our results? 187
4.1.1. Objectives and main results 187
4.1.2. Evolutionary potential of beet cyst nematodes and durability of resistances 188

4.2. Evolutionary potential of *H. schachtii* populations and co-evolution with *B. v.* ssp. *maritima* populations 190

4.3. Relationships between wild and field populations in *H. schachtii*: a missing point in the estimation of the evolutionary potential of this nematode 192

4.4. Alternative host species of *Heterodera schachtii* as a blank page for future research 193

APPENDIX 1: SEQUENCING PROTOCOL 197

REFERENCES 202

1. Introduction générale

1.1. Potentiel évolutif des agents pathogènes

1.2. Potentiel évolutif, durabilité des résistances et milieu sauvage

1.3. Etude de cas : le complexe *Heterodera schachtii*/*H. betae*/*Beta vulgaris* ssp. *Maritima*

1.4. Plan de thèse

Le parasitisme est un mode de vie répandu dans tous les écosystèmes de la planète. Il concerne une diversité exceptionnelle d'organismes différant tant par leur nature (ex. animale, végétale, unicellulaire, etc.), leurs cycles de vie, ou leurs modes de transmission (Poulin and Morand 2004; Barrett et al. 2008). En détournant les ressources de leurs hôtes à leur profit, les parasites[1] peuvent en affecter le cycle de vie, le mode de reproduction et de dispersion ou encore la survie (revue dans Gómez and Nichols 2013). Ils jouent ainsi un rôle prépondérant dans la distribution géographique et l'évolution de leurs hôtes ainsi que dans la structuration des écosystèmes (Dunne et al. 2013; Gómez and Nichols 2013). D'un point de vue évolutif, la compréhension de la dynamique des associations hôte-pathogène peut donc renseigner sur des processus aussi divers que la spéciation, le maintien de la biodiversité ou même l'évolution des génomes, lui conférant ainsi un intérêt scientifique majeur. Cet intérêt prend une dimension supplémentaire lorsque ces pathogènes s'attaquent à l'espèce humaine ou aux ressources qui lui sont indispensables, comme dans le cas des productions agricoles. L'étude de leurs capacités évolutives est alors un élément crucial dans le développement de stratégies de contrôles efficaces pour maintenir les dommages générés en dessous de seuils économiquement acceptables (Lebarbenchon et al. 2008a).

En effet, bien que les productions agricoles répondent d'abord à la nécessité d'assurer les besoins vivriers des populations humaines, elles contribuent également à la production d'énergie, de fibres textiles ou encore de molécules thérapeutiques. Dans ce contexte, la gestion des espèces pathogènes qui leur sont associées représente un enjeu socio-économique de premier ordre. Ces espèces peuvent ainsi être responsables de pertes économiques majeures, voire de famines sévères, comme celle déclenchée par le mildiou de la pomme de terre en Irlande au XIXème siècle (Goodwin et al. 1994). Pour éviter de telles situations, les modes de production du XXème siècle se sont appuyés sur le développement et l'utilisation de produits phytosanitaires qui se sont souvent révélés d'une redoutable efficacité. Par exemple, 32% des pertes de rendements enregistrées entre 1996 et 1998 sur les huit espèces les plus cultivées au monde (*i.e.* le blé, le riz, le maïs, l'orge, le soja, la pomme de terre, la betterave sucrière et le coton) étaient liées à des agents pathogènes. En absence de contrôle ces pertes auraient pu atteindre 67% (Bohan et al. 2013). Néanmoins, l'utilisation de ces produits est de plus en plus contestée en raison de leur toxicité pour les écosystèmes ou encore la santé

1 : Dans ce manuscrit, les espèces pathogènes sont définies largement comme tout organisme qui dépend d'une espèce hôte pour se nourrir, se développer et se reproduire. En ce sens, les termes « parasite », « ravageurs » et « agents pathogènes », qui peuvent parfois référer à des groupes d'espèces particuliers, sont considérés ici comme des synonymes.

humaine. Cette prise de conscience a favorisé l'émergence et le développement de méthodes de contrôle innovantes.

La lutte génétique est l'une de ces méthodes. Elle s'appuie sur l'utilisation de variétés de plantes possédant différents systèmes de défense, regroupés sous le terme de résistances. Ces résistances sont sous contrôle génétique et sont introduites dans les variétés cultivées par croisements successifs avec des espèces apparentées, issues du milieu naturel, sur lesquelles les organismes pathogènes peuvent être présents depuis des milliers d'années. Ces résistances sont donc le résultat d'un long processus d'adaptation réciproque entre parasites et plantes hôtes dans des écosystèmes naturels. Dans les agro-écosystèmes[2], les variétés portant ces résistances sont confrontées à des populations de pathogènes issues du milieu sauvage depuis plus ou moins longtemps. Il existe, par conséquent, un risque pour qu'elles ne soient que partiellement efficaces contre ces populations ou pour un laps de temps très court et ceci de façon variable, en fonction des zones géographiques où elles sont utilisées. Face au faible nombre de gènes disponibles, aux coûts et au temps nécessaire au développement de variétés cultivées génétiquement performantes, l'anticipation de ces phénomènes d'adaptation constitue une nécessité absolue, pour laquelle l'estimation du potentiel évolutif du pathogène est un facteur essentiel.

Pour tous les agents pathogènes, ce potentiel dépend à la fois de son histoire évolutive, qui s'est déroulée dans le milieu sauvage, et de certains paramètres propres à l'espèce comme ses capacités de dispersion ou la taille efficace de ses populations. Dans les agro-écosystèmes, ces paramètres peuvent être largement modifiés par les activités humaines inhérentes au milieu agricole. Il peut donc paraitre pertinent de les évaluer dans un environnement où ces perturbations sont limitées. Nous avons donc testé cette approche sur un modèle constitué par un complexe d'espèce hôtes, la betterave sucrière (*Beta vulgaris* ssp. *vulgaris*) et la betterave maritime (*Beta vulgaris* ssp. *maritima*) et deux espèces de nématodes phytoparasites, *Heterodera schachtii* et *Heterodera betae*, afin d'obtenir des informations nouvelles favorisant l'orientation et le développement de stratégies de déploiement de variétés résistantes de betterave sucrière dans les agro-écosystèmes.

2 : L'agro-écosystème est un complexe dynamique qui comprend l'ensemble des communautés naturelles, semi-naturelles, domestiquées et humaines (Loucks 1977).

1.1. Potentiel évolutif des agents pathogènes

1.1.1. Définition

Le concept de potentiel évolutif a été initialement défini par McDonald et Linde (2002) pour exprimer la capacité des pathogènes fongiques des cultures à s'adapter aux résistances génétiques utilisées pour les contrôler. Ces résistances génétiques exercent des pressions de sélection plus ou moins fortes ayant pour effets généraux la limitation ou l'arrêt du développement des pathogènes. Elles conduisent ainsi à des processus d'adaptation dans les populations de pathogènes, processus visant à contourner les mécanismes de résistance de la plante hôte. L'utilisation d'une résistance génétique implique donc la perte inexorable de son efficacité (Parlevliet 2002). La vitesse et l'ampleur de ces mécanismes de contournement de résistance dépendent néanmoins des caractéristiques évolutives du pathogène, c'est-à-dire d'un ensemble de traits d'histoire de vie incluant notamment le spectre d'hôte, les capacités de dispersion actives ou passives, la taille des populations, le mode de reproduction ou encore l'histoire de l'espèce au cours des derniers millénaires. Ces traits d'histoire de vie conditionnent en effet fortement les niveaux de diversité génétique rencontrés en population naturelle, substrat sur lequel peut agir la sélection naturelle et conduire éventuellement à une évolution d'ordre adaptatif.

La combinaison de ces caractéristiques évolutives définit donc la capacité d'un pathogène à s'adapter à une pression de sélection et peut être décrite à l'aide d'approches de génétique des populations. Ces approches permettent en effet de définir comment un variant virulent, c'est-à-dire ayant contourné un mécanisme de résistance, peut apparaitre, s'exprimer et se répandre dans un ensemble de populations. Dans leur étude, McDonald et Linde (2002) considèrent que les agents pathogènes présentant de grandes capacités de dispersion, d'importantes tailles des populations et un mode de reproduction mixte (mêlant reproduction sexuée et asexuée) sont ceux possédant les plus grandes capacités adaptatives à une résistance génétique, c'est-à-dire un grand potentiel évolutif. La description de ces caractéristiques pour une espèce pathogène a donc pour but de définir et de prédire le risque qu'elle représente pour une résistance génétique, c'est-à-dire la vitesse à laquelle cette résistance perdra son efficacité. De façon générale, un pathogène avec un potentiel évolutif élevé aura une probabilité plus importante de s'adapter à une résistance génétique portée par une variété cultivée et donc d'initier des épidémies responsables de pertes de rendement importantes.

Le potentiel évolutif représente donc un outil permettant d'estimer les capacités adaptatives d'un pathogène ou de ses populations dans le but de maximiser la durabilité et

l'efficacité des résistances. Néanmoins, bien que ce concept ait été initialement défini dans un contexte agronomique et seulement pour des espèces fongiques, les principes évolutifs sous-jacents à sa définition restent les mêmes pour toute espèce pathogène (De Meeûs et al. 2007a), à tout autre type de pressions de sélection, comme celles exercées par l'utilisation de pesticides ou d'antibiotiques. Le potentiel évolutif d'un agent pathogène ne se définit alors plus spécifiquement comme sa capacité à s'adapter aux résistances génétiques de son hôte mais plus généralement comme sa capacité à évoluer sous l'effet d'une contrainte de sélection imposée par son environnement.

1.1.2. Estimation du potentiel évolutif d'un agent pathogène au travers d'une étude de structure génétique des populations

La génétique des populations est une discipline qui étudie le fonctionnement des populations d'êtres vivants en se fondant sur la variabilité génétique existant entre les individus qui composent une population (Hartl 1988). Cette variation se traduit par une distribution différentielle des niveaux de diversité génétique à l'intérieur et entre les populations, distribution qui est le résultat de l'action simultanée de cinq forces évolutives distinctes dans le temps et dans l'espace : mutation, recombinaison, sélection, dérive génétique et flux de gènes (Encadré 1). Ces forces évolutives peuvent chacune laisser une signature génétique particulière reflétant la présence d'événements passés ou contemporains qui ont contribués à la formation des patrons actuels de distribution géographique de la diversité génétique. La génétique des populations examine donc l'histoire évolutive des populations pour comprendre leur dynamique actuelle, ce qui se révèle essentiel pour formuler des prédictions sur leur devenir.

Quel que soit le contexte évolutif, seule une partie de la diversité génétique est adaptative, c'est-à-dire, impliquée dans l'évolution en réponse à une pression de sélection. Le reste de la diversité génétique est dite neutre, car elle ne confère ni avantage ni inconvénient en termes de valeur sélective[3] aux individus (Halliburton 2004). La sélection n'ayant pas de prise directe sur la variation génétique neutre, il est plus facile d'y déceler les signatures laissées par les autres forces évolutives au cours du temps. De plus, les flux de gènes et la dérive génétique affectent à la fois la diversité adaptative et neutre. En quantifiant l'intensité de la dérive génétique et des flux de gènes, l'étude des variations de la diversité génétique neutre permet

3 : La valeur sélective d'un individu est définie comme sa capacité à survivre et à se reproduire. Les individus dont la valeur sélective est plus élevée contribuent plus que les autres à la génération suivante.

Encadré 1 : Les cinq forces évolutives *d'après Halliburton (2004)*

La **mutation** est définie comme un changement héritable de la molécule d'ADN qui apparait de façon aléatoire, et correspond à la formation de nouveaux allèles, c'est-à-dire de copies alternatives d'un même locus. Cette modification peut être bénéfique, neutre ou néfaste pour l'organisme porteur. La mutation correspond au mécanisme ultime de formation de la diversité génétique au sein des espèces. C'est un substrat généré aléatoirement sur lequel la sélection naturelle peut ensuite agir.

La **recombinaison** est une seconde source de variation génétique qui est généralement associée à la reproduction sexuée et qui crée de nouvelles combinaisons d'allèles plutôt que de nouveaux allèles. Elle peut donc générer de nouveaux phénotypes caractérisés par des valeurs sélectives variables pour lesquels la sélection peut agir.

La **sélection** naturelle correspond à l'idée que les individus les plus adaptés à leur environnement survivent mieux et se reproduisent plus que les autres. Ils laissent ainsi plus de descendants dans la génération suivante qui transmettront leurs gènes à leurs propres descendants, aboutissant à une augmentation en fréquence au cours du temps des gènes impliqués dans la valeur sélective. De façon générale, la sélection naturelle correspond à une contrainte imposée par l'environnement aux organismes à des niveaux d'organisation (populations, espèces, écosystèmes) plus ou moins élevés.

La **dérive génétique** correspond à la fluctuation stochastique des fréquences alléliques dans une population, associée au tirage aléatoire d'un nombre limité de gamètes dans une population pour former la génération suivante. Ce processus tend à éroder la variation génétique, neutre, favorable ou défavorable, au sein des populations sur le long terme. Comme ce processus micro-évolutif n'est pas déterministe, il se déroule indépendamment dans chaque population et peut ainsi favoriser leur divergence génétique.

Les **flux de gènes** correspondent au déplacement d'individus ou de gamètes depuis leur population natale jusque dans une autre où ils s'installent. Pour peu qu'ils se reproduisent, ils contribuent alors à modifier la constitution génétique de la population en question. Les flux de gènes peuvent donc représenter une source de variation pour une population quand des allèles initialement absents y sont introduits. Cet apport rend les populations plus similaires qu'elles ne le seraient en absence de flux de gènes, ce qui limite leur divergence et s'oppose donc à l'effet antagoniste de la dérive génétique.

ainsi de comprendre comment la diversité génétique, qu'elle soit adaptative ou non, est répartie dans et entre les populations, ce qui contribue à comprendre comment elles peuvent s'adapter à une pression de sélection donnée.

Dans ce cadre théorique, le potentiel évolutif dépendra de la manière dont la diversité génétique est générée, échangée et éliminée dans les populations. Ces informations sont, pour

la plupart, accessibles en décrivant la structure génétique neutre des populations. A des échelles spatiales fines et sur un nombre de génération réduit, la structure génétique des populations reflète essentiellement l'influence relative de forces antagonistes que sont la dérive génétique et les flux de gènes dans et entre les populations (McDonald and Linde 2002; Criscione et al. 2005). L'étude de cette interaction renseigne sur le fonctionnement actuel des populations, c'est-à-dire sur la façon dont les fréquences alléliques évoluent en fonction des caractéristiques biologiques propres à l'espèce considérée (ex. mode de reproduction, capacités de dispersion etc.). Cette dynamique, qui ne considère que quelques générations successives, est répétable sur de petites unités de temps (saisons, années, décennies). Sur des échelles de temps aussi courtes, les caractéristiques globales de l'environnement, comme le climat, le relief ou même les interactions inter-spécifiques, restent relativement stables et homogènes dans l'espace, ce qui peut permettre de faire abstraction de leurs influences.

En revanche, sur de grandes échelles temporelles (siècles, millénaires, périodes glaciaire…), l'environnement peut connaitre de grands bouleversements climatiques, géologiques ou structurels qui affectent les espèces qui y vivent. Ces perturbations influencent fortement les patrons de structure spatiale du polymorphisme génétique observés à grandes échelles géographiques (continentale ou mondiale). Dès lors, l'étude d'un ensemble de populations réparties sur toute l'aire géographique d'une espèce permet d'identifier les évènements historiques ayant façonné l'évolution différentielle de lignées génétiquement distinctes. L'ensemble de ces événements forgent l'histoire évolutive des populations qui est notamment étudiée au travers d'approches de phylogéographie (Criscione et al. 2005). Le potentiel évolutif d'un agent pathogène est donc constitué d'une composante contemporaine et historique qu'il est nécessaire de décrire pour appréhender le plus finement possible les capacités d'adaptation d'un agent pathogène.

1.1.3. Dynamique évolutive des populations

1.1.3.1. Dérive génétique et taille des populations

L'influence de la dérive génétique est étroitement associée aux fluctuations démographiques que peut connaitre une population. En effet, l'influence de cette force évolutive se traduit notamment en termes probabilistes qui dépendent de la taille des populations. Ainsi, plus l'effectif d'une population est réduit plus la probabilité qu'un allèle soit perdu sous l'effet du hasard est grande et ceci même si cet allèle confère un avantage sélectif à son porteur (Buri 1956; Hartl and Clark 1997). En effet, par simple ségrégation mendélienne, cet allèle est distribué au hasard et dans une partie seulement des gamètes des individus qui le possèdent.

Comme ces gamètes ne sont pas tous utilisés pour la reproduction, la transmission de cet allèle à la génération suivante sera finalement aléatoire. Par conséquent, si un allèle est en faible fréquence dans une population elle-même de taille réduite, le risque qu'il soit perdu de manière fortuite est élevé. Par ailleurs, plus l'effectif d'une population est grand, plus la probabilité d'apparition de nouveaux allèles par mutation est élevée. En effet, comme les taux de mutations sont généralement bas, la probabilité d'apparition et de maintien d'un allèle sera plus forte dans les populations de grande taille. En résumé, plus les tailles de populations sont grandes, plus la mutation pourra générer de diversité et moins la dérive génétique sera en mesure d'éliminer ces nouvelles variations. Ainsi, plus les populations d'un agent pathogène sont de grandes tailles, plus sa diversité génétique adaptative peut être importante ce qui accroit son potentiel évolutif (McDonald and Linde 2002).

En génétique des populations, l'intensité de la dérive génétique se traduit par un paramètre, appelé taille efficace des populations (*Ne* ; Encadré 2). La taille efficace décrit mieux la quantité de dérive génétique subie par une population que son effectif réel car elle tient compte du fait que tous les individus ne se reproduisent pas avec la même probabilité (Wright 1931). Ce paramètre s'interprète, néanmoins, de façon similaire : plus la taille efficace des populations est petite, plus la dérive génétique est influente. Peu d'études ont estimé rigoureusement la valeur de *Ne* dans des populations d'agents pathogène. Toutefois, des études théoriques prédisent que la taille efficace de leurs populations est probablement réduite en raison de certains traits biologiques ou critères spatiaux, comme la subdivision, l'agrégation et la contribution variable des sous-populations à la génération suivante, qui tendent à diminuer les valeurs de *Ne* en dessous des valeurs attendues pour des espèces non parasites (Price 1980; Criscione and Blouin 2005; Criscione et al. 2005). Ces hypothèses ont été confirmées par un certain nombre d'études empiriques sur différentes espèces de virus, de pathogènes fongiques et de protozoaires parasites (Sentandreu et al. 2006; Vardo and Schall 2007; Bayon et al. 2009; Zwart et al. 2011; Fabre et al. 2012).

Parallèlement, une autre partie de la littérature avance l'hypothèse que les pathogènes généralistes devraient être capables de maintenir des populations de plus grandes tailles. En effet, en absence de facteurs tels que l'allopatrie ou la différenciation de niche qui limiterait les flux entre populations de pathogène, l'infestation de plusieurs espèces hôtes pourrait rendre indépendante la dynamique des populations du pathogène de celle d'un hôte particulier (Dobson 2004; Barrett et al. 2008). Cette hypothèse a récemment été vérifiée par une étude de la taille efficace des populations d'un nématode, *Trichostrongylus axei*, parasitant six espèces d'ongulés vivant en sympatrie en Amérique du Nord (Archie and Ezenwa 2011). De façon un

Encadré 2 : Concept de taille efficace de population - N_e

Dans les populations naturelles, tous les individus d'une population ne se reproduisent pas de manière uniforme et donc ne participent pas de la même façon à la génération suivante. Il peut, en effet, exister un nombre différents de mâles et de femelles chez les individus reproducteurs, des variations des taux de reproduction en fonction de l'âge, ou encore une extrême variance dans le succès reproducteur d'un individu à un autre. En conséquence, l'évolution des fréquences alléliques ou de l'hétérozygotie au cours des générations est similaire à celle attendue pour une population d'effectif plus faible que le simple nombre d'individus recensés (Figure 1).

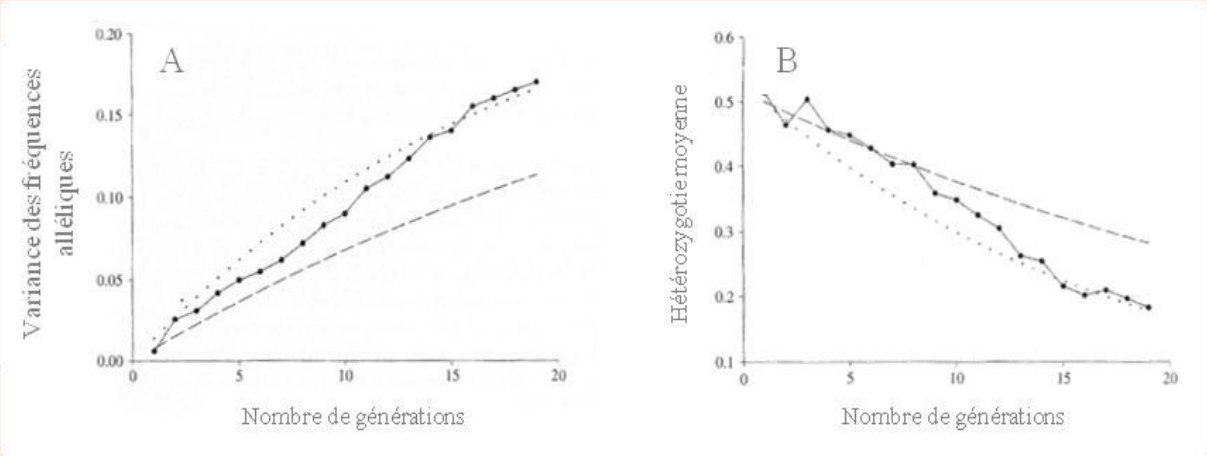


Figure 1 : Comparaison de l'évolution des fréquences alléliques (A) et de l'hétérozygotie moyenne (B) attendues et observées sur 107 populations expérimentales de 16 drosophiles sur 19 générations. Les données observées correspondent aux traits pleins, les pointillés (et les tirets) aux résultats attendus pour une population de 9 (et 16) individus sous un modèle supposant un organisme diploïde et sexué, des générations discrètes, la panmixie, une taille constante des populations, une absence de flux de gènes, de sélection et de mutation (Buri 1956; Halliburton 2004). Dans cette expérience, l'évolution de la variance des fréquences alléliques semblent plus concordante avec les résultats attendus pour une population de 9 individus, ce qui est également le cas de l'hétérozygotie après un certain nombre de générations.

Pour décrire correctement l'évolution des fréquences alléliques, Wright (1931) a alors proposé le concept de taille efficace des populations, notée Ne, qui correspond au nombre d'individus composant une population idéale, c'est-à-dire comportant autant de mâles que de femelles et dans laquelle chaque individu aurait le même nombre de descendants. Cette population idéale subirait la même intensité de dérive génétique que la population naturelle étudiée. Ce paramètre est un estimateur de la dérive génétique qui informe sur l'intensité à laquelle cette force évolutive modifie les fréquences de gènes dans une population relativement à une situation théorique reposant sur la panmixie et une taille infinie de population. Plus la taille efficace de population est petite, plus la variation des fréquences alléliques d'une génération à l'autre est forte et plus la dérive est influente. Dans un contexte de gestion des populations, ce paramètre est intéressant pour évaluer et prévenir les risques d'extinction des populations.

peu similaire, plusieurs espèces de pathogènes des cultures présentent également de grandes tailles efficaces de population (McDonald and Linde 2002; Gurung et al. 2013). Ce dernier exemple illustre bien que la dérive génétique n'a pas forcément la même influence d'un pathogène à l'autre, mais également que l'impact de cette force évolutive ne peut être correctement appréhendé sans information sur une autre force évolutive importante : les flux de gènes.

1.1.3.2. Patrons de flux géniques

La perte de diversité engendrée par la dérive génétique peut être plus ou moins compensée par l'afflux de gamètes et/ou d'individus migrants intervenant dans la constitution génétique de la population considérée. Comme les allèles apportés par les migrants sont ceux présents dans leur population d'origine, après reproduction, les deux populations seront génétiquement plus similaires qu'elles ne l'auraient été en absence de flux. Par conséquent, les flux de gènes tendent à homogénéiser les pools génétiques des populations qu'ils relient. Cette homogénéisation ne nécessite que très peu de migrants par génération (Wright 1940). Toutefois, plus les flux de gènes seront faibles, plus les populations évolueront indépendamment les unes des autres sous l'effet conjugué de la dérive génétique, de la sélection et de la mutation. En cas d'isolement total prolongé, cela peut éventuellement mener à la formation de nouvelles espèces (Slatkin 1985). Par conséquent, les flux de gènes permettent de maintenir une certaine cohésion génétique au sein d'une espèce mais également de contrebalancer les effets de la dérive en ralentissant la divergence génétique entre populations. Lorsque l'effet de la migration compense exactement les pertes occasionnées par la dérive génétique, on dit que la population est à l'équilibre migration-dérive (*cf*. Hutchison & Templeton 1999). Cette situation reste théorique, mais représente un modèle intéressant pour tester un certain nombre d'hypothèses associées à la présence de flux de gènes (Encadré 3).

Outre l'action homogénéisatrice des flux de gènes sur les pools génétiques, plusieurs études soulignent l'importance des capacités de dispersion respectives de l'hôte et du pathogène dans l'évolution des pathosystèmes[4] (Gandon et al. 1996; Thrall and Burdon 2002; Morgan et al. 2005). Un agent pathogène s'adaptera ainsi plus facilement à son hôte, localement, si ses capacités de dispersion sont supérieures à celles de ce-dernier. Dans cette situation, les flux apportent de la variation génétique sur laquelle la sélection peut agir et favorisent ainsi l'adaptation du pathogène. Cette situation a été observée dans plusieurs pathosystèmes et concorde avec l'idée que les flux de gènes sont également responsables de la

Encadré 3 : Comment mesurer la différenciation génétique des populations ?

En théorie, une espèce correspond à un groupe d'individus génétiquement « fermé » au sein duquel ils sont susceptibles de se reproduire entre eux. Néanmoins, en raison de l'hétérogénéité géographique et écologique du milieu, ces individus sont généralement distribués entre plusieurs sous-groupes, appelés populations ou dèmes, plus ou moins connectés entre eux, au sein desquels les échanges génétiques sont plus probables qu'entre individus géographiquement plus éloignés. La dérive génétique affecte de façon indépendante les fréquences alléliques de chacune de ces populations, ce qui favorise leur différenciation, c'est-à-dire leur divergence génétique. A l'inverse, la présence de flux de gènes s'oppose à cette divergence, si bien que la différenciation des populations à un instant donné est le reflet de l'influence de ces deux forces évolutives.

La mesure la plus communément utilisée pour estimer la différenciation génétique des populations, c'est-à-dire la différence des fréquences alléliques entre populations, est l'indice de fixation F_{ST} de Wright, qui est l'un des trois paramètres décrivant la structure génétique ou génotypique des populations, avec le F_{IS} et le F_{IT}. L'indice de fixation F_{ST} correspond à la corrélation entre deux gamètes tirés au hasard dans la même sous-population par rapport à la population entière et s'interprète comme la part de la réduction de l'hétérozygotie attendue sous le modèle de Hardy-Weinberg associée à la structure des populations. Il est défini par :

$$F_{ST} = \frac{H_T - H_S}{H_T}$$

où H_T représente l'hétérozygotie attendue pour la population entière et H_S, l'hétérozygotie attendue dans la sous-population. Notons qu'il existe tout un panel d'estimateurs de cet indice de fixation, non plus fondés sur des rapports d'hétérozygotie, mais fondés sur des variances de fréquences alléliques entre population (ex. Weir & Cockerham 1984 ; revue dans Holsinger & Weir 2009).

Cet indice varie entre 0 et 1. Plus sa valeur est proche de 0, plus les populations sont génétiquement proches, ce qui peut témoigner de la présence de flux de gènes important. A l'inverse, plus le F_{ST} est proche de 1 plus les populations sont génétiquement différenciées et donc potentiellement isolées en termes de flux géniques. En pratique, la différenciation génétique des populations est considérée comme importante dès que $F_{ST} > 0{,}1$.

La facilité de l'interprétation de cet indice explique en partie sa popularité dans les études de génétique des populations qui est aussi associée à la diversité des questions qu'il renseigne comme la structuration spatiale des populations, l'estimation des flux de gènes entre populations, l'histoire démographiques des populations ou encore la détection de régions génomiques sous sélection (Beaumont 2005; Holsinger and Weir 2009)

propagation d'allèles adaptés à un mode de gestion donné (résistance génétique, antibiotiques, pesticides) à large échelle (Greischar and Koskella 2007). Dans les agro-écosystèmes, les flux géniques sont alors à la fois des vecteurs d'infestation des parcelles saines, mais également des responsables de la généralisation de la perte d'efficacité d'une méthode de gestion (Bousset and Chèvre 2013). En ce sens, les agents pathogènes dont les flux de gènes sont plus intenses peuvent plus facilement se propager entre populations ce qui augmente leur potentiel évolutif.

Néanmoins, l'afflux d'allèles provenant d'autres populations peut également avoir un rôle inverse en empêchant l'augmentation en fréquences des génotypes localement adaptés aux conditions du milieu. Chez les espèces pathogènes, ces phénomènes de mal-adaptation ne sont généralement pas attendus en raison de certains de leurs traits d'histoire de vie, tels que des temps de génération courts, qui favorisent des changements de composition génétique plus rapides que chez les espèces hôtes (Kaltz and Shykoff 1998; Huyse et al. 2005). Ces phénomènes de mal-adaptation ont pourtant déjà été observés dans différentes associations hôte-pathogène (ex. Spitzer 2006; Adiba et al. 2010 ; Kniskern et al. 2011; Glais et al. 2014). Les flux de gènes peuvent donc jouer des rôle multiples et antagonistes dans l'évolution des associations hôte-pathogènes qui justifient la nécessité de les quantifier pour estimer correctement le potentiel évolutif de ces organismes.

1.1.3.3. Histoire évolutive d'une espèce

À long terme, l'action combinée des différentes forces évolutives forge l'histoire des populations. Cette histoire correspond à l'ensemble des processus évolutifs ayant affecté la structure génétique de leurs populations au cours du temps et sa reconstruction sur plusieurs dizaines de milliers d'années peut permettre d'identifier les mécanismes à l'origine des patrons géographiques observés. Par exemple, il est largement reconnu que des facteurs biogéographiques, tel que le climat ou la formation de reliefs, peuvent induire le déplacement ou le fractionnement des aires de distributions des espèces tant dans les écosystèmes marins que terrestres (Hewitt 2000; Gillespie and Roderick 2014). A plus fine échelle, de telles modifications géographiques peuvent résulter de l'isolement, l'expansion, la contraction voire l'extinction des populations. Ces phénomènes laissent une empreinte durable dans la structuration géographique de la diversité génétique des populations et définissent la composition génétique dont elles disposent à un instant donné de leur histoire.

4 : Un pathosystème correspond à un complexe d'espèces régit par une interaction parasitaire et comprend donc à la fois l'espèce hôte et le ou les pathogène(s) que lui sont associés.

Une population peut ainsi connaitre une réduction drastique de ses effectifs sous l'action de différents mécanismes comme l'arrivée d'un nouveau prédateur ou d'un pathogène, ou la dégradation de son habitat, etc. (ex. Brasier et al. 1993; Holderegger and Di Giulio 2010). Ce phénomène, appelé goulot d'étranglement, induit la perte définitive d'une partie de la diversité génétique initiale mais conduit également à un accroissement de l'influence de la dérive génétique pouvant encore accentuer l'appauvrissement du pool de gènes restant. Chez les espèces pathogènes, les goulots d'étranglements peuvent survenir lors du passage de la mauvaise saison (Tack and Laine 2014), lors de la disparition de l'hôte (Dunn et al. 2009), ou même lors des phases de transmission et de déploiement de la maladie (Gutiérrez et al. 2012; Fabre et al. 2014). De façon similaire, l'effet fondateur fait référence au développement d'une nouvelle population à partir d'un nombre restreint d'individus porteurs d'une fraction seulement de la diversité génétique présente dans la population d'origine (Wade and McCauley 1988). Dans cette situation, plus le nombre d'individus fondateurs est petit plus l'impact de la dérive génétique est important : les populations les plus récemment fondées présentent alors les niveaux de diversité génétique les plus faibles. L'effet fondateur et la perte de diversité qui y est associée sont des phénomènes souvent observés lors de la colonisation d'un nouvel habitat (Austerlitz et al. 1997; Waters et al. 2013). Ils sont ainsi caractéristiques des routes d'invasion et de colonisation prises par les espèces (ex. Fontaine et al. 2013a, b). A l'inverse, une population peut soudainement bénéficier de plus d'espace ou d'une plus grande quantité de nourriture, ce qui peut favoriser son expansion géographique et/ou démographique. Dans ce cas, l'impact de la dérive est limité et la présence d'allèles rares, caractéristique des populations en expansion, peut être favorisée (Excoffier et al. 2009). Les conséquences de ces événements sur la structure génétique des populations, notamment lors des phases de contraction, dépendent à la fois de l'intensité des flux de gènes mais aussi de leur succession dans le temps. Par exemple, des cycles successifs d'isolement et de reconnexion des populations peuvent générer des pics de diversité génétique et accroitre leurs capacités adaptatives (Alcala et al. 2013; Alcala and Vuilleumier 2014). A l'inverse, plusieurs événements de fondation consécutifs induisent un appauvrissement graduel des nouvelles populations par rapport aux anciennes. L'histoire évolutive d'une espèce peut donc se révéler complexe, notamment dans le cas d'organismes impliqués dans des interactions multipartites tels que les agents pathogènes.

Enfin, il est nécessaire de considérer certaines particularités des espèces pathogènes que sont les changements d'hôtes (« host-shift » et « host-jump »). Ces phénomènes correspondent à la colonisation soudaine d'une nouvelle espèce hôte par le parasite et sont

généralement observés dans le cadre d'études sur la cospéciation (de Vienne et al. 2013). À ce titre, ils n'influencent pas directement l'histoire évolutive des populations d'un agent pathogène car ces macro-processus se mettent en place à des échelles de temps plus longues. Néanmoins, plusieurs études montrent que les « host-jump » et les « host-shift » sont des mécanismes fréquents chez les pathogènes. La spécialisation des populations d'un agent pathogène à un hôte donné n'implique pas forcément l'isolement reproducteur de ces populations avec celles se développant sur d'autres hôtes (ex. Fournier and Giraud 2008; Spottiswoode et al. 2011; Clarkson et al. 2013), ni la perte de la capacité à infester d'autres espèces plus ou moins proches phylogénétiquement (Hoberg and Brooks 2008; Poulin and Keeney 2008). En ce sens, l'histoire évolutive d'un agent pathogène peut varier d'un hôte à l'autre, et il est donc préférable d'avoir des informations préalables sur l'étroitesse de l'association entre un pathogène et un hôte donné pour appréhender au mieux les patrons de structuration génétique observés (Stukenbrock and McDonald 2008).

1.1.4. Autres facteurs pouvant affecter le potentiel évolutif des agents pathogènes

D'autres mécanismes en dehors de la dérive génétique et des flux géniques, comme le mode de reproduction et le type de résistance rencontré, peuvent également jouer un rôle dans l'adaptation des agents pathogènes aux variétés cultivées (McDonald and Linde 2002).

1.1.4.1. Mode de reproduction

Le mode de reproduction est une caractéristique biologique propre d'une espèce qui définit la façon dont les gènes sont transmis d'une génération à l'autre. Elle peut donc influencer la distribution spatiale de la diversité génétique inter et intra-population (Hamrick and Godt 1996). L'influence des flux de gènes et de la dérive génétique peut alors s'en trouver modifiée. De façon globale, la reproduction peut être asexuée, sexuée ou un mélange des deux. Dans le cas de la reproduction asexuée, l'information génétique d'un individu ne provient que d'un seul parent. Un descendant peut être produit à partir d'une seule cellule ce qui correspond à une reproduction agamétique chez les animaux et végétative chez les plantes, ou à partir d'un œuf non fécondé (gamète femelle), ce qui correspond à de la parthénogénèse. La parthénogénèse est un mode de reproduction fréquemment observé chez les espèces pathogènes, dont il existe plusieurs variantes qui se différencient principalement par la présence/absence de méiose et de recombinaisons intra-individuelles lors de la formation des gamètes et dont les conséquences évolutives sont assez variées (de Meeûs et al. 2007b). Il est généralement admis que les populations asexuées sont moins diverses

génétiquement. Toutefois, ce type de reproduction préserve les allèles favorables apparus par mutation car ils sont moins sujets aux effets de la dérive génétique, qui ne dépend plus que de l'extinction aléatoire de certaines lignées. D'autre part, l'accumulation des allèles au cours du temps peut être à l'origine d'un excès d'hétérozygotes et créer de la diversité allélique (Judson and Normark 1996; Hansson and Westerberg 2002). Dans le cas des systèmes plantes-pathogènes, ce mode de reproduction peut être avantageux car il ne brise pas les associations d'allèles adaptées aux résistances génétiques de son hôte (Clay and Kover 1996).

La reproduction sexuée permet le transfert de la moitié de l'information génétique de chaque parent à toute leur progéniture. Il existe différent type de croisements qui s'échelonnent de la consanguinité stricte à l'allogamie obligatoire. Dans tous les cas, tous les descendants sont génétiquement différents des parents grâce à la fusion des gamètes mâles et femelles et au brassage génétique associé aux mécanismes de recombinaison intra et inter-chromosomique. La reproduction sexuée présente donc l'avantage de créer rapidement de la diversité génétique, sans avoir à attendre une mutation, et de favoriser ainsi la diversification et l'adaptation des organismes aux changements, parfois rapides, des conditions environnementales (Burt 2000). Chez les organismes pathogènes, cette hypothèse a été notamment vérifiée chez les helminthes parasites (Galvani et al. 2001; Galvani et al. 2003), mais a été bien moins étudiée chez les autres espèces parasites. Un corollaire de cette théorie est que la reproduction sexuée permet la production d'une descendance beaucoup plus variée, améliorant potentiellement les capacités de survie et de reproduction de la progéniture du pathogène malgré une forte sélection imposée par l'hôte. De fait, la reproduction sexuée devrait être favorisée par rapport à la reproduction asexuée chez les espèces parasites. Cette hypothèse a récemment été vérifiée de façon empirique dans l'ensemble du phylum Nematoda et un pathogène du blé, *Mycosphaerella graminicola* (Zhan et al. 2007; Gibson and Fuentes 2015).

Il existe néanmoins de nombreux agents pathogènes présentant des systèmes de reproduction mixte, qui peuvent à la fois développer des structures sexuées et asexuées au cours de leur cycle de vie. Beaucoup d'entre elles sont majoritairement clonales comme le puceron du pois, certains protozoaires (ex. *Trypanosoma* and *Leishmania*) ou champignons pathogènes (ex. *Candida albicans*). Mais même de faible taux de reproduction sexuée peuvent limiter, voire occulter, les effets de la reproduction asexuée sur la structure génétique des populations (Halkett et al. 2005). Pour ces espèces, il est ainsi possible de générer de nouvelles combinaisons d'allèles, par reproduction sexuée, dont les plus performantes seront conservées *via* une reproduction asexuée. Dans les cultures, cette capacité peut leur permettre

de s'adapter rapidement à leur hôte sur une large échelle spatiale, et donc d'avoir un plus grand potentiel évolutif que les espèces strictement sexuées et clonales.

1.1.4.2. Intensité de la sélection et potentiel évolutif

Les pressions de sélection imposées sur un agent pathogène par son environnement et par son hôte constituent le principal moteur du processus adaptatif du pathogène. Elles peuvent donc impacter le potentiel évolutif du pathogène, en influençant notamment la vitesse à laquelle l'adaptation à une pression de sélection peut avoir lieu. En effet, ces forces de sélection peuvent être de différentes intensités, en fonction de la façon de leur impact sur la valeur sélective de l'ensemble des individus présents dans la population. Plus le nombre d'individus favorisés par la sélection est réduit, plus la sélection est intense et plus l'adaptation sera rapide (McDonald and Linde 2002). Dans le cas des pathogènes des cultures, l'intensité de la sélection varie en fonction du nombre de variétés utilisées et du type de résistances génétiques qu'elles contiennent. La modulation de l'intensité de ces pressions de sélection exercées par les variétés est d'ailleurs l'un des leviers possible pour améliorer la durabilité des résistantes génétiques.

1.1.5. Bilan: potentiel évolutif et formation de populations virulentes

En résumé, le potentiel évolutif d'un agent pathogène, à un instant donné, est défini à la fois par son histoire évolutive et le fonctionnement de ses populations. L'histoire évolutive définit la composition génétique d'une population à un instant *t*, qui résultera de l'action combinée des différentes forces évolutives au cours du temps. Dans un contexte de gestion d'un pathogène agronomique, l'histoire évolutive détermine l'existence d'individus pré-adaptés à une résistance génétique, appelés virulents. Leur fréquence affecte directement le délai nécessaire à l'adaptation de la population (Figure 2). Si les individus virulents sont absents de la population lors de la mise en place de la pression de sélection, c'est-à-dire lors du déploiement d'une variété résistante, ils peuvent néanmoins apparaitre spontanément dans la population par mutation (ou recombinaison) ou encore provenir d'autres populations par le biais de flux de gènes. La force de la sélection exercée par la résistance génétique favorise alors leur multiplication, et à terme, la formation de populations capables de se développer même en présence du ou des gènes de résistance dans la plante hôte. L'augmentation de la fréquence des individus virulents dans la population reste néanmoins dépendante de la taille de population qui détermine (i) l'influence des flux de gènes, (ii) le nombre de mutants pouvant apparaitre dans la population au cours d'une génération et (iii) l'intensité de la sélection.

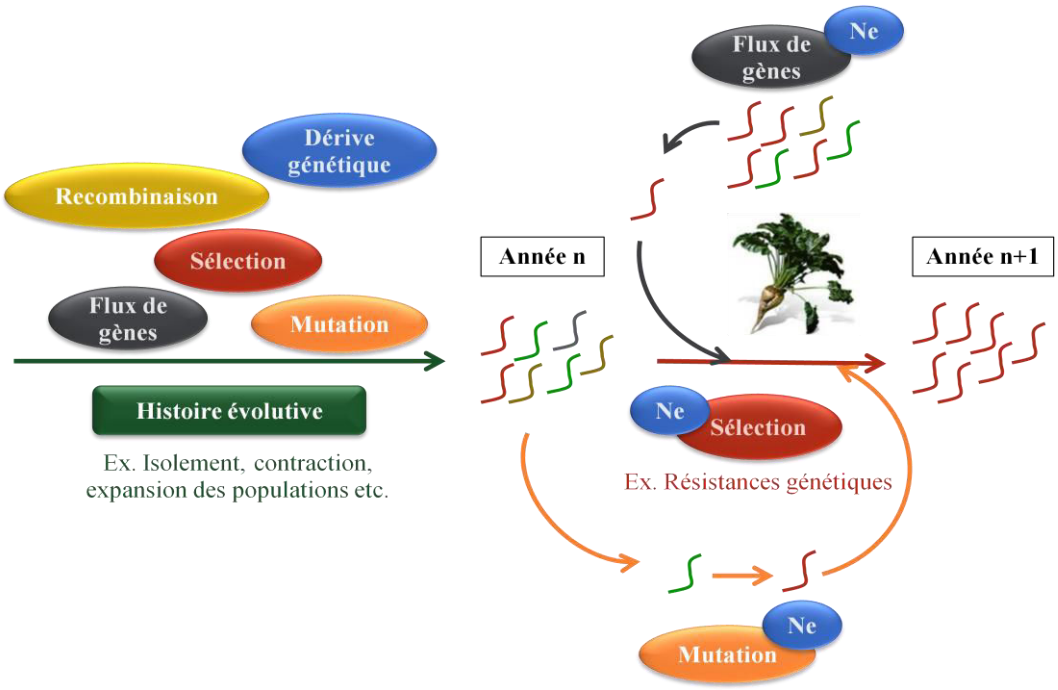


Figure 2 : Le potentiel évolutif ou l'action combinée de l'histoire évolutive et du fonctionnement des populations sur la capacité d'un agent pathogène à s'adapter à une résistance génétique. *Ne* correspond à la taille efficace des populations et représente l'impact de la dérive génétique.

1.2. Potentiel évolutif, durabilité des résistances et milieu sauvage

1.2.1. Potentiel évolutif et durabilité des résistances

L'évaluation du potentiel évolutif d'un pathogène des cultures trouve son intérêt dans son caractère prédictif de la durabilité des résistances. Les résistances correspondent, de manière générale, aux facteurs génétiques permettant de réduire la compatibilité de l'interaction entre un pathogène et sa plante hôte. Une résistance est considérée comme durable si elle reste efficace malgré son utilisation prolongée à large échelle, dans un environnement favorable à la maladie (Johnson 1984). Dans cette définition, la durabilité résulte de l'interaction entre la plante, le pathogène, l'environnement et l'homme. Ainsi, une résistance n'est pas durable en soi, mais uniquement dans un contexte précis. En considérant le nombre limité de gènes de résistances présents dans les pools génétiques des espèces cultivées et la durée nécessaire à la production d'une variété performante, la prise en compte de la dynamique de l'adaptation d'un agent pathogène est donc cruciale pour maintenir l'efficacité des variétés existantes en dépit d'une utilisation récurrente. Au cours des dernières décennies, les efforts fournis lors du processus de sélection variétale et du développement d'itinéraires techniques adaptés ont principalement été dédiés à la maximisation de l'efficacité des résistances, ce qui a échoué à

en assurer la stabilité (Bousset 2014). Ainsi, le déploiement d'un nombre restreint de variétés résistantes favorise l'invasion des populations de pathogènes par des individus virulents et mène à la perte d'efficacité de ces variétés.

Cette perte d'efficacité est appelée contournement des gènes de résistances. D'un point de vue agronomique, ce contournement survient lorsque les pertes de rendements associées aux populations de pathogène deviennent supérieures à un seuil économiquement acceptable. D'un point de vue évolutif, le contournement d'une résistance correspond à un changement de la composition génétique de la population du pathogène suite à l'application d'une force de sélection unidirectionnelle. Ce changement correspond exactement au processus d'adaptation en réponse à une pression de sélection décrit dans la section précédente. Les délais de contournement sont variables d'un pathogène à l'autre et peuvent survenir à l'issue de quelques mois ou de plusieurs décennies (ex. Johnson 1981; Parlevliet 2002; Kang et al. 2005). En plus du potentiel évolutif du pathogène, ils dépendent également d'autres facteurs comme le type de résistance déployé, les itinéraires techniques utilisés et le déploiement spatio-temporel des variétés (Hossard et al. 2010; Mundt 2014).

1.2.2. Les mécanismes de résistances chez les plantes

Les organismes parasites se caractérisent par une spécialisation extrême à leur ressource trophique, qui implique le développement de relations plus ou moins étroites avec leur hôte (Huyse et al. 2005). Cette interaction suppose que les espèces pathogènes disposent de tout un arsenal d'outils moléculaires, physiologiques ou comportementaux facilitant la reconnaissance, l'infestation et le développement au sein de cet hôte. En réponse, leurs hôtes développent tout un arsenal de défenses visant à empêcher la mise en place de la relation parasitaire ou à en réduire l'impact (Booth et al. 1993). Parmi elles, il y a par exemple le système immunitaire des mammifères, la synthèse de métabolites secondaires chez les plantes ou encore le développement de collaborations entre plusieurs espèces comme celles observées des plantes tropicales et des champignons qui réduisent les niveaux foliaires ou entre des fourmis et des bactéries dont les antibiotiques protègent les cultures fongiques (ex. Minchella 1985 ; Brunham et al. 1993 ; Currie et al. 1999 ; Mejia et al. 2014). Les espèces végétales disposent également de mécanismes de résistance qui s'appuient sur l'immunité innée présente dans chaque cellule (ex. récepteurs transmembranaires Leucin-Rich-Repeat, LRR), spécifiques au pathogène, protéines de résistance, PR, et sur les signaux systémiques (ex. hormones végétales, acide salycilique et jasmonate) émanant des sites infectés (Jones and Dangl 2006). Au niveau moléculaire, l'interaction hôte/pathogène mène donc à la mise en

place de réactions de défenses complexes, qui peuvent être spécifiques ou non à une espèce pathogène donnée, et qui découlent des résistances génétiques dont dispose la plante. Elle peuvent être de deux types, qualitative ou quantitative, et affectent différement la dynamique évolutive des populations de pathogènes.

1.2.2.1. La résistance qualitative et les interactions gène-pour-gène

La résistance qualitative discrimine les plantes-hôtes en deux catégories : celle dite « sensible », pour laquelle l'interaction avec le pathogène est totalement compatible, et celle dite « résistante » pour laquelle l'intéraction avec le pathogène est totalement incompatible. Cette variation en « tout ou rien » repose sur la reconnaissance spécifique d'effecteurs du pathogène par des récepteurs transmembranaires, généralement de types LRR, codés par des gènes particuliers (les gènes d'avirulence (Avr) pour les pathogènes et de résistance (R ou majeure) pour les plantes) qui permettent l'identification du pathogène par la plante (Flor 1971; Salvaudon et al. 2008). Selon ce modèle, il existe un gène Avr pour chaque gène R et l'absence de l'un ou l'autre de ces gènes chez l'un des partenaires de l'interaction hôte-pathogène empêche la reconnaissance du pathogène par la plante et le déclenchement des mécanismes de défense (Figure 3). La nécessité pour le pathogène d'infecter la plante sans être reconnu et celle des plantes-hôtes de reconnaitre les génotypes mutants des pathogènes favorise alors l'accroissement du polymorphisme de ces gènes chez l'hôte et le pathogène (Dodds and Rathjen 2010), ainsi que la coévolution entre les deux partenaires. La dynamique évolutive de cette interaction a été intensément étudiée et formalisée à l'aide des modèles de « course à l'armement » et de sélection fréquence-dépendante (Woolhouse et al. 2002; Gandon et al. 2008; Brown and Tellier 2011).

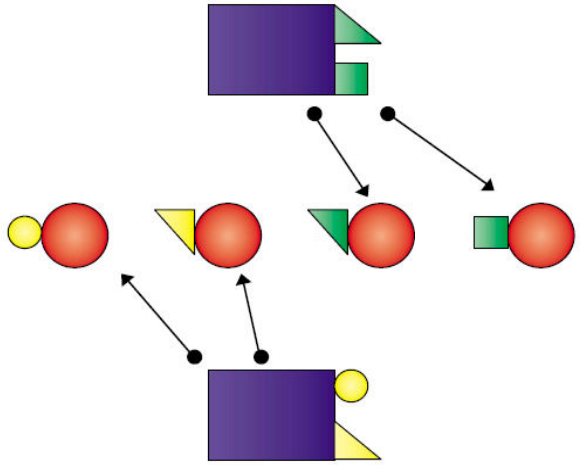


Figure 3 : Illustration du système gène pour gène entre deux hôtes (rectangles bleu) et quatre génotypes de pathogènes (rond rouges). Les triangles et carrés jaunes et verts représentent des récepteurs des hôtes et des effecteurs des pathogènes qui ne peuvent être reconnus que s'ils sont identiques. En cas d'absence des récepteurs et effecteurs compatibles, il n'y a pas d'induction des mécanismes de défense chez la plante hôte. D'après Woolhouse et al. 2002.

Chez la plante, la liaison de l'effecteur au récepteur active tout un ensemble de mécanismes physiologiques, dont la mort cellulaire programmée qui bloque l'avancée du pathogène. Avec ce type de mécanisme, appelé réaction d'hypersensibilité, les dégâts occasionnés à la plante sont minimes ce qui explique le fort intérêt que lui portent toujours les sélectionneurs. Cette résistance correspond à une pression de sélection très forte puisque seulement quelques génotypes de pathogènes sont en mesure de se développer sur les plantes qui les portent. Par conséquent, ces résistances sont généralement très efficaces mais peu durables, comme dans le cas du contournement séquentiel de chacune des variétés résistantes de blé utilisées contre la rouille dans les années 1950-1960 aux Etats-Unis (Browning and Frey 1969). Pour le pathogène, cette stratégie a mené à des cycles de réductions drastiques des effectifs des populations, suivis de la multiplication exponentiennelle des individus virulents. L'étude de ces cycles « boom-and-burst » a montré que les populations de pathogènes conservaient longtemps les gènes de virulence adaptés aux gènes R utilisés dans des cultures précédentes (Figure 4), ce qui peut avoir d'importantes conséquences pour le déploiement futur de variétés contenant un ou plusieurs de ces gènes R (Mundt 2014).

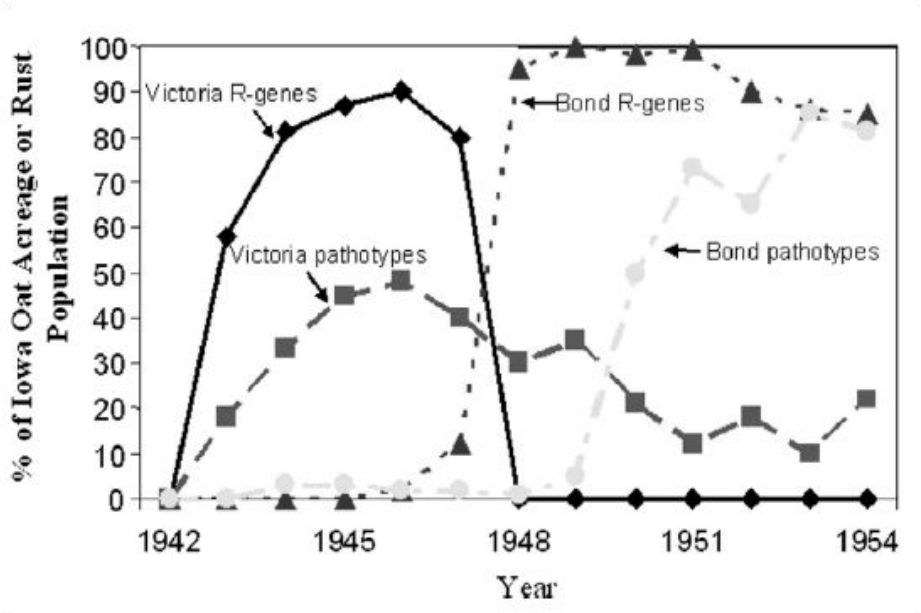


Figure 4 : Exemple de cycle « boom-and-burst » des gènes majeurs de résistances de l'avoine contre un pathogène fongique en Iowa (Etats-Unis). Les courbes correspondent au pourcentage de champ (en superficie) où les variétés Victoria et Bond étaient cultivées entre 1942 et 1954. Le déploiement d'un seul gène de résistance est suivi d'une augmentation plus ou moins rapide de la fréquence des génotypes virulents dans les populations de pathogènes. Néanmoins, malgré l'utilisation quasi-exclusive de la variété Bond à partir de 1948, les gènes de virulence contre le gène de résistance de la variété Victoria est toujours présent dans les populations six ans plus tard. D'après Mundt 2014.

1.2.2.2. La résistance quantitative

En plus des gènes majeurs de résistances, il existe également un autre type de résistance appelée résistance quantitative. Dans ce cas, l'interaction hôte-pathogène discrimine les plantes hôtes en de nombreuses catégories. Ces catégories correspondent à des niveaux de résistance quantitative qui affectent de manière graduelle la compatibilité de l'interaction en fonction des couples individu plante-individu pathogène. Avec ce mécanisme, la plupart des génotypes d'un agent pathogène sont capables d'infecter la plante, mais leur cycle de vie est limité ou ralenti par rapport à celui réalisé sur un hôte non résistant ce qui réduit leur valeur sélective. En conséquence, les résistances quantitatives sont également appelées résistances partielles, par opposition à la résistance qualitative qui est qualifiée de totale. La base moléculaire de cette résistance est moins bien connue. Elle est néanmoins polygénique et implique l'activation de différentes voies physiologiques, comme celle de l'acide salycilique ou de l'acide jasmonique. Les locus impliqués dans la variation sont appelés Quantitative Trait Loci (QTLs) et leur implication dans la résistance phénotypique est variable d'un QTL à l'autre, et même d'une variété à l'autre (Salvaudon et al. 2008; Fournet et al. 2013).

Au niveau phénotypique, l'association de ce type de caractéristiques génétiques avec les phénotypes de résistance n'est pas toujours aussi claire que pour les résistances qualitatives. Ainsi, malgré la nature théoriquement polygénique de ces résistances, certaines interactions présentent des caractéristiques similaires à celles des interactions gène-pour-gène. Par exemple, il existe une certaine spécificité de l'interaction *Linum-Melampsora* (Antonovics et al. 2011), et certains phénotypes de résistance partielle de ce pathosystèmes sont controlés par des gènes uniques (Laine et al. 2011). D'autre part, des associations entre QTLs et gènes majeurs ont été mis en évidence dans des pathosystèmes sauvages, révélant la complexité des mécanismes de résistances chez les plantes (Bevan et al. 1993; Ericson and Burdon 2009).

Ces combinaisons entre QTLs et gènes majeurs se sont également révélées intéressantes pour ralentir le contournement des résistances dans les variétés cultivées (Palloix et al. 2009; Brun et al. 2010). Un certain nombre d'études suggèrent que les résistances partielles sont plus durables en moyenne que les gènes majeurs de résistances (Parlevliet 1988; Delourme et al. 2014). Traditionnellement, cette hypothèse a été associée au plus grand nombre de gènes associés aux mécanismes de résistances, qui sont donc plus longs à contourner, et aux pressions de sélection plus faibles exercées sur les populations de pathogènes. Pourtant, aucun lien n'a encore été clairement établi entre la nature d'une résistance et sa durabilité (Lecoq et al. 2004; Moury and Simon 2011). De plus, des études aux champs et en conditions expérimentales ont montré que les résistances partielles s'érodaient avec le temps jusqu'à la

perte complète de leur efficacité (Villaréal and Lannou 2000; Montarry et al. 2012), un processus qui dépendrait notamment des compromis entre les traits d'histoire de vie, l'adaptation locale et les flux de gènes chez le pathogène (Mundt 2014).

1.2.3. Évaluation du potentiel évolutif d'un pathogène des cultures dans le milieu sauvage

Les agro-écosystèmes se caractérisent par la gestion des peuplements de végétaux par l'homme, qui en optimise l'exploitation pour ses usages. Ces activités ont pour conséquence de modifier la dynamique écologique et évolutive des communautés d'espèces qui y vivent et en particulier celle des agents pathogènes. Tout d'abord, l'apport extérieur de nutriments améliorant la croissance des plantes cultivées dans les champs représente une caractéristique des agro-écosystèmes qui rend ces milieux extrêmement productifs. Dans de tels environnements, les effectifs des populations de plantes cultivées et de leurs agents pathogènes augmentent, le coût associé à certains traits d'histoire de vie, comme la virulence, s'allègent (Lopez-Pascua and Buckling 2008; Loeuille et al. 2013), et les interactions parasitaires sont favorisées par rapport aux interactions mutualistes (Hochberg et al. 2000; Thrall et al. 2007). Ces caractéristiques de l'agro-écosystème rendent cet environnement particulièrement favorable aux agents pathogènes et peuvent faciliter leur adaptation à toute forme de pression de sélection. D'autre part, l'intensification et la simplification agricole ont mené à une perte de diversité spécifique, génétique et technique au sein des parcelles et des bassins de production. Elle se caractérise par l'utilisation d'un faible nombre de variétés (voire d'une seule) de quelques espèces végétales qui sont cultivées de façon identique sur de grandes échelles spatiales, et ceci parfois pendant plusieurs années successives (Loeuille et al. 2013). Dans ce contexte, une variété cultivée représente à la fois une ressource extrèmement abondante et une pression de sélection drastique pour les agents pathogènes, ce qui favorisent une spécialisation à la plante hôte (Loeuille et al. 2013). Enfin, le milieu agricole se caractérise également par l'utilisation de pesticides qui induit de forts taux de mortalité. Cette mortalité accrue tend à favoriser les génotypes ayant des cycles courts, à maturation et à reproduction plus précoces (Loeuille et al. 2013), mais pourrait également augmenter indirectement les densités des pathogènes en accroissant la vulnérabilité des plantes hôtes (Abrams and Matsuda 2005).

Dans l'ensemble, les agro-écosystèmes sont donc des milieux propices au développement de pathogènes spécialistes, dont les tailles de populations sont importantes et sur lesquelles les pressions de sélection sont énormes. L'accroissement de la taille des populations d'hôtes et de

pathogènes augmente leur probabilité de rencontre et diminue les probabilités d'extinction des populations de pathogènes qui pourraient ainsi avoir moins de difficultés à se maintenir localement, contrairement à ce qui est observé dans les pathosystèmes sauvages (Jousimo et al. 2014). Un tel maintien d'inoculum viable dans les parcelles favorise également l'initiation et l'expansion des épidémies (Burdon and Thrall 2008). De façon similaire, l'expansion du milieu agricole accroit les contacts entre l'agrosystème et les communautés naturelles et semi-naturelles qui peuvent représenter de véritables refuges et réservoirs pour les espèces pathogènes capables de se développer sur les espèces qui y vivent. Ces contacts peuvent favoriser les échanges génétiques entre les différentes populations de pathogènes et l'apparition de génotypes capables d'envahir les champs à proximité (Burdon and Thrall 2008). Enfin, la simplification du milieu permet l'utilisation du même matériel agricole d'une parcelle à l'autre et réduit l'hétérogénité spatiale, deux facteurs favorables à la connectivité entre parcelles et donc à la dissemination des pathogènes.

L'agro-écosystème est donc un milieu où les activités humaines intensifient les interactions entre les plantes hôtes et les agents pathogènes et modifient un certain nombre de caractéristiques évolutives des populations de ces derniers. Parmi elles, le cycle de développement, les flux de gènes ou les tailles des populations des pathogènes sont particulièrement affectés. Il est néanmoins impossible de comprendre comment les activités humaines affectent le potentiel évolutif d'un pathogène, en l'absence d'information sur sa dynamique évolutive naturelle, c'est-à-dire, dans des populations qui ne sont pas soumises aux perturbations rencontrées dans les agro-écosystèmes.

D'autre part, les pathogènes présents dans les agro-écosystème sont des organismes issus du milieu sauvage depuis plus ou moins longtemps. Ils peuvent, en effet, avoir suivi leurs hôtes cultivés dès le début du processus de domestication, avoir récemment été mis en contact avec un hôte cultivé qui était absent de leur propre écosystème et/ou avoir récemment connu un « host-jump » leur permettant de se développer avec succès sur des hôtes cultivés (Stukenbrock and McDonald 2008). Par conséquent, pour reconstruire l'histoire évolutive de l'espèce, et pas seulement l'histoire de l'invasion de la culture par le pathogène, il est nécessaire de l'étudier dans des populations se développant sur un hôte sauvage qui doit être choisi soigneusement, en fonction des connaissances concernant le pathosystème.

Face à ces caractéristiques inhérentes aux agro-écosystèmes favorisant le développement et l'adaptation des pathogènes, il est donc évident que la gestion de ces organismes doit être raisonnée dans le temps et l'espace. Estimer le potentiel évolutif de l'espèce à controler, qui est défini par l'histoire et la dynamique actuelle de ses populations, peut améliorer cette

gestion. Nous avons choisit de développer cette approche sur une culture à fort intérêt agronomique, la betterave sucrière, dont deux pathogènes, les nématodes à kystes *Heterodera schachtii* et *Heterodera betae*, parasitent également une apparentée sauvage de la betterave sucière, *Beta vulgaris* ssp. *maritima*.

1.3. Étude de cas : le complexe *Heterodera schachtii*/*H. betae*/*Beta vulgaris* ssp. *maritima*

1.3.1. Les nématodes phytoparasites

Le phylum Nematoda est un embranchement de vers non segmentés constitué actuellement de 26 000 espèces décrites (Hugot et al. 2001). Il présente une étonnante homogénéité morphologique et anatomique malgré la grande diversité des modes de vie qui caractérise les nématodes et leur présence dans tous les écosystèmes aquatiques et terrestres, à l'exception des déserts (Dorris et al. 1999). Ce phylum serait apparu au début du Cambrien vers moins 550 millions d'années (Ayala et al. 1998), probablement sous la forme d'organismes libres qui se seraient ensuite mis à utiliser de nouvelles ressources, comme des hôtes animaux ou végétaux apparus plus tardivement (Wang et al. 1999; Sanderson 2003). L'apparition du parasitisme reste néanmoins difficile à dater, car il existe peu de fossiles de nématodes. Chez les animaux, il existe des traces de nématodes parasites dans les fèces fossilisés de dinosaures datant de 240 millions d'années (Poinar and Boucot 2006; Hugot et al. 2014) ainsi que des spécimens de nématodes entomopathogènes piégés dans de l'ambre datant d'au moins 140 millions d'année (Poinar 2003). Chez les plantes, ce type de fossiles n'a pas encore été découvert, mais la distribution mondiale de la sous-famille des *Heteroderinae* laisse supposer que les ancêtres de ce groupe étaient déjà présents à l'époque de la Pangée, c'est-à-dire il y a 185 millions d'années (Ferris 1979; Veevers 2004). De nos jours, le parasitisme est un mode de vie plutôt répandu chez les nématodes, puisqu'il est présent dans les cinq clades composant le phylum *Nematoda* (Figure 5). Par ailleurs, une majorité des espèces animales et végétales a généralement une, voire plusieurs, espèces de nématodes parasites qui leur sont associées (Gilabert and Wasmuth 2013). Dans le cas des nématodes phytoparasites, le parasitisme est apparu de façon indépendante au moins à trois reprises, dans les ordres des *Triplonchida*, *Dorylaimida* et *Tylenchomorpha*, au cours de l'évolution (Baldwin et al. 2004). Ces organismes sont généralement invisibles à l'œil nu, et telluriques, bien que certains d'entre eux soient des parasites de tiges et de feuilles (ex. *Aphelenchoides* spp.). Tous se caractérisent par la présence d'un stylet, qui est une aiguille creuse et protractile située dans la partie

antérieure du nématode. Ce stylet leur permet de piquer les cellules végétales de leur hôte et de développer des structures spécifiques appelées sites nourriciers, qui leur permettent de se développer (Figure 6).

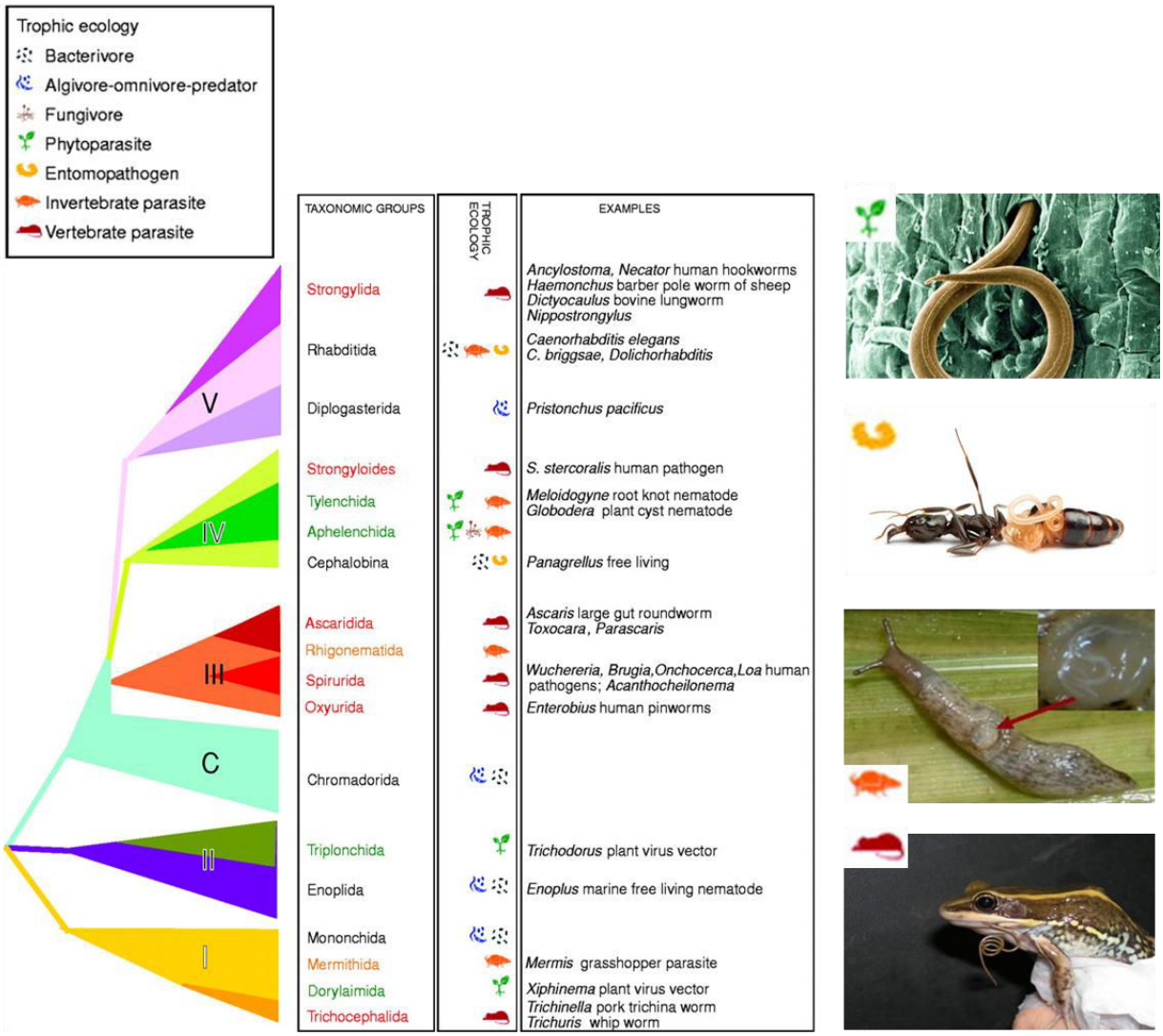


Figure 5 : Représentation de l'arbre phylogénétique du phylum *Nematoda* représentant les cinq clades numérotés de I à V et le groupe paraphylétique *Chromadorida* (C) basé sur a séquence de la petite sous-unitéde l'ADN ribosomal. Les symboles correspondent à l'écologie trophique de chaque groupe pour lesquels des exemples d'espèces sont donnés. Photos de haut en bas : *Meloidogyne incognita*, *Spinochordodes tellinii*, *Phasmarhabditis hermaphrodita*, *Paragordius tricuspidatus*. D'après Dorris et al. 1999 et Holterman et al. 2006.

La production agricole est particulièrement touchée par les espèces endoparasites sédentaires des racines. Il s'agit d'espèces qui restent fixées à la racine durant tout ou une majorité de leur cycle de développement, notamment les familles des *Meloidogynidae* et des *Heteroderinae*. Ces deux groupes ne partagent pas d'ancêtre commun récent, et toutes les similitudes dans leurs morphologies et leurs modes de vie ne sont que le résultat de phénomènes de convergence évolutive (Holterman et al. 2006). Sur les parties aériennes des

plantes, les attaques de ces nématodes se traduisent par des symptômes rarement spécifiques des espèces impliquées : les plantes se décolorent, jaunissent, se flétrissent ou ont une croissance plus lente (Oka et al. 2000). Ces plantes sont réparties en foyers qui forment des tâches au milieu des champs, qui pourraient être causées par un tout autre problème parasitaire ou agronomique. En revanche, certains symptômes, comme une forte ramification du système radiculaire ou l'observation de structures particulières développées par les nématodes sur les racines, peuvent permettre d'établir un diagnostic plus précis. Dans l'ensemble, les dégâts sont de types quantitatifs, avec une diminution du rendement des parcelles infestées, et/ou qualitatif, impliquant une baisse de la qualité du produit récolté qui peut les rendre impropres à la commercialisation.

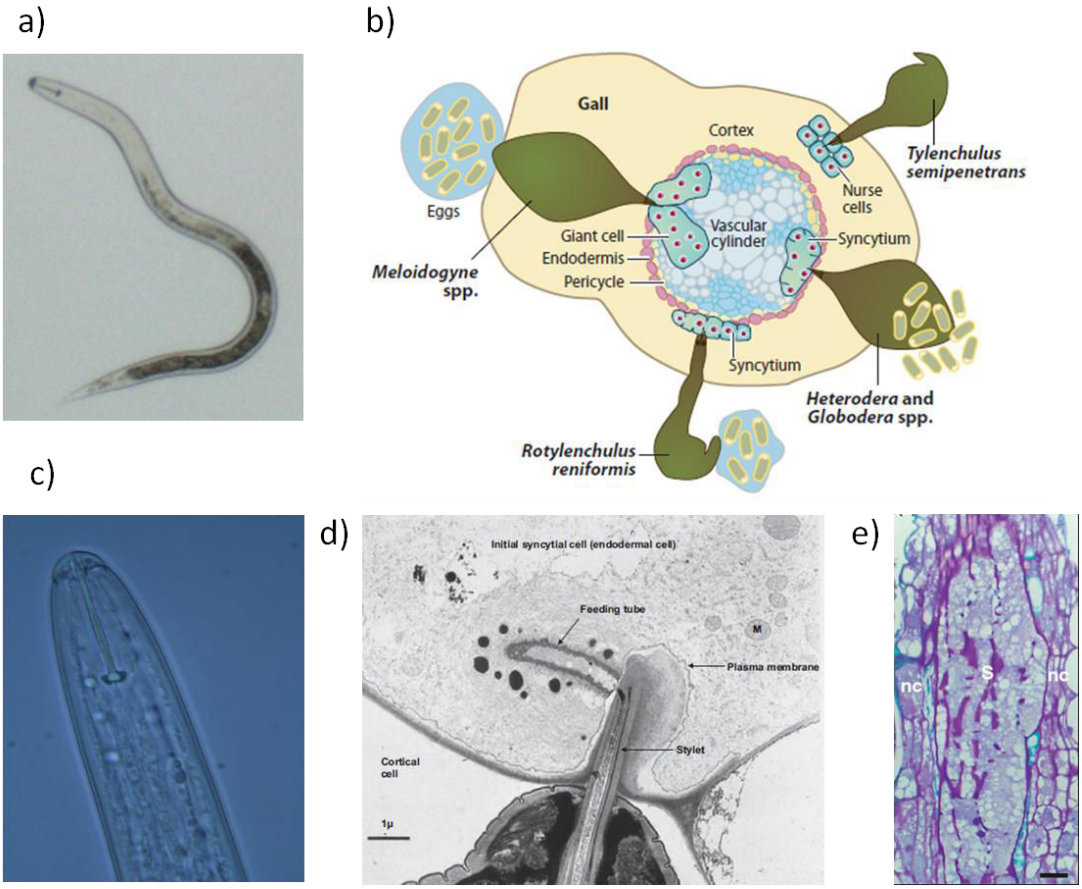


Figure 6 : Caractéristiques des nématodes phytoparasites. a. Larve d'*Heterodera schachtii*. b. Diversité des sites nourriciers développés par les espèces phytoparasites (d'après Mitchum et al. 2012). c. Stylet d'*H. schachtii*. d. Coupe transversale de la pénétration du stylet de *Rotylenchulus reniformis* dans une cellule de coton. La membrane plasmic s'invagine autour du stylet et un tube nourricier est formé dans le cytoplasme juste à côté de l'orifice du stylet pour permettre l'absorption des nutriments par le nématode (d'après Mitchum et al. 2013). e. Coupe transversale d'un syncitium de nématode à kyste (s : syncytium ; nc : neighboring cell ; d'après Gheysen and Mitchum 2011).

1.3.2. Biologie des nématodes à kystes (Heteroderinae)

Cette famille est constituée de 18 genres dont six correspondent à des nématodes à kystes dont le genre *Heterodera*, qui contient près d'une soixantaine d'espèces décrites (Subbotin et al. 2001). Cette famille de nématode se caractérise par la formation d'un stade de résistance, le kyste, qui correspond au durcissement de la cuticule du corps de la femelle après sa mort et un fort dimorphisme sexuel : les mâles sont filiformes et invisibles à l'œil nu et les femelles sont citriformes, blanches et d'une taille d'environ un demi-millimètre (Subbotin et al. 2010).

Le cycle biologique des nématodes à kystes est globalement composé de quatre stades larvaires et d'un stade adulte tous séparés par une mue (Figure 7). L'intégralité du cycle biologique se déroule dans la plante et les kystes contiennent le second stade larvaire. L'éclosion de ces larves est favorisée par la présence d'exsudats racinaires libérés dans le sol par les plantes hôtes qui les guident ensuite vers les zones d'élongation racinaire (Blok et al. 2008). La larve perce alors la paroi végétale avec son stylet et progresse à l'intérieur de la racine jusqu'au voisinage du système vasculaire de son hôte où il initie la formation d'un site nourricier, appelé syncytium, qui correspond à la fusion de plusieurs cellules végétales (Figure 6 ; Gheysen and Mitchum 2011). Ces syncytiums fonctionnent comme de véritables puits métaboliques qui détournent les ressources de la plante au profit du nématode. Celui-ci effectue ses mues successives, jusqu'au stade adulte. Les mâles quittent alors la racine pour pouvoir localiser les femelles et se reproduire, tandis que les femelles grossissent jusqu'à faire éclater l'épiderme des racines. Après reproduction, les œufs se forment à l'intérieur de la femelle, et y poursuivent leur développement jusqu'au second stade larvaire. La femelle meurt et sa cuticule durcit et brunit, ce qui forme les kystes. Ces structures peuvent conserver les larves enfermées pendant plusieurs années en cas d'absence de plante hôte. La durée du cycle est influencée par la température et certaines espèces comme *H. schachtii* ou *H. cruciferae* peuvent effectuer plusieurs générations par an (Subbotin et al. 2010).

1.3.2.1. *Heterodera schachtii*

Heterodera schachtii est une espèce sexuée et diploïde qui parasite la betterave sucrière en causant d'importants dégâts depuis le XIXème siècle. Ce nématode peut se développer sur une gamme d'hôte particulièrement étendue qui comprend de nombreuses espèces de différentes familles de plantes (*Brassicaceae*, *Chenopodiaceae*, *Amaranthaceae*, *Polygonaceae,* etc.) cultivées ou non (Subbotin et al. 2010). La présence de cette espèce a été observée en Amérique du nord, en Asie et autour du bassin méditerranéen ainsi que dans toutes les zones betteravières d'Europe, du sud de l'Espagne aux pays scandinaves. En France, les cultures de

betteraves sucrières sont particulièrement infestées autour des sucreries. Par exemple, en fonction des régions, il y 10 à 35% des surfaces de betteraves qui étaient contaminées en 2010. La distribution de ce nématode dans les écosystèmes sauvages n'est pas connue.

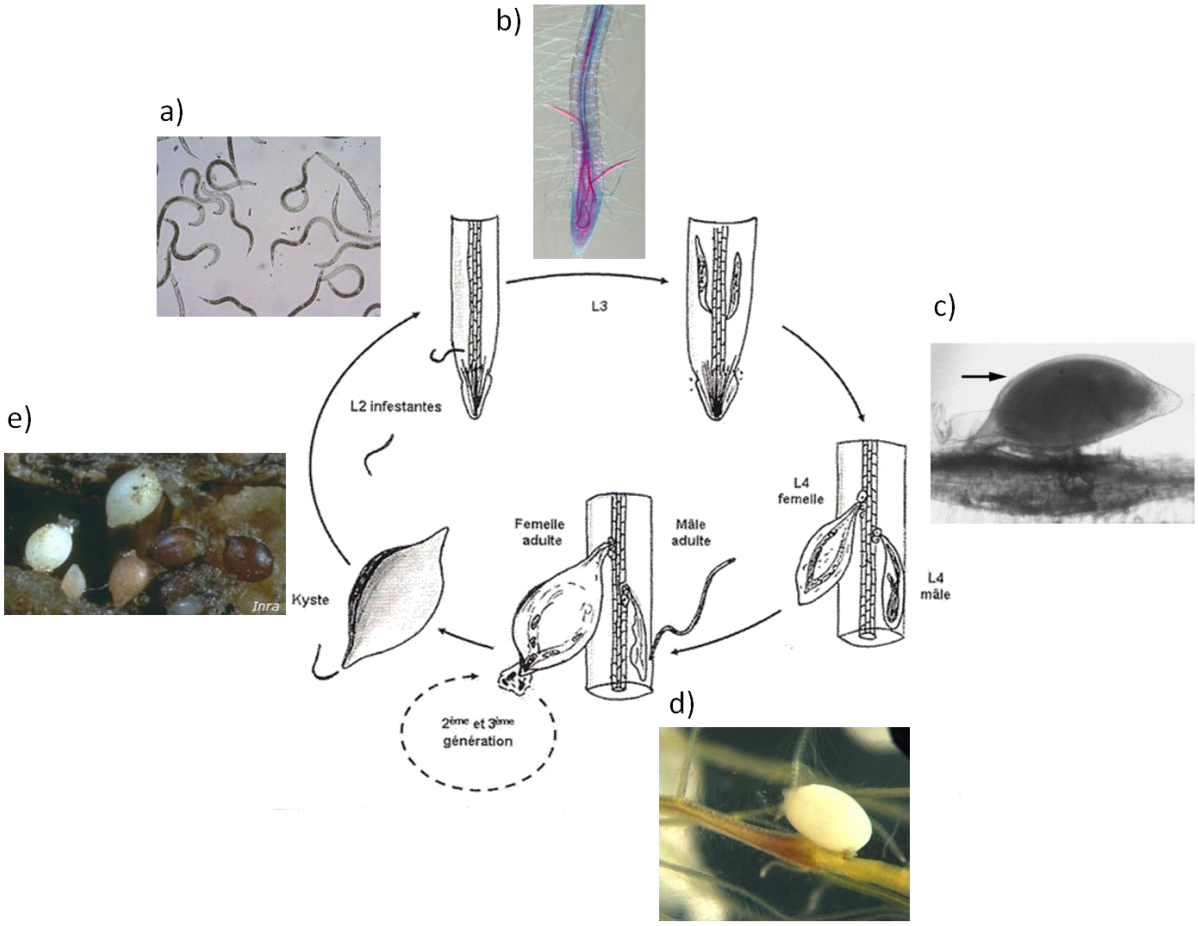


Figure 7 : Cycle de développement d'*Heterodera schachtii*. a. Larves au deuxième stade de développement (L2). b. Après l'invasion de la racine, les larves se dirigent vers la stèle de la racine (d'après Gheysen and Mitchum 2011). c. Femelle qui se remplit d'œufs (d'après Jung et al. 1998). d. Jeune femelles blanche. e. Kystes à différents niveaux de développement (les plus âgés sont les plus sombres).

Les kystes de *H. schachtii* peuvent contenir jusqu'à 600 œufs et les larves peuvent rester viables au moins pendant six ans en conditions défavorables. Comme pour tous les *Heteroderinae*, l'éclosion des larves est favorisée par les exsudats racinaires, mais cette stimulation est moins forte que pour d'autres *Heterodera* car des conditions favorables de température sont suffisantes pour provoquer une éclosion substantielle. Néanmoins, le temps d'éclosion d'un cycle à l'autre reste extrêmement variable (Zheng and Ferris 1991). Son cycle de développement peut donc commencer à tout moment de l'année, dès qu'il ne fait plus suffisamment froid pour ralentir ou arrêter le développement. En absence d'hôte, les larves

survivent seulement quelques jours dans le sol, ce qui peut fortement réduire les effectifs des populations. Au champ, deux à cinq générations sont possibles sur une même culture de betterave (Subbotin et al. 2010). Lors de forte infestation de ces cultures, les pertes de rendements atteignent 30 à 60% de la moyenne régionale.

1.3.2.2. *Heterodera betae*

Heterodera betae est une espèce qui a été découverte dans les champs de betterave sucrière du sud des Pays-Bas (Maas et al. 1982). Ces auteurs ont d'abord pensé qu'ils venaient de découvrir un nouveau biotope du nématode *H. trifolii* pour qui la betterave est un hôte particulièrement inhabituel pour cette espèce. Le cycle de développement et la gamme d'hôte d'*H. betae* sont fortement similaires à ceux de *H. schachtii* (Maas et al. 1982; Maas and Heijbroek 1982; Subbotin et al. 2010). Pourtant, ce n'est qu'en 2001 que Wouts et al. décrivent *H. betae* comme une espèce distincte d'*H. trifolii*. Cette espèce ne pose de réels problèmes agronomiques qu'en de rares circonstances. Par conséquent, la biologie et la dynamique évolutive de cette espèce ont été moins étudiées que celles d'*H. schachtii*.

Cette espèce est parthénogénétique et tétraploide, et aucun mâle ne semble avoir été observé à ce jour (Steele and Whitehand 1984). Hormis cette dernière particularité, le développement et la morphologie de cette espèce sont extrêmement similaires à ceux d'*H. schachtii*. Ces observations ont mené à l'hypothèse qu'*H. betae* pouvait avoir récemment évolué à partir d'*H. schachtii* ou d'*H. glycines* qui possèdent toutes 9 chromosomes, c'esy à dire, deux à quatre fois moins que *H. betae* (Mulvey 1957, 1958, 1959; Steele and Whitehand 1984). Depuis sa découverte initiale, cette espèce a été observée principalement dans les cultures européennes de betteraves, en France, en Allemagne, en Italie, aux Pays-Bas, en Suède et en Suisse (Subbotin et al. 2010). Comme pour *H. schachtii*, sa distribution dans les écosystèmes sauvages n'est pas connue.

1.3.3. Contrôle des nématodes à kystes de la betterave

La lutte chimique, notamment l'utilisation de fumigants, de carbamates et d'organophosphorés, a longtemps été la méthode la plus utilisée contre les nématodes à kystes. Pourtant, plusieurs de ces molécules actives, dont les plus efficaces, se sont révélées hautement nocives pour la santé humaine et l'environnement, si bien que leur utilisation est aujourd'hui interdite (Thurau et al. 2010). Parmi les stratégies de gestions alternatives qui ont été développées, la lutte culturale préconise l'allongement des rotations des cultures en fonction des sols et de la densité des populations de nématodes. Cette méthode semble prometteuse, mais reste difficile à mettre en pratique, car un grand nombre de plantes pouvant

être cultivées à la place de la betterave sucrière sont également des hôtes d'*H. schachtii*. De la même façon, les méthodes de luttes physiques comme l'inondation des sols, qui asphyxie les nématodes, ou la solarisation, qui augmente la température de surface du sol, sont aléatoires et souvent difficiles à utiliser d'une façon économiquement viable (Thurau et al. 2010). Les essais de contrôle biologique d'*H. schachtii*, notamment par l'utilisation d'un champignon parasite du nématode, s'est révélée inefficace (Meyer and Roberts 2002). L'utilisation de variétés résistantes de plantes hôtes constitue donc, dans ce système, la meilleure alternative aux nématicides.

Les résistances génétiques contre les nématodes se sont déjà révélées plus durables que celles utilisées contre les champignons ou les bactéries pathogènes (Castagnone-Sereno 2002; Molinari 2011). En effet, plusieurs espèces ne réalisent que peu de générations par an et leur petite taille ne leur confère qu'une capacité de dispersion limitée (Wallace 1968). Par exemple, le gène H1 chez la pomme de terre a été utilisé pendant 30 ans contre le nématode à kyste, *Globodera rostochiensis*, et le gène Mi-1 a, quant à lui, contrôlé pendant plus de 20 ans les nématodes à galles de la tomate avant l'apparition de quelques individu virulents en Californie (Kaloshian et al. 1996; Fleming 1998). Néanmoins, quel que soit le pathogène, aucune source de résistance n'est durable et des cas de contournement de gène majeur ont déjà été documentés pour différentes espèces de nématodes à kystes (ex. Lange et al. 1993; Lasserre et al. 1996; Dong and Opperman 1997, Fournet et al. 2013), justifiant la nécessité de développer une gestion durable des résistances génétiques.

Dans le cas des nématodes, l'efficacité d'une variété résistante se définit par son aptitude à inhiber la formation de femelles (Trudgill 1991). Plusieurs mécanismes permettent d'atteindre cet objectif : (i) le retard ou la réduction de l'éclosion des kystes par les exsudats racinaires ; (ii) une réaction d'hypersensibilité après la mise en place du site nourricier (syncytium) qui perturbe ou bloque le développement du nématode ; et (iii) la masculinisation des juvéniles qui peut être associé à un mauvais fonctionnement du site nourricier ou à la mauvaise qualité de la plante hôte (Grundler et al. 1997; Sobczak et al. 2005; Thurau et al. 2010). Chez *H. schachtii*, les mécanismes de contrôle mis en jeu dépendent de la nature de la résistance, qualitative ou quantitative, qui limite plus ou moins efficacement le développement des femelles (Figure 8). Néanmoins, les mécanismes favorisant un arrêt de développement précoce, comme la déstabilisation du site nourricier, ont, jusqu'à présent, été ceux garantissant les résistances les plus durables (Castagnone-Sereno et al. 1996; Mugniéry et al. 2007).

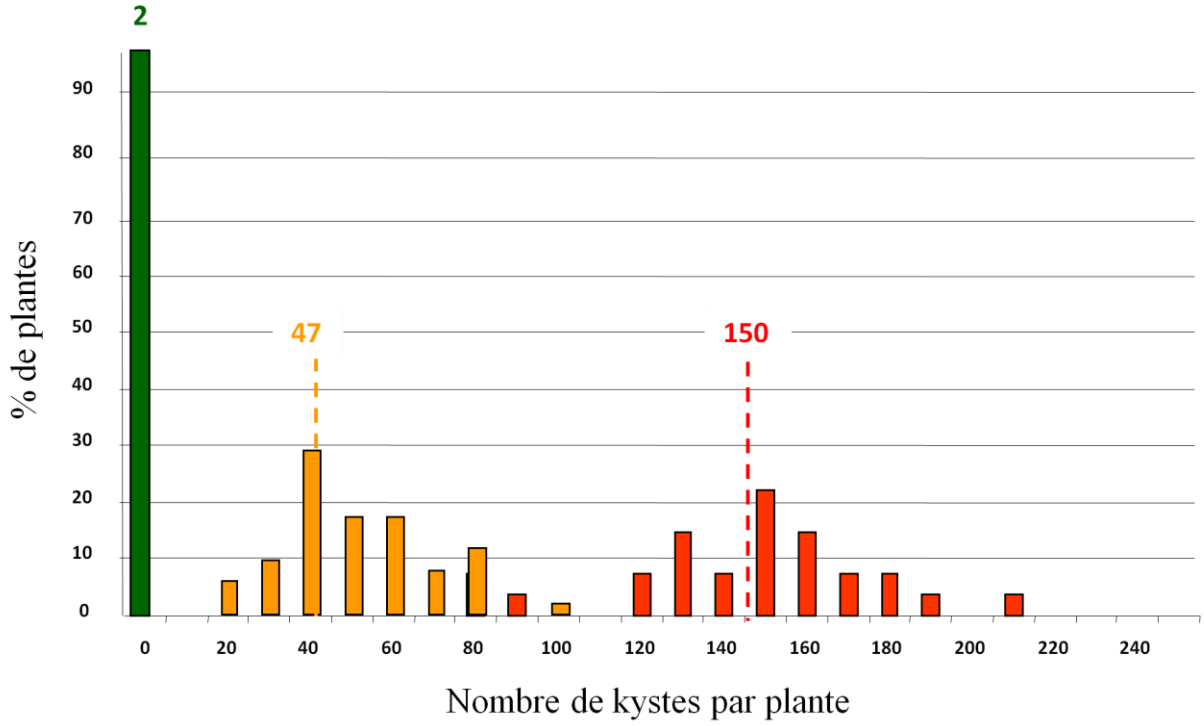


Figure 8 : Nombre de femelles (kystes) d'*Heterodera schachtii* par plant de betterave sucrière en fonction de la nature de la résistance de la plante. Rouge : variété sensible, sans résistance génétique ; Orange : variété possédant une résistance partielle (quantitative) ; Vert : variété contenant un gène majeur de résistance (qualitative). Les nombres au-dessus des barres correspondent à la moyenne du nombre de kystes obtenus par variété.

1.3.4. Betteraves sucrière et maritime

1.3.4.1. La betterave sucrière

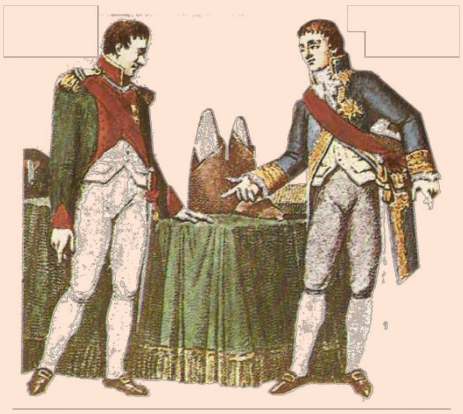
La plupart des informations connues sur *H. schachtii* et *H. betae* sont issues de l'étude de populations de champs de betterave sucrière (*Beta vulgaris* ssp. *vulgaris*) au sein desquelles ils causent le plus de dégâts. La betterave sucrière est une plante bisannuelle appartenant à la famille des *Amaranthaceae*, cultivée depuis le XVIIIème siècle pour la production industrielle de sucre (Encadré 4). Le sucre de betterave constitue, en effet, près de 20% de la production mondiale actuelle de sucre. En France, premier producteur mondial de sucre de betterave, cette culture se déroule généralement sur une année et représente près de 370 000 hectares de terres (http://www.labetterave.com/).

La sélection des variétés de betteraves a été initiée en Allemagne en 1786 par François-Charles Achard, qui ne considérait alors que des caractères morphologiques comme la taille des tubercules, la présence d'un sillon saccharifère et un enterrage normal (Bordet 1997). Puis, le processus de sélection fut ensuite amélioré avec l'avancée des connaissances en génétique moléculaire et sur la biologie de l'espèce, permettant d'augmenter significativement

Encadré 4 : Le sucre, la betterave et un peu d'histoire

1575 : Olivier de Serres décrit le jus sucré que produit la betterave lors de sa cuisson.

1789 : A la veille de la Révolution, la France est à la tête du commerce et du raffinage de sucre de canne, le seul sucre qui est cristallisable à l'époque.



1806 : Suite à la défaite de Trafalgar, Napoléon 1er institue le blocus continental de l'Angleterre, qui coupe la route des Antilles et ouvre deux fabriques de sucre de betterave à Chelles et à Saint-Ouen (Ile-de-France). Il continuera à à développer la filière de production de sucre de betterave jusqu'à la chute de l'Empire en 1814.

Figure 9 : Le ministre de l'Intérieur présente deux pains de sucre de betterave à l'Empereur. *Gravure de David*

1815-1845 : Retour du sucre de canne en France et effondrement des cours du sucre de betterave. Par la suite, différentes politiques fiscales sur les sucres coloniaux et betteraviers mènent à la fermeture d'un grand nombre de sucreries.

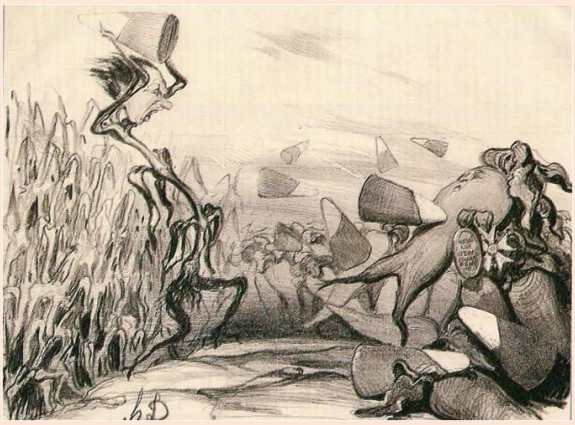


Figure 10: Tensions entre les sucres de canne et de betterave. *Gravure de Doumier est paru en 1839 dans Le Charivari n°263.*

1848 : L'abolition de l'esclavage mène à une forte augmentation du prix du sucre de canne.

1860 : Rétablissement de l'égalité des droits entre les sucres de canne et de betterave.

1870 : La France devient le premier producteur européen de sucre de betterave.

1890-1912 : Augmentation de la production de sucre, dont 60% est issu de la betterave sucrière. Pour écouler la production, une véritable campagne d'information sur les bienfaits du sucre est lancée pour augmenter la consommation des français qui perdurera jusqu'à la fin du XXème siècle.

Figure 11: Buvard plubicitaire de la campagne 1956-1957.

1914-1918 : Première guerre Mondiale : les régions betteravières françaises les plus importantes sont situées dans les zones de combats. La production chute fortement et est réquisitionnée pour l'effort de guerre.

1940 : Durant la Seconde Guerre mondiale, le sucre est à nouveau rationné pendant les combats.

1950-1975 : Développement des variétés polyploïdes et monogerme de betteraves sucrières et de la mécanisation de la culture.

1990-2000 : Développement des carburants à base d'éthanol tiré de la betterave sucrière.

les rendements. Aujourd'hui, un mètre carré de betterave suffit pour produire une boite d'un kilo de sucre (Bordet 1997). L'amélioration variétale a également concerné la gestion des espèces pathogènes. Plusieurs programmes de criblage des espèces des quatre sections du genre Beta (Beta, Nanae, Corollinae et Procumbentes) ont ainsi été mis en place pour rechercher des gènes de résistance ou de tolérance à différentes maladies comme la rhizomanie, associée à la présence du virus BNYVV (*Beet necrotic yellow vein* virus) ou encore les nématodes à kystes (Zhang et al. 2008).

Dans le cas des nématodes à kystes, au moins trois gènes de résistance différents ont été trouvés dans la section *Procumbentes*, chez les espèces *Beta procumbens*, *B. webbiana* et *B. patellaris*. Le gène *Hs1* est situé sur les chromosomes homologues 1 des trois espèces, *Hs2* est présent sur les chromosomes homologues 7 de *Beta procumbens* et de *Beta webbiana* et *Hs3* est sur le chromosome 8 de *Beta webbiana* (Lange et al. 1993; Mesbah et al. 1997; Thurau et al. 2010). Bien que le déterminisme moléculaire de ces gènes de résistance soit toujours à définir, le contournement de *Hs1* mais pas de *Hs2* par un pathotype virulent d'*H. schachtii* suggère leur implication dans différents mécanismes de résistance (Lange et al. 1993). Ces gènes ont par la suite été introduits dans des variétés de betterave sucrière par hybridation et croisement successifs, et permettent un contrôle efficace des densités de populations d'*H. schachtii* (Werner et al. 1995; Dewar 2005), mais pas des populations d'*H. betae* (Maas and Heijbroek 1982). Les rendements de ces variétés restent cependant inférieurs à ceux obtenus avec des variétés sensibles, ce qui restreint fortement leur utilisation au champ.

Des résistances partielles, ou quantitatives, ont également été identifiées chez l'apparentée sauvage de la betterave sucrière, *Beta vulgaris* ssp. *maritima*. Ces résistances n'empêchent pas l'infection de la plante par les nématodes, mais diminue le nombre de femelles formées (Thurau et al. 2010). De nos jours, la plupart des variétés commercialisées portent ces résistances partielles, et sont celles qui sont les plus souvent utilisées en présence de

nématodes à kystes. C'est pourquoi, dans le cadre de l'estimation du potentiel évolutif des populations sauvages d'*H. schachtii* et d'*H. betae*, nous avons choisi de travailler sur la betterave maritime, qui est l'hôte sauvage à la source de ces résistances génétiques et un hôte sur lequel les deux nématodes se développent(Maas and Heijbroek 1982).

1.3.4.2. La betterave maritime

Beta vulgaris ssp. *maritima* est une sous-espèce sauvage de betterave inféodée aux littoraux du pourtour méditerranéen, aux côtes atlantique de l'Europe et de quelques îles plus éloignées comme les Açores (Letschert 1993). Cette espèce peut également ponctuellement coloniser l'intérieur des terres dans les régions les plus méridionales de sa distribution, où elle peut apparaitre dans des sites rudéraux tels que des bords de route, des fossés ou des friches. Dans certains cas, comme dans le sud-ouest de la France, elle est alors en contact avec les zones de production de semences de betteraves cultivées, avec lesquelles elle est totalement interfertile et peut générer des hybrides (Dijk et al. 2004; Fénart et al. 2008; Arnaud et al. 2009; Arnaud et al. 2011).

La betterave maritime est une espèce pérenne et itéropare, dont la durée de vie peut varier entre deux et dix ans en fonction de la stabilité des conditions environnementales (Hautekèete et al. 2002). De la même façon, l'âge de la première floraison peut survenir dès la première année au sud de son aire de répartition, alors qu'au nord, la première floraison n'a généralement lieu que lors de la deuxième année. Ce polymorphisme est associé à un besoin de vernalisation déterminé par un gène majeur, nommé *B* (pour « bolting »), dont les allèles retardent ou non l'induction de la floraison (Boudry et al. 1994). Cette plante est également anémophile et auto-incompatible (Owen 1942). Ses fleurs sont groupées par paquets de deux à huit le long des axes floraux, fusionnent au sein de ces paquets pour former un fruit sec et non charnu, le glomérule, qui contient un nombre de graines égal au nombre de fleurs initialement présentes (Figure 12). Les graines produites peuvent être dormantes et constituer des banques de graines pérennes dans les populations naturelles ou dans les cultures de betteraves sucrières (Arnaud et al. 2010).

Les populations naturelles de *Beta vulgaris* ssp. *maritima* peuvent être gynodioïques, et contenir à la fois des individus femelles et des individus fonctionnellement hermaphrodites, notamment le long des côtes françaises (Biancardi et al. 2012). Le sexe des individus est déterminé par une interaction entre des gènes cytoplasmiques de stérilité mâle et des allèles nucléaires de restauration de la fonction mâle (Cuguen et al. 1994; Fénart et al. 2006). Il existe quatre principaux gènes cytoplasmiques de stérilité mâle qui peuvent induire un

génotype femelle, dont la fréquence, ainsi que celle de leur restauration, varient d'une population à l'autre (Dufay et al. 2009). La valeur sélective des différents génotypes résultant de ces interactions (femelles, hermaphrodites restaurés et hermaphrodites normaux) n'est pas équivalente, ce qui influence le sex-ratio local, et donc la structure des populations naturelles de betteraves maritimes (De Cauwer et al. 2010; De Cauwer et al. 2012).

Figure 12 : Morphologie de *Beta vulgaris* ssp. *maritima*. a. Individu adulte. b. Fleur d'un individu femelle dont les étamines sont réduites et vides. c. Fleur d'un individu hermaphrodite avec des étamines jaunes et remplies de pollen. d. Grains de pollen après coloration alexander, distinguant les grains viables (violet) et non viables (vert). e. Glomérules (fruit). f. Plantule en population naturelle. D'après De Cauwer 2010.

1.4. Plan de la thèse

L'objectif de ce travail de thèse est d'évaluer le potentiel évolutif de deux nématodes à kystes de la betterave sucrière, à travers l'étude de la structure génétique locale et de l'histoire biogéographique de leurs populations. Les résultats attendus doivent apporter des informations utiles au développement de stratégies d'utilisation durable des résistances

génétiques utilisées pour les contrôler. Ce travail a été entièrement réalisé sur des populations de nématodes se développant sur des plantes hôtes sauvages de *Beta vulgaris* ssp. *maritima*. Il s'est scindé en deux axes principaux :

(1) Le premier axe se concentre sur la reconstruction et la compréhension de l'histoire évolutive des populations d'*H. schachtii* et d'*H. betae* sur la betterave maritime. Pour réaliser ce travail, il a tout d'abord été nécessaire de localiser et caractériser écologiquement et génétiquement les populations de ces deux pathogènes dont la distribution dans les écosystèmes terrestres n'était que peu documentée. Pour cela, nous nous sommes appuyés sur un échantillonnage hiérarchique de la côte atlantique européenne s'étendant du Détroit de Gibraltar en Espagne jusqu'en Suède. Le génotypage des populations de betterave maritime et de nématodes nous ont ensuite permis d'examiner les patrons de recolonisation post-glaciaires respectifs des trois espèces.

(2) Dans un second temps, nous nous sommes attachés à décrire la structure génétique des populations d'*H. schachtii* à une échelle plus locale, en nous concentrant plus particulièrement sur la double influence de l'action de la dérive génétique et des flux géniques. Ce travail a été réalisé à l'aide d'un échantillonnage temporel réalisé sur deux années consécutives et permet de mieux comprendre la dynamique locale du pathogène en situation naturelle. Il apporte également des éléments de compréhension sur l'influence des activités humaines sur la structure génétique des populations de nématodes localisés au sein des agro-écosystèmes.

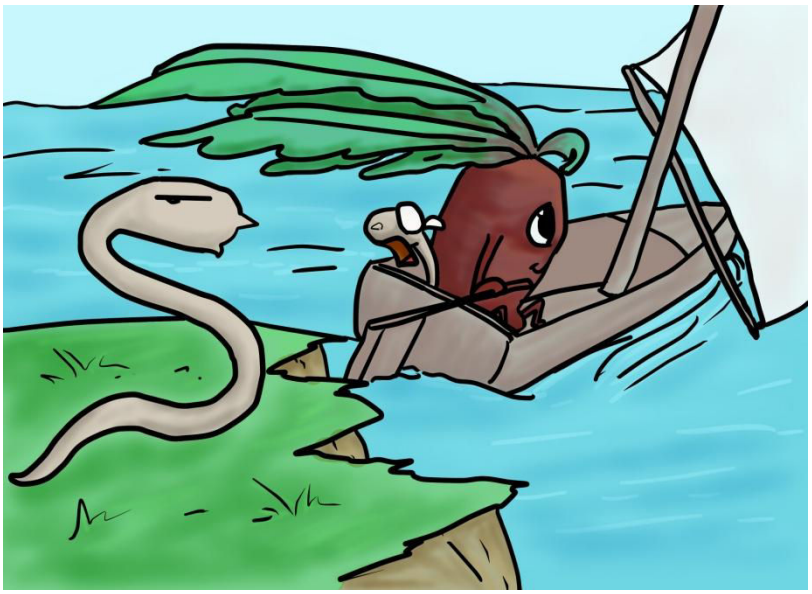
2. Geographical occurrence and patterns of genetic diversity of two cyst beet nematodes and its associated host-plant, the sea beet

2.1. Characterization of the geographical distribution of nematode populations in the wild

2.2. Spatial distribution and basic ecology of *Heterodera schachtii* and *H. betae* wild populations developing on sea beet, *Beta vulgaris* ssp. *maritima*

2.3. Comparative phylogeography of host/parasite wild populations: wild beets and cyst nematodes as a case study

2.4. What is new and what to do next



2.1. Characterization of the geographical distribution of nematode populations in the wild

2.1.1. A comprehensive sampling on the putative species distribution area as a prerequisite

The first step was to describe the large-scale geographical distribution of nematodes and host-plant species along with their patterns of spatial genetic structure. This survey may help to localize the species of interest in poorly investigated habitats and to define the extent of their geographical distribution range. This information may be particularly useful for the monitoring of endangered species or of economically important species like crop pathogens. Additionally, precise knowledge of a species' phylogeography may help to contextualize results performed on fine spatial scales where micro-evolutionary processes are likely to dominate.

Population genetic structure is mainly shaped by the interaction of two fundamental micro-evolutionary forces over space and time: genetic drift and gene flow. Dispersal events implying effective gene flow are generally not random in space and can have important consequences in the dynamics of genes, in shaping population demography and in determining the spatial and temporal scale at which populations can respond to selection. Genetic drift is another evolutionary force with antagonistic influence to gene flow. The equilibrium between gene flow and genetic drift is thus a central process in shaping the spatial distribution of genetic diversity within and among populations, in determining species cohesion and how they can extend their range. Interestingly, populations are often composed of distinct breeding units that can display hierarchical genetic patchiness due to spatially restricted gene flow. Gene flow and genetic drift thus govern the set-up of fine-scale genetic structure and the size of local genetic neighborhoods within which selective processes will take place. However, present-day species diversity and geographical distribution are also the products of past historical and ecological factors occurring over large periods of time. Beyond studying local patterns of genetic structure, understanding the underlying mechanisms of range shifts and characterizing large-scale patterns of historical gene flow are therefore key elements to understand species cohesion and evolutionary potential of their populations. The scale of investigation is then inherent to specific questions on evolutionary trajectories of populations/species.

In this respect, co-evolutionary and co-adaptive interactions among host and pathogen species are long-lasting evolutionary processes which take place over the lifespan of host and

pathogen populations. To detect such evolutionary processes, it is necessary to go back enough in time and a continental study sounds as a suitable scale to investigate co-adaptive interactions. However, the possibility to detect the footprint of past events in a given species also depends on tools and approaches. For example, genetic markers can trace back past events depending on their evolution rate. Fast-evolving markers, such as neutral DNA microsatellites, cannot apprehend geological processes. In contrast, using a molecular clock, mitochondrial DNA can be a high-resolution tool for the investigation of prehistoric demographic events, such as founder effects and population expansions. In the same way, the generation time is important because it also impacts how far a molecular marker can go back in time to trace genetic signatures of past historical events. For example, microsatellites of a perennial species such as trees should be able to detect older events than in annual plants because trees produce less generations on the same period of time.

In the case of sea beet populations, previous work showed that microsatellite markers were enough informative to describe past evolutionary processes occurring since the last Glacial Maximum, suggesting these markers are suitable for the investigation of the history of this plant (Leys et al. 2014). However, for nematode species such information was not available. Because parasites are expected to evolve faster than their hosts (Huyse et al. 2005; Nieberding and Olivieri 2007), we decided to used both microsatellites loci and mitochondrial sequences to increase our chances to detect signature of biogeographic factors. Moreover, all the work presented in this section was based on a hierarchical sampling performed along the European Atlantic coastline, from the North of Morocco to the strait of Kattegat in Sweden. This sampling design, composed of several nested scales, may be particularly relevant to give *a posteriori* information on the relevant geographical scale to consider in the study of evolutionary processes occurring in *Heterodera schachtii* and *H. betae*.

2.1.2. Sampling wild nematodes populations is a hard task

As for many crop pathogens, almost nothing was known on the occurrence of *H. schachtii* and *H. betae* in natural ecosystems at the beginning of the project. Indeed, wild populations of cyst nematodes, and *Heterodera* species in particular, were only described in coastal foredunes and grasslands (van der Putten et al. 2006). However, for both ecosystems nematode identification was generally limited to the genus definition, and there were no clear references to *H. schachtii* or *H. betae* occurrence (Verschoor et al. 2001; Verschoor et al. 2002; van der Putten et al. 2005; Van der Stoel et al. 2006). Additionally, experimentations in controlled conditions showed that visual symptoms induced by cyst nematodes on wild host-

plants may be limited, or similar to symptoms caused by abiotic stresses in natural conditions (Brinkman et al. 2005). Thus, the presence of wild populations of cyst beet nematodes is difficult to diagnose and may limit the development of adequate sampling designs. Individuals of *Heterodera* spp. are dominant in the soil nematode community of foredunes, where the species *Heterodera arenaria* has been extensively studied (see references in van der Putten et al. 2006). Similarly, other studies reported the occurrence of *Heterodera avenae* and more recently of *Heterodera mani* in sub-humid coastal plains and in foredunes (Mokabli et al. 2001; Mateille et al. 2014). This suggests that coastal environments may be particularly suitable for the development of a large spectrum of *Heterodera* species including our species of interest.

Based on this literature, we expected to find three *Heterodera* species in soil samples: *H. arenaria*, *H. avenae* and *H. schachtii*. Indeed, they all have been observed in the coastal habitat and *H. schachtii* has been found in cultivated fields of all countries located along the Atlantic coastline (Subbotin et al. 2010). This may indicate that these latitudes are beneficial for its development in the wild. In contrast, *H. betae* has mainly been reported from cultivated fields of northeastern Europe. As a result, *H. betae* was not initially expected in wild soil samples. However, its ability to damage sugar beet fields, its large occurrence in all the investigated area (cf. § 2.2) and cross-amplification of microsatellite markers between *H. schachtii* and *H. betae* encouraged us to include the study of *H. betae* in this project.

Beta vulgaris ssp. *maritima* is a wild relative species of sugar beet (Beta vulgaris ssp. vulgaris) occurring along the Atlantic and Mediterranean shorelines, which is known as a suitable host for both *H. schachtii* and *H. betae* (Maas and Heijbroek 1982). This plant has the particularity to be the source of most of the sugar beet resistant varieties used to manage *H. schachtii* in the fields (Thurau et al. 2010). Studies on wild cyst nematode populations developing within sea beet populations may thus give insights on the evolutionary potential of field nematode populations (McDonald and Linde 2002). This species occur on the Atlantic shorelines since the Last Glacial Maximum, suggesting wild beet cyst nematodes populations may have had a long interaction (Leys et al. 2014). As a result the sea beet was suspected to support large populations of nematodes containing virulent individuals which may represent a potential danger for sugar beet fields. *Beta vvulgaris* ssp. *maritima* is sensitive to competition and restricted to the shoreline, just at the upper level of high tides where salt and drought conditions limit the density of competitor plants in the neighborhood (Coons 1954; Biancardi and De Biaggi 1979; Biancardi et al. 2012). These characteristics make the sea beet a host plant of choice for the development of a workable sampling procedure. Moreover, the coastal

environment has the advantage to be relatively narrow and homogenous along the shoreline, which limits the spatial area to survey. Hence, among all alternative host species for both nematodes, the sea beet appeared as an excellent candidate for a first survey of geographical distribution of wild populations in cyst beet nematodes.

2.1.3. Preliminary results on geographic structure of genetic diversity of the three species, hypotheses and objectives

The sampling and molecular analyses of host plant and nematode populations started before my PhD. The evolutionary history of the sea beet was described for populations distributed from Morocco to Brittany. Sea beets persisted in latitudes lower than the North of Portugal during the Last Glacial Maximum, and expanded northward through postglacial migrations (Leys et al. 2014). Phylogeographic information from sequences data of two nuclear genes for both nematode species showed a total absence of geographical structuring among *H. schachtii* populations and evidenced a phylogeographical pattern in populations of *H. betae* (Voldoire et al. unpublished data).

Two main scenarios emerged from these preliminary results. First, nematodes and sea beet populations could have interacted for a long time, resulting in common phylogeographic histories that favor the development of co-adaptation or co-evolutionary processes. In this case, population genetic structure of the host-plant and the parasitic nematode should show some congruence in their genetic signatures (Avise 2000; Huyse et al. 2005). Second, the nematodes could not interact preferentially with the sea beet, which was infested as any other suitable host-species along the shoreline. In that case, the host and the parasites may have different evolutionary trajectories and may exhibit different phylogeographical patterns. Observed differences between the two nematode species may result from very different biological traits like their reproductive mode, their host-range or their dispersal capabilities. It may also be an artifact related to the use of limited number of nuclear genes. Indeed, genealogies result from the interaction between different evolutionary forces and demographic variations. Random processes may slightly be different for each locus and may not reflect the true history of the species. As a result, in phylogeographical studies, the combination of data from several markers is strongly recommended to give a good support to a particular biogeographical scenario of species' history (Hickerson et al. 2010).

In this context, my work was devoted to confront the observed geographical distribution of the two nematodes species with the literature, particularly for *H. betae* which occurred at higher frequencies than previously expected. Next, I genotyped *B. v.* ssp. *maritima*, *H.*

schachtii and *H. betae* populations with existing microsatellite loci. Finally, I developed new sequence markers (nuclear and mitochondrial genes for nematodes and a mitochondrial gene for the sea beet) together with sequencing procedures (Roche 454 sequencing technology) for nematode genes. These data were subsequently used to analyze the occurrence of the two nematodes in the wild and to develop a comparative phylogeographical study of the three species. Results were grouped in the two articles presented in this section.

1. In the first one, presence/absence data for geographical occurrence of the three species were used to (i) describe their respective distribution along the Atlantic coastline, (ii) investigate whether nematodes were or not randomly distributed in space (iii) identify potential environmental factors that may drive their distributions. This work has been published in *Nematology* in 2014 (vol. 16, p. 797-805).

2. In the second article, the genetic diversity of the three species was investigated to trace back and compare their phylogeographical patterns. Specifically, this study aimed at (i) determining whether there is congruence between genetic structures of *B. v.* ssp. *maritima* and of the two nematodes or not, (ii) gaining further insights into the post-glacial recolonisation history of the sea beet in its northern part of species distribution area and (iii) identifying factors influencing the large-scale spatial genetic structure in the three species. A particular focus will be made on the effects of climatic fluctuations or marine hydrodynamic. This work is still in progress and will be submitted for publication later.

2.2. Spatial distribution and basic ecology of *Heterodera schachtii* and *H. betae* wild populations developing on sea beet, *Beta vulgaris* ssp. *maritima*

Cécile GRACIANNE, Eric J. PETIT, Jean-François ARNAUD, Catherine PORTE, Lionel RENAULT, Didier FOUVILLE, Claudia ROUAUX, Sylvain FOURNET

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ABSTRACT

Most populations of crop pathogens have wild relative populations from which they can originate but for which basic knowledge on their ecological requirements *in natura* is scarce. However, the study of spatial distribution and ecology of wild pathogen populations may help control them in crops through a better understanding of the environmental factors driving population dynamics. Here, we focused on *Heterodera schachtii* and *H. betae*, two cyst nematodes that cause severe damage to sugar beet (*Beta vulgaris* ssp. *vulgaris*) crops and can develop on a wild beet relative, the sea beet (*Beta vulgaris* ssp. *maritima*). We investigated the occurrence of both nematode species in the wild and explored some environmental factors that may influence their geographical distribution. To do so, we sampled the wild host *B. v.* ssp. *maritima* along the European Atlantic and North Sea coastlines. Results showed that *H. schachtii* mainly occurred in the colder environments of northern Europe, whereas *H. betae* was preferentially distributed in the warm environments of southern Europe. It was previously established that *H. betae* only recently appeared in The Netherlands, which are in the North of Europe. Thus, our results do not support this hypothesis. Overall, this study accurately documents the geographical occurrence of two nematode crop pest species in the wild and helps identify the main environmental factors affecting their distribution range.

Keywords: cysts nematode; environmental factors; geographical distribution; wild beet; wild crop pathogen populations.

Yield reduction due to crop plant pests is a worldwide concern causing several billion euros of economic losses annually and can sometimes even lead to famine. Pest management has thus been a challenge for agronomic research for several decades (Matthews 1984). Recently, increasing restrictions on the use of pesticides has led to the development of new environmentally friendly control methods, among which resistant varieties often appear as one of the most cost-effective alternatives. In most cases, resistance comes from the wild relatives of cultivated species and results from long co-evolutionary interactions between wild host plants and wild pathogen populations (Cook 2004). Also, field pathogen populations can be assumed to originate more or less recently from wild pathogen populations and to exchange some individuals with wild reservoirs (according to their dispersal features). As a result, the efficiency of introduced resistance in crop plants depends on the similarity of genetic characteristics between field and wild pathogen populations. Thus, any knowledge about the spatial distribution, ecology and genetics of wild pathogen populations will give us a better understanding about the dynamics of those species and may shed lights on the management of field pathogen populations by improving crop resistance deployment strategies (van der Putten et al. 2006).

There is little information currently available on the distribution and the basic ecology of wild populations of most plant-parasitic nematodes associated with major crops. This is particularly true for *Heterodera schachtii* and *H. betae*, two species of cyst nematodes that cause severe annual damage to sugar beet (*Beta vulgaris* ssp. *vulgaris*). In sugar beet fields, the use of resistant varieties developed from the wild beet relative, the sea beet (*Beta vulgaris* ssp. *maritima*), has recently become the only way to control *H. schachtii* populations or to minimise their effects on yield. This nematode is an amphimictic species that is widely distributed throughout the world and has a lot of alternative host species. Resistance to *H. schachtii* bred in certain sugar beet varieties has also helped to control *H. betae* populations, although to a lesser extent. *Heterodera betae* is considered as a true species (Wouts et al. 2001) and has been found in Europe and Morocco(Amiri et al. 2002; Subbotin et al. 2010), although its reproductive mode and host range remain to be confirmed.

Heterodera schachtii and *H. betae* can reproduce on *B. v.* ssp. *maritima* in the laboratory (Maas and Heijbroek 1982; Steele et al. 1983). This wild host plant is widely distributed all along the European Atlantic and Mediterranean coastlines (Biancardi et al. 2012) on a narrow area, very close to the sea. Ecosystems where sea beet occurs may thus be considered as

potential natural reservoirs of field populations of both nematodes species. *Beta vulgaris* ssp. *maritima* is an ideal candidate for a study on the geographical occurrence and basic ecology of wild populations of *H. schachtii* and *H. betae* in Europe. Here, we carried out a large-scale study to define the geographical distribution of both nematodes, to test the influence of environmental factors on their respective distributions, and to give a description of the favourable wild habitats for two major nematode crop pests.

MATERIALS AND METHODS

Biological material and sampling procedures

Sampling was designed according to previous population genetic analyses on *H. schachtii* field populations, which demonstrated that gene flow can occur among populations of nematodes occurring in *B. vulgaris* ssp. *vulgaris* fields separated by distances of up to ~100 km (Plantard and Porte 2004). To select distinct populations, we sampled 33 different locations separated by distances of 150 km. They were distributed along the Atlantic coastline, from northern Morocco to southern Sweden (Fig. 1). At each location, a minimum of three different sites were sampled along 20 km of the coastline. At each site, one population of *B. v.* ssp. *maritima* was characterised by selecting at least 10 live plants from which fresh leaves were collected for molecular identification. Soil samples surrounding the roots of each plant in these populations were collected, pooled and stored in a single plastic bag. Each bulk soil sample taken from a site was then considered as a population of *H. schachtii* and/or *H. betae* if nematodes were present. Cysts of *Heterodera* spp. were manually collected in every soil sample after extraction using a Kort elutriator. Cysts were then stored in moistened sand at 4°C before molecular characterisation.

Molecular characterisation of nematodes

Heteroderidae species (*H. schachtii*, *H. betae*, *H. ciceri*, *H. diverti*, *H. galeopsidis*, *H. glycines*, *H. medicaginis* and *H. trifolii*) can be discriminated by RFLP polymorphism of ribosomal DNA (Subbotin et al. 2000, 2001). In our case study, the digestion of PCR products by *Mva*I (2 h at 37°C) leads to specific restriction profiles for the *H. schachtii* and the *H. betae* ITS sequence, allowing accurate identification of these nematodes (Amiri et al. 2002) (see Fig. 2).

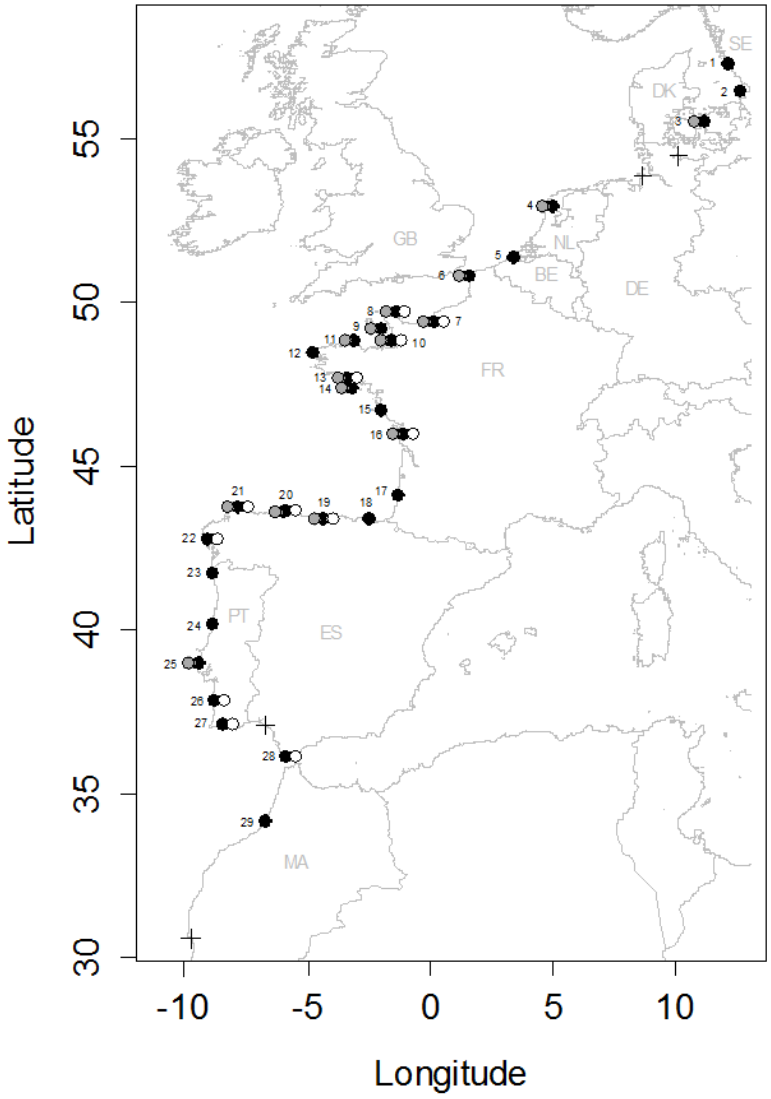

Figure 1: Geographical distribution of *Heterodera schachtii*, *Heterodera betae*, and their common host, *Beta vulgaris* ssp. *maritima*, along the Atlantic coastline. Black circles represent locations where *B. vulgaris* ssp. *maritima* plants were found. Gray and white circles correspond to locations where *H. schachtii* and *H. betae*, respectively, were observed. Black crosses correspond to locations where *B. vulgaris* ssp. *maritima* was not found. Numbers refer to the location code used in Table 1.

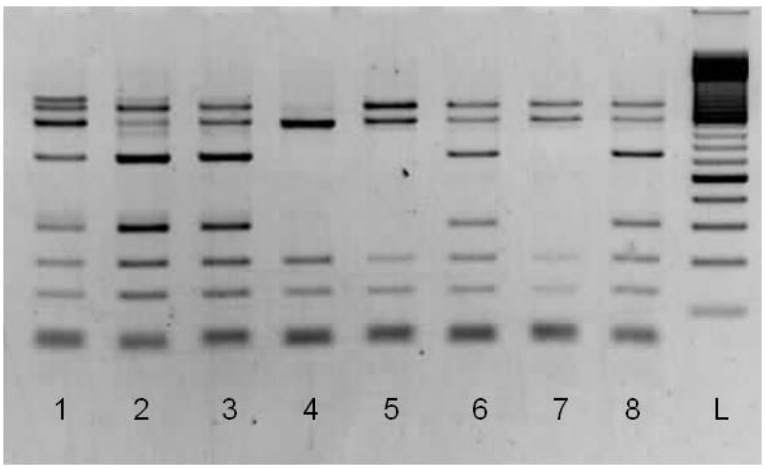

Figure 2: Restriction patterns of ITS amplified fragments. The amplification and the digestion of the ITS region were used to discriminate *H. schachtii* and *H. betae* individuals. 1, 2, 3, 6, 8: *Heterodera schachtii*. 4, 5, 7: *Heterodera betae*. L: ladder.

For each population and for a minimum of 20 cysts, cysts were manually crushed and one juvenile from each cyst was transferred to a tube containing 20 µl of NaOH (0.25 M) overnight. Samples were then incubated at 99°C for 2 min. A buffer (0.25 M HCl, 0.5 M Tris HCl (pH 8), 2% Triton X-100) was added to samples that were then incubated for 2 min at 99°C. Proteinase K (5×) was subsequently added and samples were held at 55°C for 1 h. Two primers (5'-TTGATTACGTCCCTGCCCTTT-3' and 5'-TTTCACTCGCCGTTATAAGG-3') targeting the nematode ITS region were used for PCR amplification. The cycling procedure for PCR amplification was as follows: 1 min at 94°C, 35 cycles with 1 min at 94°C, 50 s at 60°C, 1 min at 72°C, and a final step at 72°C for 5 min.

Genetic characterisation of *Beta vulgaris* ssp. *maritima*

To avoid any possible confusion with other species of the *Beta* genus, the multilocus genotypes of plants collected in sampled locations were characterised individually to ensure subspecies identity and reveal another potential host plant species, the closely related self-fertilising species, *Beta macrocarpa*. This genotyping is described elsewhere (Leys et al. 2014).

Habitat analysis

The overall degree of differentiation or exclusion of the two nematode species was evaluated with the kappa statistic κ (Cohen 1960), estimated as described in Guelat et al. (2008). This statistic is an analogue of the correlation coefficient for categorical variables. In our study, this index was used to evaluate whether the spatial distributions of *H. schachtii* and *H. betae* were correlated, *i.e.,* whether they preferentially co-occurred. κ was considered to differ from random expectations if the 95% confidence interval of the bootstrap (1000 replicates) distribution did not include zero.

Generalised linear models (GLM) were also used to test for the effects of habitat variables on the geographical distributions of the two nematode species. The habitat variables we considered included abiotic and biotic factors (see Table 2 for details). GLM are an extension of classical multiple regressions and are particularly suitable for analysing spatial distribution of species because they can handle binomial presence-absence data and non-normal response variables (Guisan et al. 2002). For *H. schachtii* (or *H. betae*), GLM were fitted considering *H. betae* (or *H. schachtii*) as an explanatory variable.

Correlation tests were performed to remove redundant covariables and simplify models. All analyses were performed with R software version 2.15.2 (R Development Core Team 2012).

RESULTS

Molecular characterisation of the sampled sea beet plants ensured that all soil samples were taken from *B. v.* ssp. *maritima* or its closely related species, *B. macrocarpa* (Leys et al. 2014). *Beta v.* ssp. *maritima* was present in all locations surveyed along the Atlantic and North Sea coastlines, except in Germany and one Moroccan population composed of *B. macrocarpa*, totalling 90 populations of *B. v.* ssp. *maritima* from 29 locations (Fig. 1, Table 1). ITS characterisation revealed 27 populations of *H. schachtii* distributed in 15 (52%) of the sampled locations. *Heterodera schachtii* was mainly observed in northern Spain and in northwestern France. It also occurred sporadically in Portugal, Denmark and The Netherlands. ITS characterisation also revealed 21 populations of *H. betae* in 12 (41%) locations. Ten populations of *H. betae* were found in association with *H. schachtii* (Table 1). *Heterodera betae* populations were mainly observed in France, Spain and Portugal and were absent from northern Europe (Fig. 1).

The two species occurred together significantly more frequently than expected by chance, but the low κ value suggests that distributions of both species do not completely overlap (κ = 0.29; 95% CI (0.07, 0.49)). Results of GLM showed that *H. schachtii* was preferentially distributed at high latitude values (northern Europe) ($P < 0.001$) and where *H. betae* was present ($P < 0.01$). Occurrence of *H. betae* occurred preferentially at low latitudes (southern Europe) where *H. schachtii* was present ($P < 0.001$) and when the probability of presence of alternative hosts plants was high ($P < 0.05$). There were no significant effects of the distance to the nearest cultivated fields or the nearest river estuary for either species.

DISCUSSION

This study investigated environmental factors influencing the spatial distribution of wild populations of two crop nematodes along the Atlantic and North Sea coastlines. Results showed that nematodes are widely distributed in Europe, with a large area of overlapped distribution, and are influenced by the latitude, a proxy of temperature, and the presence of other host species for *H. betae*.

Table 1: Location and number codes of nematode populations. Presence (x) of *H. schachtii* and/or *H. betae* was determined by ITS characterization.

Location code	Country	Region	Site	Population code	Presence of *H. betae*	Presence of *H. schachtii*
1	Sweden	Skalderviken	Torekov	Su1-1		
			Torekov	Su1-2		
2		Batfjorden	Venner	Su2-1		
3	Denmark	Kerteminde	Nordskov	Dan1-1		
			Nordskov	Dan1-2		
		Kalunborg	Reerso	Dan1-3		x
4	The Netherlands	Den Helder	Den Helder	Hol2-1		
		Wieringen	Den Oever	Hol2-2		x
5	Belgium	West-Vlaanderen	Zwin	Hol1-1		
6	France	Nord-Pas de Calais	Groffiers	Fra10-1		
			Etaples	Fra10-2		x
			Wimereux / Slack	Fra10-3		x
			Audresselles	Fra10-4		x
7		Haute Normandie	Honfleur	Fra9-1		x
			Pennedepie	Fra9-2		
			Pennedepie	Fra9-3	X	
8			Fermenville	Fra8-1		x
			Cosqueville	Fra8-2	X	
			Gatteville le Phare	Fra8-3		
			Montfarville	Fra8-4	X	x
9	United Kingdom	Jersey	Gouray	Gb1-1		x
10	France	Basse Normandie	Granville	Fra7-1	X	x
			Granville	Fra7-1No	X	x
			Carolles	Fra7-2		x
			Genêts	Fra7-3		
			Saint Léonard	Fra7-4	x	x
11			Paimpol	Fra6-1		
			Loguivy	Fra6-2		
			Ile à bois	Fra6-3		
			Île à bois	Fra6-4		x
12			Aber Ildut	Fra5-1		
			Pors Gored	Fra5-2		
			Pors Gored	Fra5-3		
13		Bretagne	Gâvres	Fra4-1		x
			Gâvres	Fra4-2		x
			Maguero	Fra4-3	x	
			Barre d'Etel	Fra4-4		x
			Nestadio	Fra4-5		
14			Port Jean	Fra4-bis1		x
15		Pays de Loire	Saint Gilles Croix de Vie	Fra3-1		
			Saint Gilles Croix de Vie	Fra3-2		
			La Sauzaie	Fra3-3		
			La Sauzaie	Fra3-4		
16		Poitou-Charentes	Baie d'Yves	Fra2-1		
			Fouras	Fra2-2		x
			Île Madame	Fra2-3	x	x
			Port des Barques	Fra2-4		
			Marennes	Fra2-5	x	
17		Aquitaine	Contis plage	Fra1-1		
18	Spain	Euskadi	Lekeitio	Esp1-1		
			Laga	Esp1-2		
			Kanala	Esp1-3		
19		Cantabria	Oyembre	Esp2-1	x	x
			San Vicente de la Barquera	Esp2-2		
			San Vicente de la Barquera	Esp2-3	x	x
			San Vicente de la	Esp2-4	x	x

			Barquera			
			San Vicente de la Barquera	Esp2-5	x	x
			San Vicente de la Barquera	Esp2-6		
			San Vicente de la Barquera	Esp2-7		
20		Asturias	Verdicio	Esp3-1		
			Verdicio	Esp3-2	x	
			Xago	Esp3-3		x
21		Galicia	Celtigos	Esp4-1		
			Celtigos	Esp4-2	x	x
			Espasante	Esp4-3		
22			Texueiro	Esp5-1		
			San Francisco	Esp5-2		
			San Francisco	Esp5-3	x	
23	Portugal	Viana do Castelo	Paço	Por1-1		
			Areosa	Por1-2		
			Areosa	Por1-3		
24		Coimbra	Praia da Tocha	Por2-1		
			Quiaios	Por2-2		
			Cabedelo	Por2-3		
25		Lisboa	Ericeira	Por3-1		x
			Ericeira	Por3-2		
			Lizandro	Por3-3		
26		Setúbal	Samoqueira	Por4-1		
			Porto Covo	Por4-2		
			Pessegueiro	Por4-3	x	
27		Faro	Carvoeiro	Por5-1		
			Carvoeiro	Por5-2	x	
			Marinha	Por5-3	x	
			Armacao de Pera	Por5-4		
28	Spain	Andalucía	Chiclana de la Frontera	Esp6-1		
			Sancti Petri	Esp6-2		
			Roche	Esp6-3		
			Barbate	Esp7-1	x	
			Zahara	Esp7-2	x	
			Zahara	Esp7-3		
29	Morocco		Sidi Boukdanel	Mar1-1		

Table 2: Description of environmental variables used in the habitat use analysis. All habitat variables were estimated with the measure tool of the 7.1.1 Google Earth software.

Habitat variable	Justification
Latitude	Proxy of temperatures which is known to Influence larval development in laboratory conditions
Distance to the nearest cultivated field	Possible exchanges between fields and sea beet plants
Distance to the nearest river estuary	Possible facilitation of the surface runoff carrying cysts from inland zones to the coast by the presence of river drainage basins
Presence of alternative hosts	Probability of having alternative host-plant species for both nematode species coded as a binary variable (0 or 1) based on the observation in the immediate vicinity of sampled plants. 0: no contact between sea beet and potential alternative host plants (Goodey et al 1965) ; 1: presence of potential alternative host plants close to sea beet

Geographical distribution of nematodes in wild populations of the sea beet

Heterodera schachtii was found in 52% of its wild host locations. Its presence was previously reported in cultivated fields in all European countries involved in this study (see Subbotin et al. 2010 for a review). This is the first work that documents its geographical occurrence throughout Europe on one of its wild hosts. *Heterodera schachtii* seemed to be relatively rare in southern Spain and in Portugal, whereas it was more frequent on the northern coast of Spain, in France, in The Netherlands and in Denmark. These observations were supported by the analysis of the effect of habitat variables, showing that *H. schachtii* occurred preferentially in northern Europe, therefore in cooler habitats. These results were in accordance with several studies on the impact of temperature on the biology of *H. schachtii*. Indeed, cyst survival, maximal reproduction, root penetration and juvenile development occur preferentially between 10 and 28°C, and the development of juveniles and females is inhibited at temperatures greater than 30°C (Wallace 1955; Thomason and Fife 1962; Griffin 1981; Maas and Heijbroek 1982). *Heterodera schachtii* is thus better adapted to temperate and cold climatic conditions, explaining its presence only in the irrigated cultivated fields of the warmest regions of the southern Iberian Peninsula.

Heterodera betae can also develop successfully on sugar beets in the field and on wild beets in laboratory experiments (Steele et al. 1983; Wouts et al. 2001). This species was found in 41% of the sampled locations. It has recently been documented in cultivated fields in Sweden, The Netherlands, Belgium, Germany, Lithuania, Switzerland, France and Italy (Subbotin et al. 2010), but our study is the first that describes its distribution in wild habitats. Moreover, its occurrence in Spain and Portugal has never been reported before. In several locations, *H. betae* co-occurred with populations of *H. schachtii*. Previous studies have indicated high similarities in the host ranges of *H. betae* and *H. schachtii* (Maas et al. 1982; Steele et al. 1983), but this is the first time, to our knowledge, that mixed populations have been described. The univariate κ statistic was significant, suggesting that it is more probable to find both species co-occurring than either species alone. This suggests that, while both species were found alone in areas matching their typical habitat requirements (*i.e.,* cold conditions for *H. schachtii* and warmer conditions for *H. betae*), they preferentially co-occur in natural areas with favourable environmental conditions for both species. As *B. v.* ssp. *maritima* is a particularly suitable host for both species, they occur in the same host populations in a given location.

The distribution of *H. schachtii* was not influenced by the presence of other host species in areas surrounding sampled sites. This nematode has a wide host range including several

species with a wide geographical distribution in disturbed habitats (Goodey et al. 1965), such as urban areas. By contrast, the distribution of *H. betae* was influenced by the presence of other host species in the surrounding area. According to the literature, this nematode has a host range that is as large as the host range of *H. schachtii* (Maas and Heijbroek 1982; Andersson 1984). However, understanding clearly how the presence of alternative hosts influences the distribution of *H. betae* and *H. schachtii* requires further investigation.

Finally, the distribution of these nematode species was not related to the distance to the nearest estuary or cultivated fields. This suggests that the presence of nematodes on wild sea beet is independent of their potential occurrence in cultivated fields. The detection of effective exchanges of individuals among wild and field populations needs further population genetics investigations.

The natural history of *Heterodera betae* revisited

Our results challenge what was previously known for *H. betae*. This species was discovered in Dutch beet fields in 1975 and was first considered as a biotype of the clover cyst nematode, *H. trifolii*, able to parasitise a non-usual host, *B. v.* ssp. *vulgaris* (Maas et al. 1982). More recently, molecular and morphological characterisation established that *H. betae* was distinct from the *H. trifolii* complex and constituted a true species (Wouts et al. 2001). Interestingly, *H. betae* has 35, possibly 36, chromosomes and reproduces by mitotic parthenogenesis indicating that this species is potentially tetraploid (Steele and Whitehand 1984). More specifically, it was suggested early on that *H. betae* may have evolved very recently from *H. schachtii* or *H. glycines* because both species have nine chromosomes (Mulvey 1957, 1958, 1959; Triantaphyllou and Hirschmann 1964; Steele and Whitehand 1984; Subbotin et al. 2010). However, *H. glycines* was absent from Europe until 2008, which brings into question a common ancestry with *H. betae* (Steele and Whitehand 1984; Greco and Inserra 2008). Comparative morphological studies show that *H. betae* and *H. schachtii* are similar but have minor differences in size. Eggs and juveniles of *H. betae* are longer than those of *H. schachtii* (Steele and Whitehand 1984; Wouts et al. 2001; Amiri et al. 2003). In nematodes, differences in ploidy are often associated with differences in individual size (Hirschmann 1956; Hirschmann and Triantaphyllou 1979). Thus, the differences and similarities between those two species could be the result of speciation by polyploidy, whereby *H. betae* derives from *H. schachtii*.

After its discovery, *H. betae* was documented only in Dutch regions for 10 years. It was thus assumed that its native area was The Netherlands (Steele and Whitehand 1984).

However, we did not find any occurrence of this nematode on *B. v.* ssp. *maritima* in northern European countries (Germany, Sweden or Denmark), whereas it was detected in cultivated fields of the same countries (Subbotin et al. 2010). Moreover, our habitat use analysis showed that *H. betae* was preferentially located in warm habitats of southern Europe (Spain, Portugal and France). These results may be due to our sampling design: the 20 km coastline segments were randomly defined and it is possible that locations of *H. betae* were missed. Nevertheless, the systematic presence of the nematode in western France and on the Iberian Peninsula does not suggest that there was any sampling bias. Moreover, the observed *H. betae* distribution was consistent with the biology of this nematode: the hatching rate of juveniles from cysts is higher at warm temperatures (between 25 and 30°C) and close to zero for temperatures below 15°C (Maas and Heijbroek 1982). Unlike *H. schachtii*, *H. betae* may thus be better adapted to warm climatic conditions and its presence in northern Europe may result from the cultivation of sugar beet crops in favourable climatic conditions. Finally, our results do not support the assumption of a very recent appearance of this species and suggest a more complex evolutionary history in natural environment, unless *H. betae* arises recurrently from its amphimictic relative *H. schachtii*. Thus, The Netherlands, which is located in northern Europe, is probably not the center of origin of *H. betae*.

CONCLUSIONS

Our study demonstrated that *H. schachtii* and *H. betae* are widely distributed along the Atlantic coastline, with a large area of overlapped distribution. Temperature appeared to influence their relative spatial distribution. Our results raise many questions on the origin and evolution of those nematodes and on their coexistence in *B. v.* ssp. *maritima*. The centre of origin of both nematode species is still unknown. Determining its location could provide interesting clues for breeders selecting new resistant beet varieties. The relationship between *B. v.* ssp. *maritima* and both nematode species is also an interesting issue. The wild beet shows a south-to-north phylogeographical pattern attesting to post-glacial recolonisation (Leys *et al.,* in press). Both nematode species should thus exhibit the same recolonisation history, unless alternative hosts may have played a major role in their evolutionary history. This hypothesis requires further phylogeographic analyses. Finally, the significant presence of both nematodes species in natural environment also raises questions as to the contamination risk of cultivated fields.

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2.3. Comparative phylogeography of host/parasite wild populations: wild beets and cyst nematodes as a case study

Cécile GRACIANNE, Michael HICKERSON, Jaanus REMM, Sylvain FOURNET, Catherine PORTE, Sylvie BARDOU-VALETTE, Eric J. PETIT, Jean-François ARNAUD

INTRODUCTION

Comparative phylogeography aims at investigating the effects of past history and biogeographic events on evolutionary trajectories among co-distributed species. Signatures left in spatial genetic structure are particularly relevant to identify shared environmental, past historical factors or anthropogenic disturbances explaining the current patterns of population genetic structure (Bermingham and Moritz 1998; Avise 2000; Knowles 2009). Indeed, large-scaled factors may induce similar demographic variations or genetic differentiation within and among populations of phylogenetically distant species sharing similar geographical distributions. Occurrence of concordant phylogeographical patterns among multiple co-distributed species is considered as a signature of common evolutionary histories, which is commonly used to understand large-scaled responses of species to climate change (Avise 2000; Hickerson et al. 2010).

Whether concordant phylogeographical patterns arise has also received great attention in host-pathogen systems (de Vienne et al. 2013). These associations are characterized by an intimate interaction between the parasite and the host, which may favor co-evolutionary processes and thus concordance of phylogeographical patterns of pathogens and host (Huyse et al. 2005). Intrinsic features of pathogens such as host specificity, vertical transmission, short generation time, high mutation rate and limited dispersal abilities may make pathogen evolutionary history suitable to infer ecology or history of the host (Whiteman and Parker 2005; Nieberding and Olivieri 2007), even sometimes giving insights into the history of associated species like host predators or parasitoids (Barbosa et al. 2012; Marske et al. 2013). Conversely, host range-shifts and biogeographical patterns also give clues into habitat history of the pathogen. It allow a better understanding of the pathogen's history which may be blurred due to (i) the occurrence of multiple co-distributed host species (Nieberding et al. 2008) or (ii) to a quicker loss of demographic and/or colonization genetic signatures because of shorter generation time (Léotard et al. 2009; Silva-Iturriza et al. 2012). Therefore, properties of host-pathogen systems favored the development of comparative phylogeographic studies devoted to various evolutionary issues: host-pathogen interactions

and calibration of a parasite molecular clock (Nieberding et al. 2004), impact of recombination rate on pathogen genetic diversity (Roe et al. 2011), estimation of the level of specificity of a pathogen for different host species (Glennon et al. 2008), description of host population functioning and dispersal (McCoy et al. 2005), or the role of human activities on pathogen histories (Morgan et al. 2012). Most of these studies showed that concordant patterns were not necessary the rule among host and pathogen species and highlighted the importance of species-specific characteristics like dispersal, life-history traits or ecological requirements (Clayton et al. 2004; Toon and Hughes 2008; Bagley and Johnson 2014) beyond host specificity and biogeographical factors (Crandall et al. 2008; Stefka et al. 2011; du Toit et al. 2013; Werth et al. 2013). Consequently, comparative phylogeography may thus allow inferring crop pathogen histories which are generally complex because of (i) the involvement of different evolutionary mechanisms such as host-tracking, hybridization or host-shift and of (ii) anthropogenic influence on migration at all spatial scales (Stukenbrock and McDonald 2008). Phylogeographical approaches have already been useful to trace back field pathogen population history on cultivated hosts (*e.g.* Picard et al. 2007; Ceresini et al. 2007; Zaffarano et al. 2009; Boucher et al. 2013), and on wild hosts (*e.g.* Brewer 2010; Tamm 2013). Indeed, crop pathogens generally emerged from wild pathogen populations evolving on wild hosts, suggesting that evolutionary histories should be inferred on wild populations (Stukenbrock and McDonald 2008).

In this respect, studying biogeographical patterns of wild host/pathogen systems would be valuable to better understand the impact of wild populations in crop epidemiology. Numerous studies have evidenced low levels of genetic differentiation among pathogen populations from wild and cultivated host species, reflecting the occurrence of gene flow among wild and field pathogen populations (Fournier and Giraud 2008; Stukenbrock and McDonald 2008; Kiros-Meles et al. 2011; Clarkson et al. 2013). Wild populations of crop pathogen were thus considered as reservoirs of genetic diversity and/or inoculums. Consequently, the role they may play in crop pathogens diversity and epidemiology is receiving increasing attention (Burdon and Thrall 2008; Leroy et al. 2014). However, the evaluation of the impact of wild populations on genetic diversity of field populations requires first to investigate the genetic legacy of wild pathogen populations in field pathogen populations.

The phylum Nematoda contains almost 4,100 plant parasitic nematodes species, and several of which are major soilborn crop pests causing worldwide economic losses (Jones et al. 2013). To date, there is few available data on the evolutionary relationships between plant-parasitic nematodes and their hosts. One study performed on the subfamily Anguinidae has

shown that gall-forming plant parasitic nematode that belong to the same taxa were generally associated with host plants belonging to the same or related systematic groups, suggesting some host specificity among nematode species developing on the same gall-type (Subbotin et al. 2004). Phylogeographical histories of plant-parasitic nematodes were only described for three nematodes species, *Globodera pallid*, *Globadera rostochiensis* and *Bursaphelenchus xylophilus* (Picard et al. 2007; Boucher et al. 2013; Mallez et al. 2014). The two first species are cyst nematodes originating from South America and were subsequently introduced in Europe and North America. Beyond highlighting origin and invasion spread, both studies gave valuable information on the type of genetic resistance that may be suitable to efficiently manage these species in potato fields according to their geographical origin.

Other cyst nematodes species, namely *Heterodera schachtii* and *Heterodera betae*, are involved in economic losses worldwide in sugar beet crops (*Beta vulgaris* spp. *vulgaris*). Management strategies involve crop rotation, and an increasing use of resistant varieties due to the toxicity and the progressive ban of traditional chemicals (Thurau et al. 2010). Genetic resistances may be overcome more or less quickly according to the nematodes' abilities to evolve, but there is no information available on their evolutionary potential so far. *H. schachtii* and *H. betae* are also able to develop on the wild coastal relative species of sugar beet, the sea beet (*Beta vulgaris* spp. *maritima*). Sea beets are the source of most genetic resistances used in sugar beet resistant varieties to manage field nematode populations. Sea beet is widely distributed along the European Atlantic and Mediterranean coastlines. This species is thought to have persisted in the Southwest of Europe during the last Glacial Maximum and to have recolonized the Atlantic coastline northward by sequential founder events (Leys et al. 2014).

Because pollen of *B. v.* ssp. *maritima* is wind-dispersed and seeds can be drifted by marine currents, phylogeographical patterns in this species are expected to display similar genetic discontinuities than those observed in marine and coastal species. In contrast, active dispersal capabilities in the two nematodes are extremely limited (Wallace 1968), and the extent of passive dispersal is still unknown in the wild. However a study performed on field populations of *H. schachtii* suggested passive gene flow among fields distant of 150 km from each other (Plantard and Porte 2004). Assuming equivalent dispersal capabilities among wild populations, some long-distance episodes of gene flow may occur among distant populations and may lead to the appearance of similar phylogeographical break as observed in the host. However, the shorter generation times of nematodes and the frequent extinction/recolonization events occurring in sea beet populations may favor the development

of more spatially-structured patterns of genetic structure (De Cauwer et al. 2012). Similarly, the differences in reproductive mode, sexual for *H. schachtii* and parthenogenic for *H. betae*, may also result in more structured patterns of genetic differentiation in the latter species. In this study, we explored and compared the large-scaled spatial genetic structure of the two nematodes species along with those observed in sea beet populations. Specifically, (i) we searched for concordant phylogeographical patterns in host and phytoparasitic nematode species, (ii) we examined the influence that climatic variations of the Pleistocene may have had on their patterns of population genetic structure and (iii) we discussed the potential role of micro-evolutionary processes in shaping the genetic structuring at both global and regional scales. In particular, the role of water current drifting host seeds and/or cyst nematodes and the presence of northern refugia are questioned. We also discuss the effect of the poor dispersal capability of *H. schachtii* and *H. betae* in enhancing drift effects and bluring genetic signatures of past colonization events.

MATERIAL AND METHOD

Sampling

Geographical occurrence of host and cyst nematode populations in the western part of species' range was described in Gracianne et al. (2014) using a hierarchical sampling along the European Atlantic coastline. This survey was performed from Morocco to southern Sweden to get soil and host leave samples from 91 sites separated by distances ranging from 300 m to 150 km. Samples used in this study comprised all sites where nematodes occurred on host sea beets (*B. vulgaris* spp. *maritima*). However, we discarded populations located in United Kingdom and in French islands owing to likely independent settlement events and independent evolutionary histories from that of mainland populations. We also included sites free of nematode species to get a comprehensive sampling of the sea beet in its western range margin. Finally, a total of 38 populations of *Beta vulgaris* spp. *maritima*, 25 populations of *Heterodera schachtii* and 21 populations of *H. betae* were collected and analyzed for molecular variation (Fig. 1A). Populations of sea beets corresponded to a maximum of 20 different plants living in one sampled site. Populations of nematodes corresponded to a pool of soil samples collected around roots of sea beets. Leaves of sea beets were dried and stored at room temperature. Soil samples were filtrated using homemade sieves (250µm and 800µm) before manual examination of filtrates to extract cysts of *H. schachtii* and *H. betae*, and storage in moistened sand at 4°C.

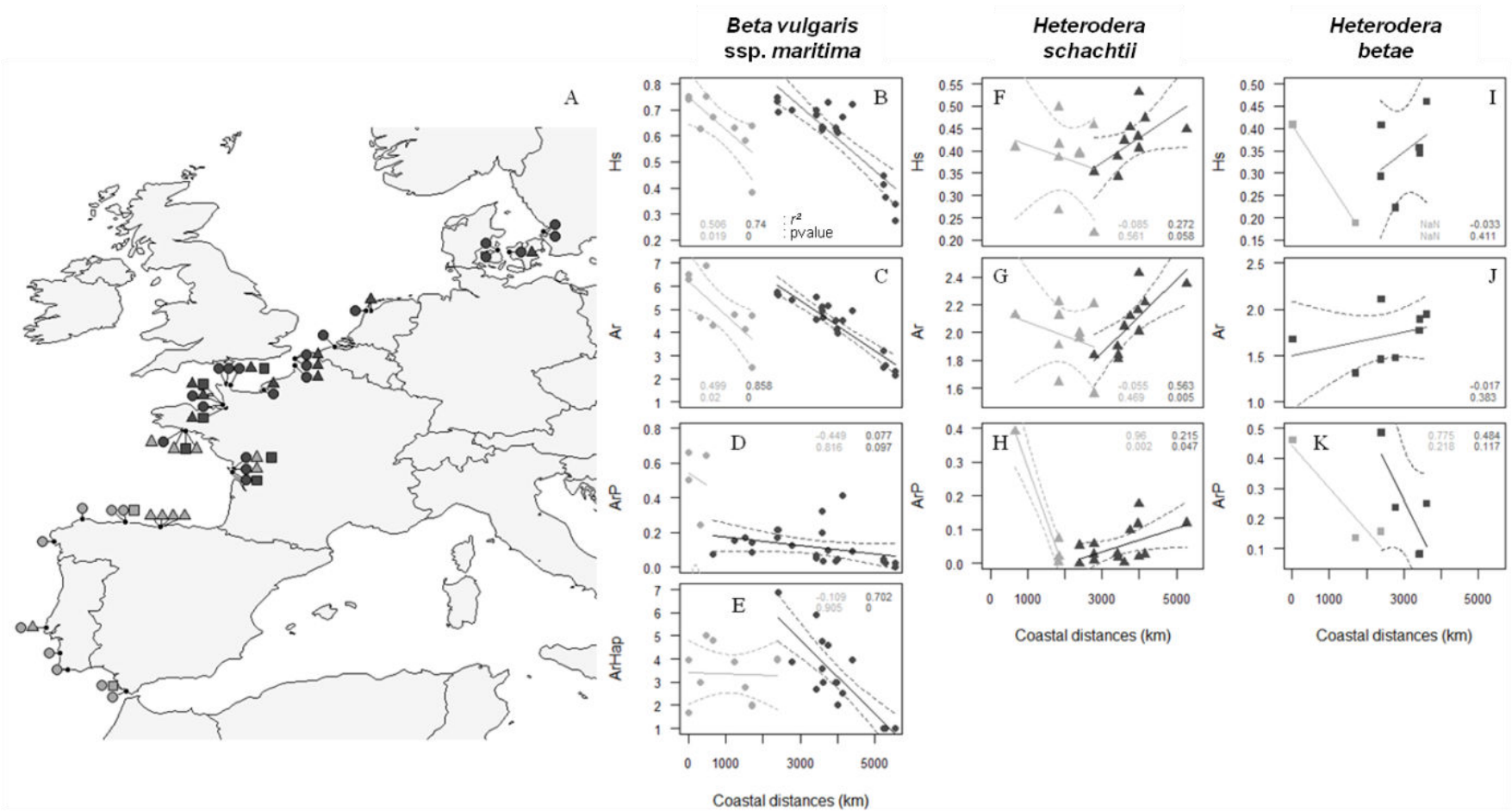

Figure 1: Best piecewise regression model of genetic diversity along costal distances and results from simple linear regression analyses. Circles: *Beta vulgaris* ssp. *maritima*; Triangles: *Heterodera schachtii*; Rectangles: *Heterodera betae*. Light grey: southern group; dark grey: northern group, as defined by the expected heterozygosity in each species. Hs: expected heterozygosity; Ar: allelic richness; ArP: private allelic richness; ArHap: haplotypic richness. A. Distribution of the populations of the three species along the Atlantic coastline. B to K: linear regression of genetic diversity along coastal distances; B, C, D, E: *Beta vulgaris* ssp. *maritima*; F, G, H: *Heterodera schachtii*; I, J, K: *H. betae*. Numbers within regression figures: first line: r^2; second line: p-value of the regression model.

Molecular characterization and genotyping: nematodes

To limit sibling relationships related fine-scaled genetic structuring in *H. schachtii*, only one larva per cyst was manually extracted for molecular identification and genotyping. The distinction of *Heterodera* species found in soil samples was based on restriction profiles of the ITS region using the MvaI enzyme. DNA extraction and PCR amplification were performed following protocols described in Amiri et al. 2002. Both nematodes species were genotyped at eight nuclear microsatellites, named Hs33, Hs36, Hs55, Hs56, Hs68, Hs84, Hs111, and Hs114, initially developed for *H. schachtii*, that also successfully cross-amplified in *H. betae*. Development, primers, and PCR amplifications are described in Montarry et al. (2015). Electrophoresis and analyses of PCR products were performed with an ABI Prism® 3130xl sequencer (Applied Biosystems). Allele sizes determination was performed with automatic binning procedures implemented in GeneMapper v4.1 (Applied Biosystems) and manual checking. To check stability of results, irregular genotypes, rare alleles, and missing data, the complete procedure of genotyping, from PCR amplification to the electrophoresis, was performed twice for 10% of individuals in each species. All multilocus genotypes with more than two missing data were removed from the final dataset. Finally, 441 (18±1.5; mean per population ± standard error, see Table 1) and 168 individuals (8±1.7; mean per population ± standard error, see Table 1) were successfully genotyped in *H. schachtii* and *H. betae*, respectively.

Molecular characterization and genotyping: sea beet

Beta vulgaris ssp. *maritima* DNA was extracted and purified from dried leaves using the NucleoSpin® 96 Plant II (Macherey-Nagel) and following the standard extraction guidelines outlined in the handbook protocol. Samples were first genotyped using eight nuclear microsatellites, FDSB1027 (McGrath et al. 2007), Bvm3 (Mörchen et al. 1996), Caa1, Gtt1 (Viard et al. 2002), SB04, SB06, SB07, and SB15 (Richards et al. 2004). Primers, PCR amplifications, and allele sizing were performed as described in Fénard *et al.* (2007). Additionally, cytoplasmic polymorphism of samples was also characterized using four mitochondrial minisatellites, TR1, TR2, TR3, and TR4 (Nishizawa et al. 2000) whose primers and PCR amplification conditions were described in Fénart *et al.* (2008). Electrophoresis and analysis of PCR products were conducted on a 3130xl Genetic Analyzer (Applied Biosystems). Allele sizing was performed as above and 10% of all dataset was genotyped twice to remove irregular results and missing data. Mitochondrial genome of *B. vulgaris* ssp. *maritima* is inherited as a single linkage unit. We therefore considered the combination of the

Table 1: Genetic diversity and summary statistics for host (*Beta v.* ssp. *maritima*) and nematodes populations sampled in 2012 and 2013. Significant values of F_{IS} are highlighted in bold. n: number of genotypes; Hs: expected heterozygosity; Ar: allelic richness; Private Ar: Private allelic richness; An: number of alleles; Nb Haplo: number of haplotypes; Hapr: haplotypic richness.

						Beta maritima									*Heterodera schachtii*						*Heterodera betae*					
						Nuclear microsatellites						**Cytoplasmic minisatellites**			**Nuclear microsatellites**						**Nuclear microsatellites**					
Country	**Region**	**Label**	**Population Code**	**Latitude**	**Longitude**	**n**	**H_S**	**A_r**	**Private A_r**	**A_n**	**F_{IS}**	**Nb haplo**	**Hap_r**	**A_r**	**n**	**H_S**	**A_r**	**Private A_r**	**A_n**	**F_{IS}**	**n**	**H_S**	**A_r**	**Private A_r**	**A_n**	**F_{IS}**
Sweden	Batfjorden	1	Su2.1	12.122832	57.277643	3	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Skalderviken	2	Su1.1	12.622655	56.426298	2	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		3	Su1.2	12.635224	56.435320	19	0.275	2.177	0.022	2.500	-0.003	1	1.000	1.000	-	-	-	-	-	-	-	-	-	-	-	-
		4	Su1.3	12.633433	56.433767	20	0.339	2.315	0.000	2.750	0.119	1	1.000	1.000	-	-	-	-	-	-	-	-	-	-	-	-
Denmark	Kerteminde	5	Dan1.1	10.589471	55.612467	14	0.448	3.206	0.044	3.625	0.004	1	1.000	1.000	-	-	-	-	-	-	-	-	-	-	-	-
		6	Dan1.2	10.605702	55.605096	17	0.413	2.502	0.033	2.875	0.052	1	1.000	1.000	-	-	-	-	-	-	-	-	-	-	-	-
	Kalundborg	7	Dan1-3	11.119194	55.519011	19	0.365	2.592	0.021	3.125	0.027	1	1.000	1.000	21	0.448	2.354	0.120	3.500	**0.207**	-	-	-	-	-	-
The Netherlands	Den Helder	8	Hol2-1	4.778965	52.964750	10	0.720	4.947	0.090	5.125	-0.008	4	3.968	1.750	-	-	-	-	-	-	-	-	-	-	-	-
	Wieringen	9	Hol2-2	4.985470	52.931222	1	-	-	-	-	-	1	-	-	22	0.474	2.219	0.028	3.250	**0.302**	-	-	-	-	-	-
Belgium	West-Vlaanderen	10	Hol1-1	3.369367	51.365333	23	0.673	4.540	0.410	5.750	0.039	3	2.524	1.631	-	-	-	-	-	-	-	-	-	-	-	-
France	Nord-Pas de Calais	11	Fra10-4	1.6272	50.5198	20	0.633	4.526	0.037	5.375	-0.007	3	2.990	1.498	20	0.532	2.429	0.176	3.375	**0.360**	-	-	-	-	-	-
		12	Fra10-3	1.606983	50.789567	20	0.621	4.143	0.043	4.875	-0.080	3	2.995	1.499	22	0.406	2.012	0.021	2.375	**0.312**	-	-	-	-	-	-
		13	Fra10-2	1.59455	50.818483	20	0.612	3.963	0.047	4.625	-0.053	2	1.998	1.499	22	0.432	2.162	0.117	3.000	**0.271**	-	-	-	-	-	-
	Haute Normandie	14	Fra9-3	0.159	49.40955	20	0.728	5.175	0.095	6.375	-0.022	5	4.599	1.900	-	-	-	-	-	-	2	-	-	-	-	-
		15	Fra9-1	0.2134	49.424833	2	-	-	-	-	-	2	-	-	23	0.453	2.121	0.098	2.750	**0.185**	-	-	-	-	-	-
		16	Fra8-1	-1.456083	49.691367	15	0.635	5.122	0.197	6.000	0.062	4	3.582	1.646	2	-	-	-	-	-	-	-	-	-	-	-
		17	Fra8-2	-1.40455	49.702683	14	0.623	4.940	0.323	5.875	0.012	5	4.775	2.188	-	-	-	-	-	-	3	-	-	-	-	-
		18	Fra8-4	-1.2419	49.646067	12	0.630	4.633	0.035	5.000	0.026	3	2.987	1.497	23	0.423	2.045	0.003	2.750	**0.409**	9	0.461	1.949	0.250	2.375	0.181
	Basse Normandie	19	Fra7-4	-1.445125	48.654778	-	-	-	-	-	-	-	-	-	23	0.342	1.843	0.020	2.375	**0.128**	20	0.344	1.896	0.082	2.375	**0.285**
		20	Fra7-2	-1.572704	48.750652	20	0.699	4.584	0.049	5.250	0.007	3	2.671	1.500	1	-	-	-	-	-	-	-	-	-	-	-
		21	Fra7-1	-1.568002	48.803328	20	0.682	5.540	0.069	7.250	0.067	7	5.889	2.127	24	0.343	1.813	0.018	2.500	0.072	5	-	-	-	-	-
		22	Fra7-1No	-1.569374	48.806191	-	-	-	-	-	-	-	-	-	23	0.387	1.903	0.029	2.375	0.100	9	0.358	1.776	0.081	2.375	0.026
		23	Fra6-4	-3.075539	48.8283	-	-	-	-	-	-	-	-	-	6						-	-	-	-	-	-
	Bretagne	24	Fra4-1	-3.359300	47.687899	18	0.700	5.420	0.124	7.125	**-0.094**	4	3.892	1.723	14	0.353	1.839	0.058	2.250	**0.330**	-	-	-	-	-	-
		25	Fra4-2	-3.3576	47.693033	-	-	-	-	-	-	-	-	-	23	0.217	1.559	0.010	2.125	**0.481**	-	-	-	-	-	-
		26	Fra4-3	-3.241350	47.662483	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	14	0.223	1.482	0.236	1.375	**-0.813**
		27	Fra4-4	-3.213583	47.645483	-	-	-	-	-	-	-	-	-	21	0.457	2.209	0.028	3.125	**0.409**	-	-	-	-	-	-
	Poitou-Charente	28	Fra2-2	-1.068333	45.955383	20	0.692	5.637	0.213	7.875	-0.011	9	6.882	2.735	21	0.396	1.998	0.000	2.625	**0.169**	-	-	-	-	-	-
		29	Fra2-3	-1.111200	45.953467	19	0.732	5.671	0.170	7.250	0.037	5	3.987	1.920	21	0.392	1.961	0.053	2.500	0.107	23	0.408	2.112	0.486	3.000	**0.483**
		30	Fra2-5	-1.139200	45.821883	16	0.747	5.763	0.215	6.875	0.028	4	3.947	1.737	-	-	-	-	-	-	20	0.293	1.465	0.157	1.750	**0.428**
Spain	Cantabria	31	Esp2-1	-4.329317	43.38925	3	-	-	-	-	-	2	-	-	18	0.415	2.125	0.020	2.875	0.039	1	-	-	-	-	-
		32	Esp2-3	-4.37125	43.391217	5	-	-	-	-	-	1	-	-	20	0.267	1.646	0.003	2.000	0.050	2	-	-	-	-	-
		33	Esp2-4	-4.37205	43.390683	1	-	-	-	-	-	1	-	-	23	0.385	1.909	0.005	2.375	**0.192**	6	-	-	-	-	-
		34	Esp2-5	-4.373417	43.38995	4	-	-	-	-	-	1	-	-	21	0.498	2.222	0.074	3.000	-0.002	1	-	-	-	-	-
	Asturias	35	Esp3-2	-5.879117	43.624783	15	0.385	2.485	0.142	2.750	0.118	2	1.963	1.241	-	-	-	-	-	-	21	0.189	1.315	0.135	1.125	**-0.761**
		36	Esp3-3	-5.91265	43.608683	14	0.639	4.744	0.086	5.500	**0.128**	2	2.000	1.250	4	-	-	-	-	-	-	-	-	-	-	-
	Galicia	37	Esp4-2	-7.794517	43.725417	15	0.581	4.138	0.168	4.750	**0.139**	3	2.790	1.645	3	-	-	-	-	-	5	-	-	-	-	-
		38	Esp5-3	-9.06135	42.760383	14	0.630	4.778	0.155	5.500	-0.081	4	3.861	2.249	-	-	-	-	-	-	1	-	-	-	-	-
Portugal	Lisboa	39	Port3-1	-9.419167	38.961967	14	0.672	4.303	0.074	4.750	0.044	5	4.791	2.397	20	0.408	2.126	0.390	3.125	0.092	-	-	-	-	-	-
	Setúbal	40	Port4-3	-8.791217	37.829617	9	0.752	6.875	0.643	6.875	**0.120**	5	5.000	2.750	-	-	-	-	-	-	1	-	-	-	-	-
	Faro	41	Port5-2	-8.454083	37.091367	14	0.629	4.662	0.245	5.375	**0.158**	3	2.997	1.749	-	-	-	-	-	-	1	-	-	-	-	-
		42	Port5-3	-8.413067	37.090733	5	-	-	-	-	-	1	-	-	-	-	-	-	-	-	3	-	-	-	-	-
Spain	Andalucia	43	Esp7-1	-5.908133	36.18435	20	0.751	6.302	0.500	8.750	-0.025	2	1.671	1.336	-	-	-	-	-	-	19	0.409	1.678	0.462	2.75	**0.389**
		44	Esp7-2	-5.851783	36.139833	14	0.742	6.499	0.662	7.750	0.026	4	3.949	1.737	-	-	-	-	-	-	2	-	-	-	-	-

four minisatellite loci as a single haplotype in subsequent analyses (see Fievet et al. 2007; De Cauwer et al. 2010; Leys et al. 2014). Overall, a total of 511 individuals were genotyped for both kind of markers (13±1.1; mean per population ± standard error, see Table 1).

Sequencing

Increasing the number and kinds of molecular markers improve the accuracy of inferences of phylogeographical histories (Hickerson et al. 2010; Robinson et al. 2014). We thus sequenced additional mitochondrial and nuclear loci in both nematode species, and chloroplastic and mitochondrial loci in the sea beet (Appendix 1). We did not include sequence data in this chapter because the 454 sequencing of nematode samples was delayed to December 2014.

Data analyses

The number of sampled cysts in *H. schachtii* and *H. betae* ranged from 1 to 24 per population (Table 1). Similarly, sea beet population samples comprised 1 to 20 individuals. Small sample size can result in artifacts in frequency-based analyses. Therefore, we only considered populations with a minimum of nine individuals for frequency-based analyses, while using all individuals in other analyses like Bayesian clustering. Besides, because the sea beet has a coastal distribution, we considered its habitat to be unidimensional at the scale of our investigation. In all analyses, geographical coordinates of populations thus corresponded to their costal distance to the southernmost sampled population, for which we arbitrarily set to zero its coordinate.

Genetic diversity characterization

Genetic variation was investigated using classical descriptive summary statistics. Number of alleles (Na), allelic richness (Ar) and expected heterozygosity (He) were computed using the software FSTAT version 2.9.3.2 (Goudet 1995). Linkage disequilibrium among all pair of loci and departures from Hardy-Weinberg (HW) equilibrium were also tested using permutations procedures implemented in FSTAT. Departures from HW expectations were assessed using the estimation of intra-population fixation index (F_{IS}). F_{IS} significance was assessed through a randomization procedure applying sequential Bonferroni corrections for multiple comparisons (Rice 1989). Additionally, private allelic richness was also computed for all populations considering the rarefaction method implemented in the ADZE software version 1.0 (Szpiech et al. 2008).

We tested of the relationship between estimates of genetic diversity and coastal distances using both simple linear and piecewise regression models that combined two linear regressions. All possible piecewise regression models were built by dividing the data set into two parts, each containing different combination of populations. The threshold delimiting the two geographical data sets varied among models: the first model combined a linear regression computed with the two southernmost populations and a linear regression computed with all other populations; the second model was computed for a combination of a regression with the three southernmost populations and a regression computed with all other populations, and so on. These regressions were performed using the R software version 3.1.1 (R Development Core Team 2014). In addition, likelihood-ratio tests were also implemented in R to test whether each piecewise regression model better fit the data than the simple linear regression.

Spatial distribution of genetic diversity

Geographical distribution of genetic diversity among populations was first investigated using spatial Principal Component Analysis (sPCA). sPCA is a powerful exploratory tool which is not based on any population genetic assumptions like departures from HW or linkage equilibrium (Jombart et al. 2008). This method summarizes genetic variation into synthetic variables maximizing the product of the variance of those synthetic variables and of the Moran's *I* index (*i.e.* spatial autocorrelation). A network based on relative neighbors was used for calculating Moran's *I*. Indeed, this type of network gives the highest values of spatial autocorrelation in the three species and picture the most faithfully connections among nematode and sea beet populations given their low dispersal capabilities. Synthetic components can be positive or negative reflecting respectively global, with positive spatial autocorrelation among genetic units, or local structures, with negative spatial autocorrelation among genetic units. To draw a comprehensive synthetic representation of sPCA scores, each of the first two principal component scores were simultaneously represented into a channel of colors, as described in Menozzi et al. (1978). All analyses and significance of global and local structures were performed using the R package ADEGENET (Jombart 2008a).

Large-scale population genetic structure was also investigated using the Bayesian clustering algorithm implemented in Structure 2.3.3 (Pritchard et al. 2000). This method probabilistically assigned individuals to one or several genetic clusters according to allelic frequencies of each locus and ancestral origins of individuals (Falush et al. 2003; Hubisz et al. 2009). No *a priori* assumption on individuals' population affiliation was made. For each species, *K* values ranging from 1 to the maximum number of populations were tested using

runs with 3.10^6 iterations and a burnin period of $1.\ 10^6$. Admixture ancestry with correlated allele frequencies was assumed and 10 independent runs were performed for each *K* tested to check consistency of results. CLUMPP v1.1 was used to find the best alignment over all runs (Jakobsson and Rosenberg 2007). Mean values of log-likehood, L(K), and the ΔK statistic (Evanno et al. 2005) were both used to determine the optimal number of genetic clusters. Results were visualized using DISTRUCT (Rosenberg 2004).

Hierarchical population genetic differentiation

Hierarchical analyses of population structure were performed to quantify levels of genetic differentiation based on population grouping featured by piecewise regressions, sPCA and Bayesian clustering. In each case, the genetic differentiation was investigated among groups (geographical areas and genetics clusters), among populations within groups, and within populations. The estimation of variance components of each hierarchical level and the test for their statistical significance were performed with the R package Hierfstat (Goudet 2005), as described in De Meeûs and Goudet (2007).

Search for isolation by distance

Isolation by distance (IBD) testing may also give information on micro-evolutionary processes underlying population genetic structure such as gene flow and genetic drift (Holsinger and Weir 2009). Analyses were performed among all sampled populations and among populations within groups defined by piecewise regressions. Because the Atlantic coastline can be considered as a one dimensional habitat, we performed a linear regression between pairwise F_{ST} estimates and coastal distances among populations (see Rousset 1997). Mantel tests were used to evaluate the statistical significance of IBD patterns, as described in Smouse et al. (1986).

Genetic discontinuities in depicted IBD

Genetic discontinuities in population structure are generally associated with areas favoring or limiting dispersal of individuals among locations. Genetic discontinuities were identified in each species with a spatially explicit approach, called DresD (*i.e.* distribution of residuals dissimilarity, Keis et al. 2013). This analysis seeks geographically consistent patterns in the distribution of residuals in IBD, which are explored by regressing pairwise genetic distances over pairwise geographical distances. The DresD procedure is able to identify geographic areas where local genetic differentiation was too high (barriers) or too low (corridors) when compared with patterns expected under a pure isolation by distance model.

Concordance of population genetic structure among the three species

We used the statistical procedure described in Legendre and Lapointe (2004) to estimate the congruence among depicted genetic structure in sea beet and nematode populations. The CDAM (congruence among distance matrix) test is an extension of Mantel test allowing for comparison of more than two distances' matrix. We thus computed the genetic distance of Nei (1972) among pairwise populations for each species and tested the significance of congruence among all matrix using CDAM implemented in the ape package of the R framework (Legendre and Lapointe 2004). Owing to the variation in the co-distribution of species, subsets of eight populations and four populations were selected for analyses between the sea beet and *H. schachtii* and *H. betae*, respectively. This analysis was also performed between the two nematodes considering only four common populations.

RESULTS

Spatial distribution of levels of genetic diversity

After Bonferroni correction, no significant linkage disequilibrium were detected in the 16,240, the 11,200 and the 4,480 comparisons performed among all loci and all populations of *B. vulgaris* spp. *maritima*, *H. schachtii* and *H. betae*, respectively. For sea beet populations, 6% (2%) out of 232 single-locus F_{IS} values were significantly positive (negative). These departures from HW equilibrium were not associated to particular loci or populations suggesting spurious effects of genetic drift and/or genetic sub-structuring. Overall mean multilocus F_{IS} estimate was significant ($F_{IS} = 0.020$; $P < 0.05$) and mean single-locus values ranged from -0.094 to 0.158 (Table 1).

In *H. schachtii* (*H. betae*) populations, F_{IS} values ranged from -0.002 (-0.813) to 0.481 (0.483), also suggesting departures from panmixia (Table 1): 26% (2%) out of 160 single-locus F_{IS} values of *H. schachtii* were significantly positives (negatives), and 19% (8%) out of 60 single-locus F_{IS} values estimated in *H. betae* were significantly positive (negative). Overall mean multilocus F_{IS} values were also significantly positive (*H. schachtii*: F_{IS}=0.197; *H. betae*: F_{IS}=0.135; all: $P < 0.05$), and higher as compared to sea beet populations.

For sea beet populations, a total of 172 alleles were observed for nuclear loci, ranging from 8 (Gtt1) to 36 (SB07) alleles (Table 2). Mean multilocus He was of 0.612 (Table 1). For mitochondrial minisatellite loci, a total of 31 haplotypes were observed; 8 of which represented 80.55% of the total number of haplotypes. All estimators of nuclear and mitochondrial diversity showed a significant northward decrease from southern Spain to Sweden (H_S: r^2=0.308; Ar: r^2=0.390; ArP: r^2=0.397; all at $P < 0.001$; ArHap: r^2=0.137; $P <$

0.05). For nematode species, nuclear polymorphism was much lower with 48 and 50 alleles observed in *H. schachtii* and *H. betae*, respectively, ranging from 2 (Hs36) to 9 or 10 (Hs84), (Table 2). There was no significant correlation between any measures of genetic diversity and coastal coordinates in nematode species.

For the three species, piecewise regressions between most of estimators of genetic diversity and coastal coordinates better explained the data than a simple linear regression (Figure 2). For sea beet populations, samples from the Iberian Peninsula were separated from all other populations regarding nuclear allelic and mitochondrial haplotypic richness (Fig. 2B, D). The same separation was observed with the analysis on expected heterozygosity, but only with the third best piecewise regressions model. The first model separated Danish and Swedish populations from all other populations (Fig. 2A). Piecewise regressions performed on private allelic richness separated the four most southern populations from the rest of European populations (Fig. 2C). For populations located in the Iberian Peninsula, allelic richness and expected heterozygosity decreased significantly northward (H_S: r^2=0.506; Ar: r^2=0.499; all at $P < 0.05$), but not cytoplasmic genetic diversity and private allelic richness. In contrast, populations distributed from the West of France to Sweden showed a significant decrease of nuclear and cytoplasmic genetic diversity northward (H_S: r^2=0.740; Ar: r^2=0.858; ArHap: r^2=0.702; all: $P<1.10^{-4}$; Fig. 1B, C and E).

Piecewise regressions split genetic diversity of the two nematodes into groups that were similar to groups defined for the sea beet. Spatial trends in levels of allelic richness and expected heterozygosity in *H. schachtii* separated populations in the North of Brittany from all populations located in the South of this peninsula. Piecewise regressions for private allelic richness show similar patterns except that part of Spanish populations split from the rest of populations (Fig. 2E, F and G). Splitting of the dataset occurred thus in a similar geographical area for the three estimators. In the most southern group, genetic diversity did not show any correlation with increasing northward coastal coordinates, except for private allelic richness which significantly decrease northward (ArP: r^2=0.96, $P < 0.01$; Fig. 1H). In contrast, in the second group of populations, allelic richness and private allelic richness showed a significant southward increase and the same pattern was almost significant for expected heterozygosity (H_S: r^2=0.272, P=0.058; Ar: r^2=0.563; ArP: r^2=0.215, both: P<0.05; Fig. 2E, F and G).

In *H. betae*, piecewise regressions performed on expected heterozygosity and private allelic richness split the datasets into two groups that were similar to what was observed for both *B. v.* ssp *maritima* and *H. schachtii* (Fig. 2H and J). In contrast, there was no model significantly better than the global linear regression for allelic richness (Fig. 2I). No

significant correlation was observed between all genetic diversity estimators and coastal coordinate in all of these groups (Fig. 1I, J and K).

Table 2: Genetic diversity and Weir and Cockerham (1984) F-statistics estimated over all *Beta vulgaris* subsp. *maritima* (n = 29), *H. schachtii* (n = 20) and *H. betae* (n=8) populations for eight nuclear microsatellites and four cytoplasmic minisatellite loci.

Locus	An	Hs	F_{ST}	F_{IS}
B. v. spp. maritima				
FDSB1027	17	0,562	0.204	0.030
Bvm3	32	0.740	0.168	0.005
Caa1	34	0,716	0.201	0.033
Gtt1	8	0,378	0.126	-0.031
Sb04	17	0,584	0.234	**0.097**
Sb06	11	0,647	0.166	-0.007
Sb07	36	0,665	0.210	**0.056**
Sb15	17	0,606	0.139	-0.037
Average	21.5	0,612	0.185	**0.020**
H. schachtii				
Hs55	3	0,238	0,269	-0.009
Hs68	8	0,533	0,196	**0.522**
Hs33	7	0,253	0,279	0.062
Hs36	2	0,269	0,166	0.009
Hs56	4	0,327	0,147	0.076
Hs84	9	0.540	0,328	**0.399**
Hs111	7	0,561	0,219	0.020
Hs114	8	0,515	0,255	**0.168**
Average	6	0,405	**0,241**	**0.197**
H. betae				
Hs55	10	0,328	0,569	-0.351
Hs68	5	0,304	0,657	**0.446**
Hs33	3	0,134	0,735	-0.092
Hs36	2	0,209	0,527	0.163
Hs56	4	0,166	0.740	**0.324**
Hs84	11	0,557	0,397	0.462
Hs111	6	0,407	0,474	**0.125**
Hs114	9	0,418	0,572	-0.107
Average	6.3	0,315	**0,577**	**0.135**

Population genetic structure

sPCA analyses performed on populations of sea beet revealed a significant global structure ($P < 0.001$), but no local structure ($P = 0.137$) for nuclear data. Neither global ($P = 0.053$) nor local structure ($P = 0.821$) was found for mitochondrial data. Global patterns were captured by the first two axes of sPCA ($I = 0.658$, var = 0.230 and $I = 0.695$, var = 0.123, respectively). Four different geographic entities can be identified on the resulting synthetic map of principal component scores: (1) the Swedish and Danish coasts, (2) from the North Sea coasts to the

South of Brittany, (3) the rest of the Bay of Biscay and (4) the South of the Iberian Peninsula (Fig. 3A). No significant global and local structures were detected in *H. schachtii* and *H. betae*. In *H. schachtii*, most of variability was captured by the two first axis of the sPCA which allowed the identification of two geographic entities: (1) from the Danish coastlines to the South of Brittany and (2) the rest of the Bay of Biscay with the Iberian Peninsula (Fig. 3D). In *H. betae*, only the first axis accounted for the observed genetic variability, which revealed two geographical entities: (1) all the French coastline together with the North-East of Spain and (2) the West and South of the Iberian Peninsula (Fig. 3G).

Likewise, Bayesian clustering analysis identified 4, 3 and 2 clusters in the whole datasets of *B. v.* spp. *maritima*, *H. schachtii* and *H. betae*, respectively. The evolution of log-likelihood probability of the data and the ΔK statistics can be visualized in Figure S1. For sea beet populations, genetic clusters were characterized by low levels of admixture and corresponded to the four distinct geographical areas identified by sPCA analyses (Fig. 3B, 3C). In nematode species, genetic clustering did not match any clear geographical areas (Fig. 3E, 3F and 3H, 3I). However, populations of both species showed rather low levels of genetic admixture. This observation was particularly true for *H. betae* for which only two populations showed potential admixture.

Population genetic differentiation, isolation by distance, and genetic discontinuities

For sea beet populations, hierarchical analyses showed discrepancies between the genetic variance partitioning resulting from piecewise regressions, sPCA and Bayesian clustering analyses. For piecewise regressions, most of the genetic differentiation occurred among populations ($F_{Populations/Groups}$=0.166, $P < 0.001$; Table 3). In contrast, population structure analyses partitioned populations into groups which had the strongest significant genetic differentiation observed among all hierarchical levels (sPCA: $F_{Groups/Total}$=0.119; Bayesian clustering: $F_{Clusters/Total}$=0.124; both at $P < 0.001$; Table 3). For nematode species, hierarchical analyses showed that most of the genetic variation occurred among populations of the two species, independently of the global partitioning of populations (Table 3), suggesting a very strong influence of local processes on the evolution of populations. *H. betae* showed higher average value of *F*-statistic among populations than *H. schachtii* (mean value: *H. betae*: $F_{Populations/Groups}$=0.510; *H. schachtii*: $F_{Populations/Groups}$=0.217), suggesting a stronger structuring

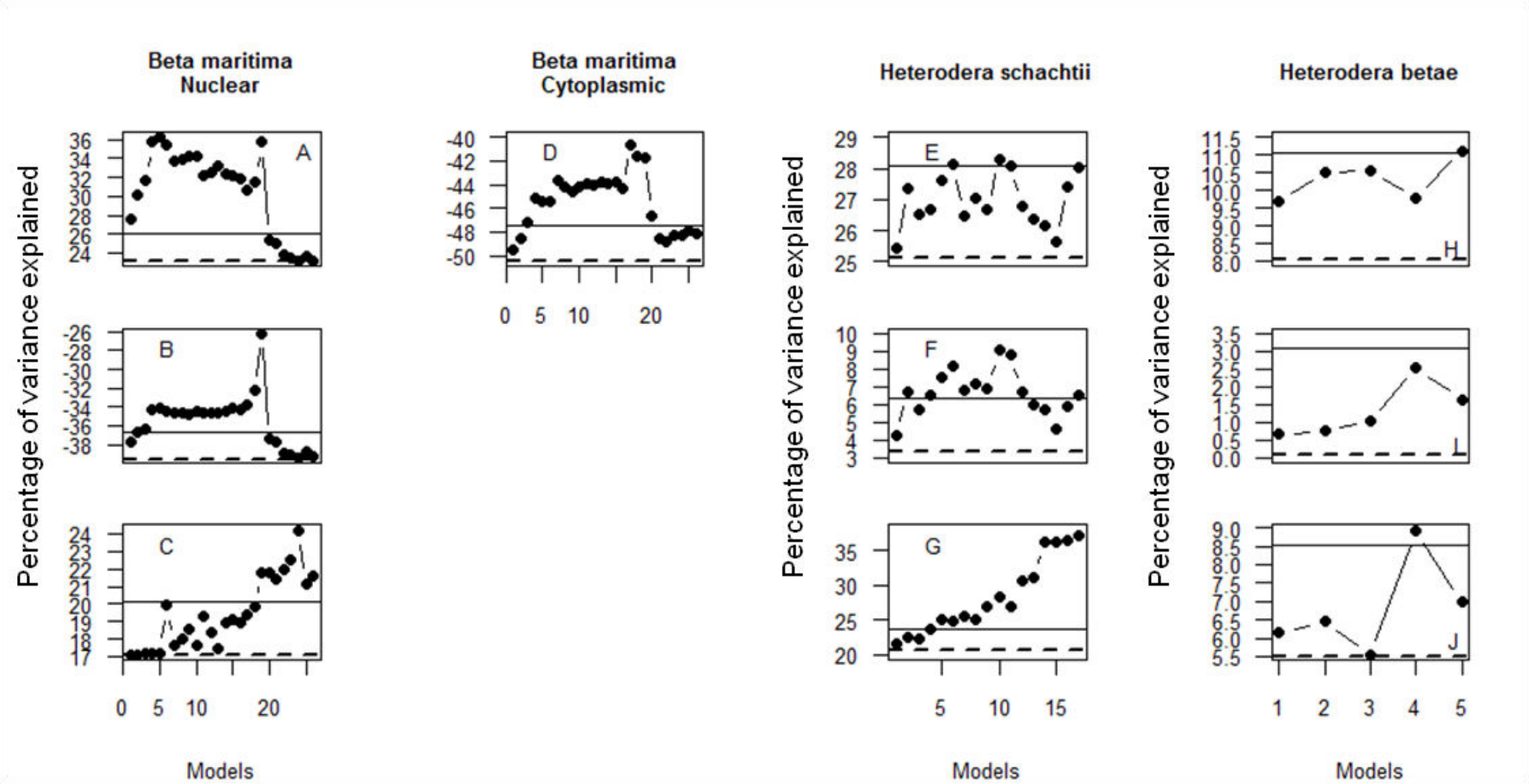

Figure 2: Percentage of variance explained by models of piecewise regressions of genetic differentiation versus coastal distances. Model 1 gives the variance explained by a regression computed with the two southernmost populations and a regression computed with all other populations; Model 2 gives the variance explained by a regression computed with the three southernmost populations and a regression computed with all other populations and so on. Dashed lines represented the percentage of variance explained by a simple linear regression, without splitting the dataset. Plain lines corresponded to the upper limit of the 95% confidence interval of the percentage of variance of the simple regression: piecewise regression models that were significantly better than the simple regression model are above these lines (likelihood-ratio tests; d.f. = 2; $P < 0.05$).

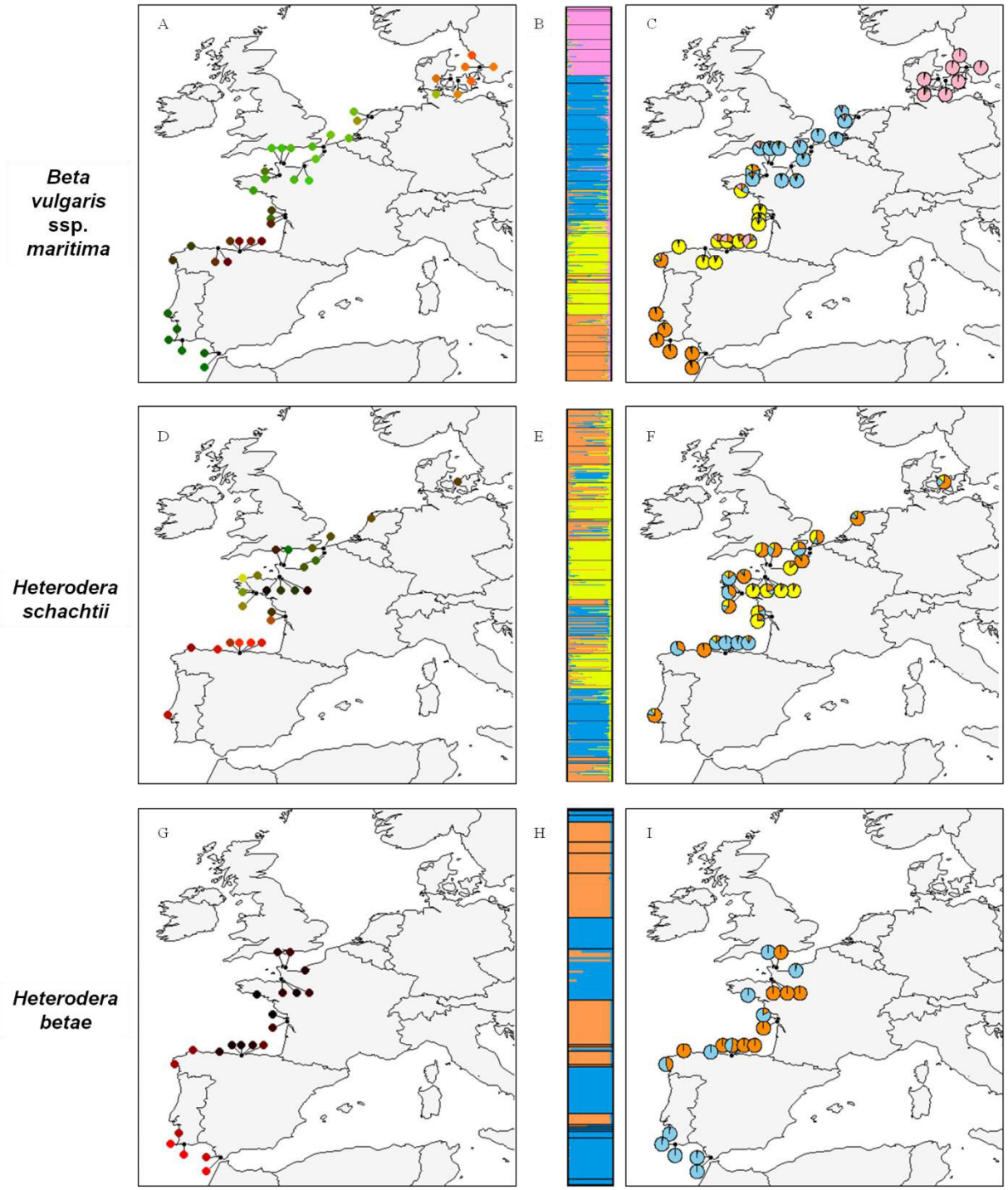

Figure 3: Population genetic memberships for *Beta vulgaris* ssp. *maritima*, *Heterodera schachtii* and *Heterodera betae*. A, D, G: synthetic maps of first sPCA scores. B, E, H: Barplots of individual probabilities of memberships. C, F, I: Map of mean population membership probabilities for the modal K clusters. A, B: *Beta vulgaris* ssp. *maritima*; C, D: *H. schachtii*; E, F: *H. betae*.

of populations of *H. betae*. Nevertheless, in contrast to *H. schachtii*, variance partitioning at the upper hierarchical level in *H. betae* varied among the different analyses (Piecewise regressions: $F_{Groups/Total}$=0; sPCA: $F_{Geographical\ groups/Total}$=0.119; Bayesian clustering: $F_{Clusters/Total}$=0.420). The highest differentiation value corresponded to a partitioning which did not take the geographical localization of populations into account. This suggests that distant populations of *H. betae* can be either extremely similar or extremely different.

In *Beta vulgaris* spp. *maritima*, DresD analysis identified significant departures from pure isolation by distance model in three geographical areas (Fig. 4A). Populations of the North West of the Iberian Peninsula and populations of Swedish and Danish coasts were characterized by a higher genetic differentiation than expected under a pure IBD model assuming migration/drift equilibrium. These geographical areas may probably correspond to barriers of gene flow among sea beet populations. In contrast, populations of the North West of France showed a lower genetic differentiation than expected under IBD. As a result, this region should correspond to a corridor for dispersal. DresD analyses did not identify any genetic discontinuities for nematode species. However, *H. schachtii* and *H. betae* showed a negative and a positive relationship with coastal distances which suggest two different colonization routes of European beaches for the two nematodes species (Fig. 4B and C).

These observations were concordant with IBD analyses. Indeed, in sea beet populations, a significant IBD was observed for nuclear and cytoplasmic data among populations of the Iberian Peninsula (rz = 0.494; rz = 0.322; all at $P < 0.05$; Fig. 5A and C). For populations distributed from France to Sweden, a significant IBD patterns was observed for nuclear data (rz = 0.645, $P < 0.01$; Fig. 5B), but not for cytoplasmic data (Fig. 5D). A significant IBD pattern was also observed among populations of *H. schachtii* distributed in the North of Brittany (rz=0.497, P = 0.01; Fig. 5F), but not among populations located in the South of this Peninsula (Fig. 5E). Global IBD pattern among all populations of *H. betae* was almost significant (rz = 0.70; P = 0.064; Fig. S2). Similarly, IBD pattern among populations of the West and the North-West of France was close to be significant (rz = 0.39; P = 0.051; Fig. 5G). There were not enough populations of *H. betae* to investigate IBD patterns in the most southern group of populations determined by piecewise regressions.

Table 3: Analysis of molecular variance according to populations partitioning by each analysis of genetic diversity structure.

Species	Analysis	Source of variation	Components of variance	Percent of variation	*F*-statistics
Beta v. spp. *maritima*	Piecewise regressions	Between groups	0.305	4.96	**0.050**
		Between populations within groups	0.972	15.8	**0.166**
		Within populations	0.099	1.61	**0.020**
		Total	4.773		
	sPCA	Between geographical groups	0.737	11.9	**0.119**
		Between populations within geographical groups	0.570	9.22	**0.105**
		Within populations	0.099	1.60	**0.020**
		Total	4.773		
	Bayesian clustering	Between genetic clusters	0.763	12.4	**0.124**
		Between populations within genetic clusters	0.536	8.69	**0.099**
		Within populations	0.099	1.6	**0.020**
		Total	4.773		
Heterodera schachtii	Piecewise regressions	Between groups	0.200	4.57	**0.046**
		Between populations within groups	0.928	21.2	**0.221**
		Within populations	0.646	14.7	**0.198**
		Total	2.611		
	sPCA	Between geographical groups	0.254	5.72	**0.057**
		Between populations within geographical groups	0.922	20.8	**0.221**
		Within populations	0.647	14.6	**0.198**
		Total	2.611		
	Bayesian clustering	Between genetic clusters	0.244	5.59	**0.056**
		Between populations within genetic clusters	0.863	19.8	**0.209**
		Within populations	0.647	14.8	**0.198**
		Total	2.611		
Heterodera betae	Piecewise regressions	Between groups	0.000	0	**0**
		Between populations within groups	3.377	57.7	0.577
		Within populations	0.363	6.20	0.146
		Total	2.116		
	sPCA	Between geographical groups	0.748	11.9	**0.119**
		Between populations within geographical groups	3.066	48.7	0.553
		Within populations	0.363	5.77	0.146
		Total	2.116		
	Bayesian clustering	Between genetic clusters	2.957	42.0	**0.420**
		Between populations within genetic clusters	1.632	23.2	0.400
		Within populations	0.330	4.69	0.134
		Total	2.118		

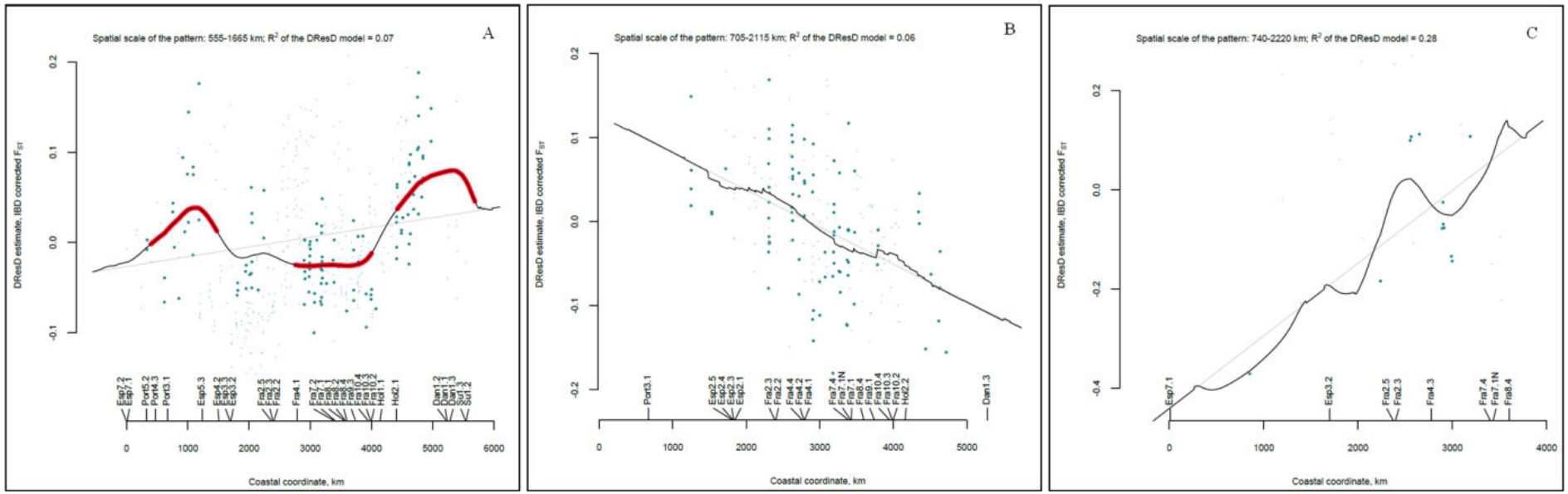

Figure 4: DresD residuals along coastal distances in *Beta vulgaris* ssp. *maritima*, *Heterodera schachtii* and *Heterodera betae*. Dispersion of IBD residuals (equivalent to IBD corrected FST) are represented according to population locations on the x axis. The plain line corresponded to a basal isolation by distance model. Red parts corresponded to geographical area which significantly departed from the basal model. Barriers can be suspected above the basal model and corridors can be suspected below the basal model. A: *Beta vulgaris* ssp. *maritima*; B: *H. schachtii*; C: *H. betae*. No significant phylogeographical breaks were observed in the two nematode species. *Heterodera schachtii* and *H. betae* also showed contrasting global differentiation patterns.

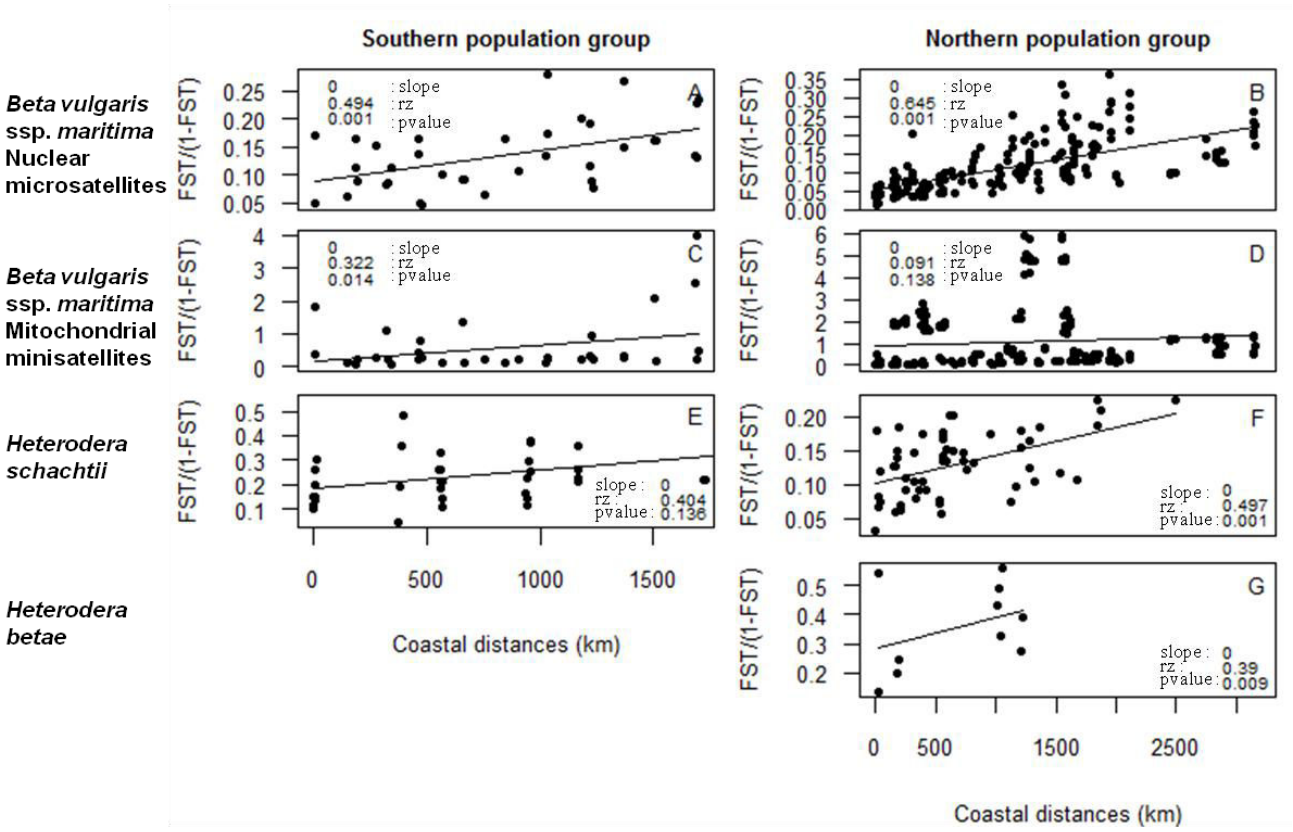

Figure 5: Isolation by distance in southern and northern populations groups defined by piecewise regressions in *Beta vulgaris* ssp. *maritima*, *Heterodera schachtii* and *Heterodera betae*. A, C, E: IBD over populations in the southern areas; B, D, F, G: IBD over populations in the northern groups. A, B: *Beta vulgaris* ssp. *maritima*, nuclear markers; C, D: *Beta vulgaris* ssp. *maritima*, cytoplasmic markers; E, F: *H. schachtii*; G: *H. betae*. slope: slope of the linear regression model; rz : rz Mantel coefficient; pvalue : p-value of the Mantel test.

Concordance of population structure among the three species

We tested for congruence among genetic distance matrix of the three species using CDAM. Global CDAM tests between matrix of *B. v.* spp. *maritima* and *H. schachtii* and between *B. v.* spp. *maritima* and *H. betae* did not reject the null model of incongruence among matrix, suggesting different patterns of population genetic structure for each species.

DISCUSSION

This study aimed at investigating and comparing the phylogeography of two phytoparasitic crop nematodes and their wild host plant to determine whether these species share some part of their evolutionary histories. Our data strengthen the postglacial northward recolonization scenario of the shoreline observed in a previous work on sea beet populations distributed from Morocco to Brittany (Leys et al. 2014). Our results also suggest that the history of recolonization may have been more complex than expected. Results on *H. schachtii* show very different patterns of population genetic structuring than those observed among sea beet

and *H. betae* populations. Overall, this study emphasized the contrast between the large-scale geographical structuring of genetic diversity observed in the host plant and the lack of concordant patterns in population genetic structures of both nematodes which are probably influenced by local and regional micro-evolutionary processes.

A southern origin for nematodes and sea beets?

Analyses of genetic diversity levels and spatial structure split the Atlantic coastline into southern and northern homogeneous groups, which are approximately identical among the host plant and the two nematode species. This suggests that similar biogeographical events may have influenced the spatial genetic structure of the three species.

In sea beet populations, the southern group is composed of populations of the Iberian Peninsula and is characterized by a significant northward decrease in nuclear genetic diversity and a significant IBD pattern. Such decreasing pattern of diversity may result from sequential founder events along the shoreline implying a small number of colonizers. However, IBD could either result from this colonization process or from short-range dispersal among sea beet populations (Ibrahim et al. 1996; Austerlitz et al. 1997; Puechmaille et al. 2011). This suggests a gradual northward recolonization of the Iberian Peninsula, as reported in previous work (Leys et al. 2014). In contrast, cytoplasmic genetic diversity did not show any northward decrease. First, the influence of genetic drift is stronger on cytoplasmic markers due to their lower effective population size and may erase any genetic signatures of leading edge expansions. Second, previous work evidenced that kin-structured founder events and recurrent arrival of seeds from distant populations may also strongly affect the spatial distribution of cytoplasmic genetic diversity within populations (De Cauwer et al. 2010). Therefore, at large spatial scale, the combination of these processes over time may lead to the faster disappearing of the colonization signal in mitochondrial genomes.

In nematodes, the southern group is also composed of populations from the Iberian Peninsula, but also includes populations from southern-western France. This split is not always exactly the same depending on the genetic diversity estimators used. Additionally, the small number of populations considered in analyses based on allelic frequencies may have lead to a reduced statistical power of piecewise regression analyses. Nevertheless, this split is supported by the concordant opposition of Spanish populations and populations of southern-western France with the rest of the dataset for both nematode species. In this southern biogeographical nematode entity, the genetic diversity did not show any correlation with coastal coordinates nor isolation by distance. Considering the northern limit of species

distribution ranges and their temperature optima, it can be assumed that the two nematodes species were distributed farther in the South during the last glaciations (Thomason and Fife 1962; Griffin 1981; Maas and Heijbroek 1982; Gracianne et al. 2014). Therefore, two potential colonization scenarios of northern European areas can be put forward.

First, nematodes may have followed the sea beet post-glacial spread and recolonized Europe along the coastline. In this case, a faster evolution of nematode populations would explain the lack of reduced genetic diversity expected for front-edge populations experiencing serial bottlenecking (see Waters et al. 2013). Indeed, this is in agreement with other work that reported substantial influence of genetic drift on local genetic structure of populations of *H. schachtii* due to limited dispersal, short generation time and small effective population size (Jan et al. in review; Gracianne et al. in review). These life-history traits may have led to independent evolution of populations, which favors the loss of post-glacial recolonization signal over time. Precise knowledge on biological traits for *H. betae* is still lacking. However, this species is thought to be parthenogenetic, which may explain the stronger genetic differentiation observed among populations of *H. betae* than among *H. schachtii* populations.

Second, the nematodes did not follow their host's post-glacial recolonization route but recolonized Western Europe using alternative hosts distributed along the shoreline. In this case, independent colonization events of sea beet populations may have occurred along the shoreline and/or inland. This resulted in non-genetically related coastal nematode populations that depart from biogeographical patterns observed in sea beets. Limited dispersal events along with moderate effective population sizes may then have favored a mosaic of genetically differentiated populations with divergent evolutionary trajectories.

In *H. schachtii*, the second colonization scenarios may be supported by results of population structure which did not evidence any clear spatial genetic structure. Likewise, hierarchical analyses of genetic differentiation showed that the apportionment of genetic variation may be explained by evolutionary processes occurring among populations, *i.e.* at local and/or regional spatial scale. It should be noted that spatial genetic structure in *H. betae* did not show clear patterns of genetic structure probably because of the smaller number of populations sampled in this study. On the other hand, higher private allelic richness was observed in populations of the southern group, supporting a potential origin and/or an older colonization of southern areas. Concordantly, sPCA and Bayesian analyses featured a common ancestry among Portuguese and Andalusian populations, which may favor the first colonization scenario.

A two-step recolonization of Europe?

In the northern group of sea beet populations, nuclear and cytoplasmic genetic diversity significantly decreased northward and this pattern was associated with a significant isolation by distance, suggesting a northward recolonization by sequential founder events. The occurrence of IBD in cytoplasmic data suggests this recolonization of northern areas was more recent.

Surprisingly, a highly localized hotspot of genetic diversity was found in Brittany. High levels of genetic diversity may be produced by admixture between genetically differentiated colonizing lineages in northward part of sea beet distribution range. Such an increase of genetic diversity was unexpected because sequential founder events generally result in a decrease of genetic diversity, particularly when they are associated with limited gene flow among populations (Austerlitz et al. 1997). A recent study reported high levels of genetic diversity soon after the colonization of a new habitat, *i.e.* after a drastic founder event involving adaptive evolution through rapid population growth over a very short evolutionary time scale (de Bruyn et al. 2014). Nevertheless, such a scenario may be unlikely in sea beet populations because it requires the "right environmental conditions which are potentially difficult to gather" (de Bruyn et al. 2014). Alternatively, the reestablishment of gene flow among populations which were disconnected during a prolonged period may result in the observed increase of genetic diversity on the French West coast. Indeed, specific alleles may accumulate in isolated populations and be spread after the end of isolation. A simulation study showed that this phenomenon creates a peak of diversity which can be conserved for a long time in populations (Alcala et al. 2013).

The isolation of sea beet populations distributed in the West of France may have happened owing to climatic oscillations observed after the Last Glacial Maximum (LGM). 13,000 years ago, climate warmed favoring the retreat of polar fronts and probably the northward expansion of southern species (Bonifay 1992). Nevertheless, climatic conditions were then degraded during the periods of the Bölling-Alleröd (-12,700; -11,900 years before present (BP)) and of the Younger Dryas (-10,950; 10,150 years BP) where temperatures dropped dramatically (Isarin and Bohncke 1999; Fig. S3). During these periods, polar fronts progressed southward, but not as far as during the LGM suggesting the southern limit of permafrost was localized more in the North than during the LGM (Bonifay 1992; Waters et al. 2013). Thus, sea beet populations may have persisted at upper latitudes than during the LGM. Isoclines reconstruction of the Younger Dryas supports this hypothesis and shows that temperatures reached a maximum of 14°C in July in the South of Brittany in the West France

(Isarin and Bohncke 1999; Fig. S3). This temperature corresponds to the isotherm observed at the current northern range limit of sea beet and suggests that sea beet populations were able to persist in this region during the Young Dryas period. This long decrease in temperature at global scale may have disturbed long-distance gene flow among sea beet populations which may have temporary isolated Iberian and French populations of sea beet. As a result, the West coast of France may have played the role of a cryptic northern refugium for the sea beet during the periods of the Bölling-Alleröd and the Younger Dryas.

Refugia are defined as locations where populations survived periods of regionally adverse climate, and should correspond to a contraction of the whole species range (Stewart et al. 2010). For the sea beet, range contraction may have occurred because climate cooling probably led to the extinction of populations which were able to reach higher latitudes than Brittany during the climate warming at the beginning of the Bölling-Alleröd (Stewart et al. 2010). Nevertheless, the presence of refugia should be independently inferred from fossil record, phylogeographical and species distribution modeling studies (Gavin et al. 2014). Radiocarbon studies on soil macrofossil charcoal evidenced that the South of the Bay of Biscay was a cryptic refugia for European beech during the Older Dryas (-16,300; -12,700 years BP), which was one of the coldest interval of the last glacial period (de Lafontaine et al. 2014a; de Lafontaine et al. 2014b).These findings of cryptic refugia north of well-established southern peninsula refugium is consistent with results on various plant species (Tzedakis et al. 2013). This suggests that in warmer periods such as the Bölling-Alleröd and the Younger Dryas, populations of sea beet may also have persisted in the West of France. This area was already reported as potential refugia in several phylogeographical studies on terrestrial species such as plant pathogenic fungus, orchid and hair-grass, which give additional supports to this refugia hypothesis (Pfeifer et al. 2009; Vercken et al. 2010; Harter et al. 2014).

Nematode data are also compatible with a potential influence of past climatic fluctuations of the Bölling-Alleröd and the Younger Dryas on their population genetic structures, as evidenced by the split between southern and northern *H. schachtii* populations groups observed in the sPCA. The weaker signal of this event in nematode genetic data may be related to a lack of diversity of microsatellite markers and/or to the quicker loss of the genetic signature of this event due to marked influence of local genetic drift. However, our results supported different recolonization scenarios for *H. schachtii* and *H. betae*. The genetic diversity of the northern group of *H. schachtii* populations significantly increased northward and was associated to a strong IBD pattern. This pattern is concordant with the northward increase of genetic differentiation evidenced by the DresD analysis, but may be counter-

intuitive. Indeed, it suggests a northern origin of migrants, which is in opposition with the potential southern origin of nematodes. Additionally, the IBD pattern suggests some genetic relationships between the closest populations, which are in contradiction with the unstructured distribution of population membership inferred by Bayesian clustering along the Atlantic coastline. A northward recolonization scenario, assuming an inland distribution of an alternative host-plant species with a subsequent infestation of coastal populations of sea beet, and occasional migration between inland and coastal nematode populations, may generate such patterns. None of these hypotheses can be tested with our dataset, because genetic information for nematodes populations distributed on inland alternative hosts is lacking. However, this scenario implies a recent infestation of sea beet populations, which may be consistent with the presence of an IBD signal occurring under migration-drift equilibrium.

In contrast with patterns observed in *H. schachtii*, the private allelic richness of *H. betae* increased in populations distributed in western France, and tended to decrease northward. It is worth noting that neither this pattern nor the related IBD were significant. The lack of significant results clearly evidenced low statistical power of analyses in this species. This may be related (i) to the small number of population included in allelic frequencies based analyses and/or (ii) to a lack of genetic diversity induced by the parthogenetic reproduction of this species or (iii) to ascertainment bias due to cross-species microsatellite amplification. However that may be, *H. betae* data showed concordant trends with patterns observed in the sea beet populations.

Marine currents and coastal dispersal

Spatial and clustering analyses evidenced additional genetic structuring in the northern and southern populations groups defined by piecewise regressions, which was particularly evident for the sea beet. Most locations of genetic changes correspond to well-known phylogeographical boundaries, and were clearly identified as barriers or corridors to gene flow among sea beet populations. Some of these genetic breaks also appear in nematode genetic structures.

A first genetic discontinuity was located on the Spanish Cape Finisterre in the North-West of the Iberian Peninsula and was described as a barrier to gene flow among sea beet populations (Leys et al. 2014). It is also identified in *H. betae*, but not in *H. schachtii*, which may be related to a stronger isolation of coastal populations from inland populations for *H. betae*. Although no genetic discontinuity is detected using the DresD analysis, spatial analyses and the Bayesian clustering performed on *H. betae* populations clearly separated populations

of the West and the Southwest of the Iberian Peninsula from the rest of populations. The North-West of the Iberian Peninsula was previously reported as a phylogeographical break in several marine species. Indeed, this area corresponds to the contact zone of two different water-masses which creates two distinct biogeographical areas in the North and the South of the Spanish Cape Finisterre (Piñeira et al. 2008; Fig. 6). Marine currents act as a physical barrier to dispersal in various marine species such as algae, marine snails, seahorses or fishes (Lundy et al. 1999; Piñeira et al. 2008; Woodall et al. 2011; Neiva et al. 2012), but also in another coastal plant species, *Eryngium maritimum* (Kadereit et al. 2005). Our results thus suggest that hydrodynamic characteristics of the Spanish Cape Finisterre may also influence population genetic structure of *H. betae* and its host plant, the sea beet, while it remain unclear for *H. schachtii*.

Another barrier to gene flow has also been detected among sea beet populations from the Channel and North Sea and populations from the East of the Danish peninsula. This genetic discontinuity was absent in both nematodes species, which was consistent with the absence of genetic structuring observed in *H. schachtii* and with the absence of *H. betae* populations in this area. Although incoming water masses from the North Sea make a counterclockwise loop in the Skagerrak (Fig. 6), this region has generally not been associated to a geographical break. Several studies have reported gene flow and low genetic differentiation among fish and coastal plants populations of the North Sea and the regions of Skagerrak and of Kattegat (Knutsen et al. 2004; Kadereit et al. 2005). However, further work evidenced that environmental specificities (*e.g.* weak marine current and lower salinity, Fig. 6) of this region lead to the establishment of a transition zone characterized by specific genotypes which have few exchanges with populations from the Baltic and the North Sea (Nielsen et al. 2004; Johannesson and André 2006; Limborg et al. 2012). As a result, ecological properties of this area may limit gene flow among marine populations from the North Sea and from the Kattegat.

Because the sea beet is a terrestrial coastal plant species, it should be not affected by this non-physical barrier. The genetic differentiation observed among North Sea populations and Kattegat populations would then result from the use of an alternative colonization route by Kattegat populations, as observed in *Triglochin maritima*. Indeed, one genetic group of this coastal plant species independently recolonized the Denmark and Sweden coasts using an inland route before the opening of the Baltic Sea (Lambracht et al. 2007). However, in our case study, this scenario is unlikely because inland sea beet populations are only found in human-made habitats in Mediterranean areas (Biancardi et al. 2011). Moreover, as observed

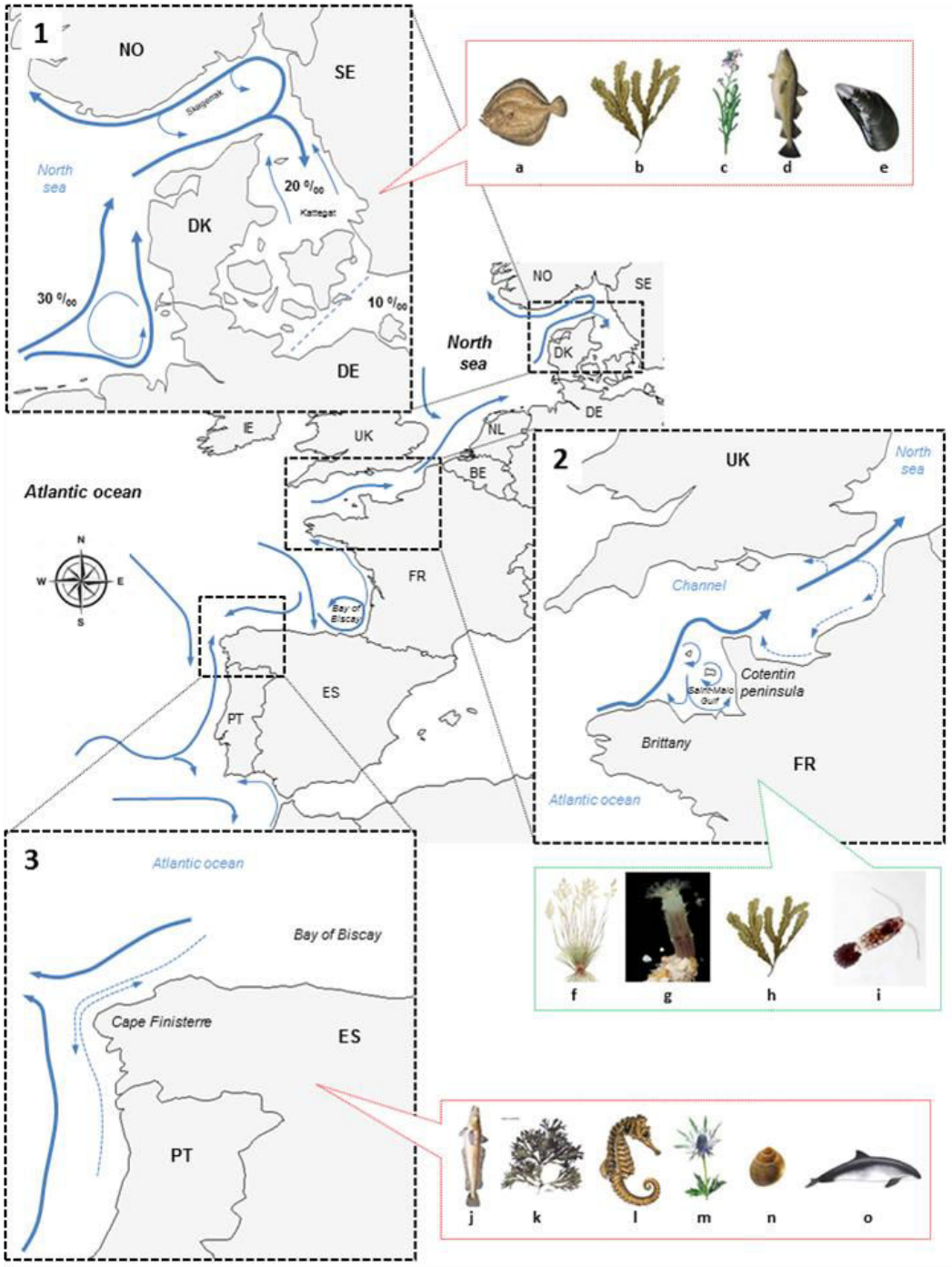

Figure 6: Main marine currents along the European Atlantic coastline and phylogeographical breaks observed in the sea beet and the two nematodes (after Lee and Ramster 1968; Salomon and Breton 1993; Krause et al. 1995; Koutsikopoulos and Le Cann 1996; Danielssen et al. 1997; Lazure and Jegou 1998; Barnay et al. 2003; Peliz et al. 2005; Johannesson and André 2006; Piñeira et al. 2008). Pictures corresponded to species evidencing similar barriers (in red) and corridor (in green) to dispersal when compared to sea beet genetic structure. Arrows with solid lines represented dominant currents. Arrows with dotted lines represented temporary currents associated with specific conditions. a: Scophthalmus maximus (Nielsen et al. 2004); b, h: *Fucus serratus* (Johannesson and André 2006); c: *Cakile maritima* (Kadereit et al. 2005); d: *Gadus morhua* (Nielsen et al. 2003); e: *Mytilus edulis* (Johannesson and André 2006); f: *Salsola kali* (Kadereit et al. 2005); g: *Owenia fusiformis* (Barnay et al. 2003); i: *Eurytemora affinis* (Winkler et al. 2011); j: *Merluccius merluccius* (Lundy et al. 1999); k: *Fucus ceranoides* (Neiva et al. 2012); l: *Hippocampus hippocampus* (Woodall et al. 2011); m: *Eryngium maritimum* (Kadereit et al. 2005); n: *Littorina saxatilis* (Piñeira et al. 2008); o : *Phocoena phocoena* (Fontaine et al. 2007).

for sea beet, most marine species of the Baltic Sea show very low levels of genetic diversity. These patterns were related to relatively recent range expansions in these areas due to the opening of the Baltic Sea, only 8,000 years ago (Björck 1995; Johannesson and André 2006). The subsequently isolation of the Baltic Sea populations promoted the effect of genetic drift which may have prevented genetic diversity recovering (Johannesson and André 2006). Finally, another plant species, *Cakile maritima*, that recolonized the Atlantic shoreline by the coast, showed genetically distinct populations from the North Sea and the Baltic Sea (Kadereit et al. 2005). To summarize, sea beet dispersal seems to be strongly related to marine currents, but also to ecological characteristics of the shoreline.

In addition to phylogeographical barriers, the DresD analysis evidenced the presence of a corridor of gene flow among sea beet populations in northwestern France, along the Channel. This result may also be associated to marine currents. Atlantic water masses enter in the West of the Channel and flow eastward to the Dove Strait. This current hits the North of the Cotentin peninsula and isolates the Saint-Malo gulf which is characterized by several gyres induced by tidal movements (Salomon & Breton 1993). In this region, marine currents may facilitate gene flow through seed drifted among sea beet coastal populations located along the Channel (Fievet et al. 2007). This hypothesis is also supported by a fine-scale investigation of the population genetic structure of the sea beet on both sides on the Channel which evidences a concordant eastward and parallel recolonization pattern on both the English and French shorelines (Arnaud, unpublished data).

We did not find any evidence for a corridor of gene flow in the two nematode species. Populations distributed in the West of the Saint-Malo gulf are highly structured and show low levels of admixture. At the regional scale, the strongest gyres of the Gulf of Saint-Malo are able to persist in a wide range of climatic conditions, and split the gulf into two parts, isolating the western part of the gulf from the rest of the Channel (Salomon and Breton 1993; Barnay et al. 2003). Marine currents may thus isolate nematode populations of the Gulf of Saint-Malo and generate high genetic structuring. This hypothesis was supported by a similar situation observed in *H. schachtii* and *H. betae* populations of Cantabria, in Northern Spain, a region also characterized by a long-term persisting marine gyre (Météo France, data not shown). Similarly, the modeling of dispersal in polychaete larvae has shown that coastal water movements may improve dispersal along the Channel eastward, but also westward (Barnay 2003; Fig. 6). Such water movements may facilitate westward gene flow among close nematode populations resulting in the IBD pattern and the southern decrease of genetic diversity observed in *H. schachtii* populations in northern populations. As a result, our data

suggest that marine currents may influence nematode dispersal at local and regional spatial scales. This kind of dispersal mode was never considered before for cyst nematodes species, because these species are generally studied in fields. As a result, water current-mediated cyst dispersal was only documented for an endoparasitic plant nematode, *Pratylenchus* ssp., which is able to survive to seawater immersion inside the rhizome of its host before developing on a new host. Thus, exceptional events, such as storms, may assist long-distance dispersal of this nematode (de la Peña et al. 2011). Such a scenario is equally likely for *H. schachtii* and *H. betae* that make most of their development cycle inside the roots of their hosts. Storms may disperse cysts, which are dormant structure able to stay alive for years in soil. However, we cannot conclude on this hypothesis, because no information about cyst survival in seawater is available so far.

Finally, our data suggest that both global processes such as climatic fluctuations of the Bölling-Alleröd and of the Younger Dryas and more local processes like marine hydrodynamic may have a strong influence on the genetic structure of coastal populations for the three species. However, this influence varied among species depending on their biological specificities, and also according to their respective evolutionary histories.

CONCLUSION

Results from this study suggest that the sea beet and the two nematodes may have persisted in southern Europe during the last Glacial Maximum. At the end of glaciations, they expanded northward, but their spreading was probably disturbed by climatic fluctuations of the Bölling-Alleröd and the Younger Dryas period, which may have affected them differently (Figure 7). The current sea beet genetic population structure probably results from a two-step coastal northward recolonization of the Atlantic coastline which may have been driven by physical and ecological properties of the coastal marine environment. Interestingly, microsatellite data indicate that *H. schachtii* may have recolonized Europe through inland alternative hosts, rather than by strictly following the sea beet. This recolonization may have, however, also occurred in two steps due to the climate cooling during the Bölling-Alleröd Younger Dryas. Thus, *H. schachtii* and the sea beet did not share the same evolutionary histories, and thus did not co-evolve together over long evolutionary time-scale. In contrast, concordant trends with the genetic population structure of the sea beet may suggest a coastal northward recolonization of Europe for *H. betae*. However, *H. betae* biological requirements may have prevent its persistence in a northern cryptic refugia (Figure 7). Further investigations, based on a more significant sampling or based on additional markers, will be required to give

reliable conclusions on this species. Finally, this study shows that the investigation of the evolutionary history of a host may improve the understanding of spatial genetic patterns observed in its parasites.

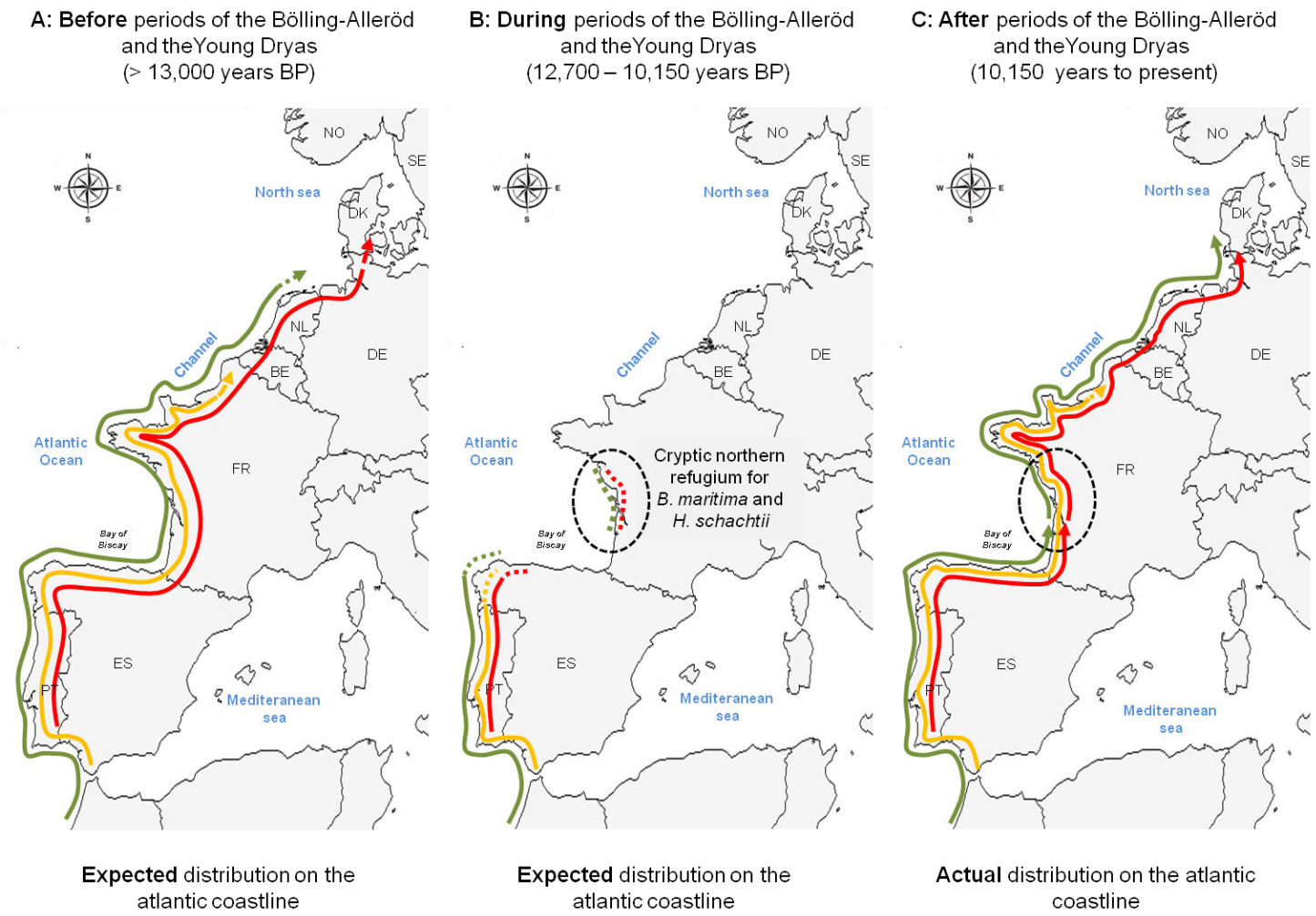

Figure 7: Summary of the recolonization scenario of the European Atlantic coastline by the sea beet and the two nematodes. A: climate warming favored northward range expansions in the three species. B: Climate cooling during the periods of the Bölling-Alleröd and of the Yougner Dryas isolated sea beet and *H. schachtii* populations. Biological requirements of *H. betae*, which preferred warmer temperatures, potentially prevented the persistence of this nematode at upper latitudes. Prediction of species distributions are based on their current northernmost distribution and the isothermal reconstruction of Isarin and Bohncke (1999). Green arrow and dot lines: sea beet; red arrow and dot lines: *H. schachtii*; yellow arrow and dot lines: *H. betae*.

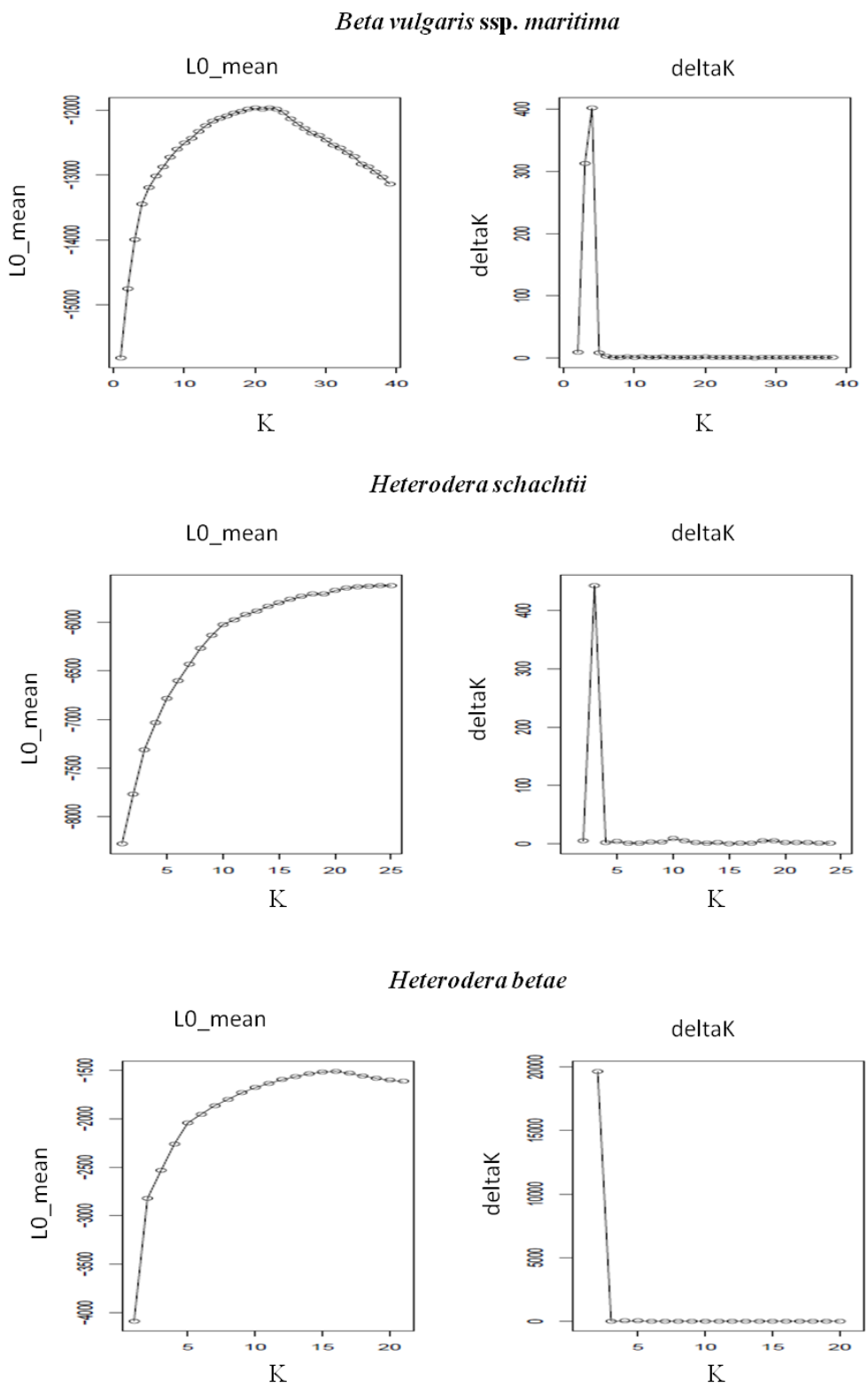

Figure S1: ΔK statistic and the log-likelihood obtained for each K value tested in *Beta vulgaris* ssp. *maritima*, *Heterodera schachtii* and *Heterodera betae*.

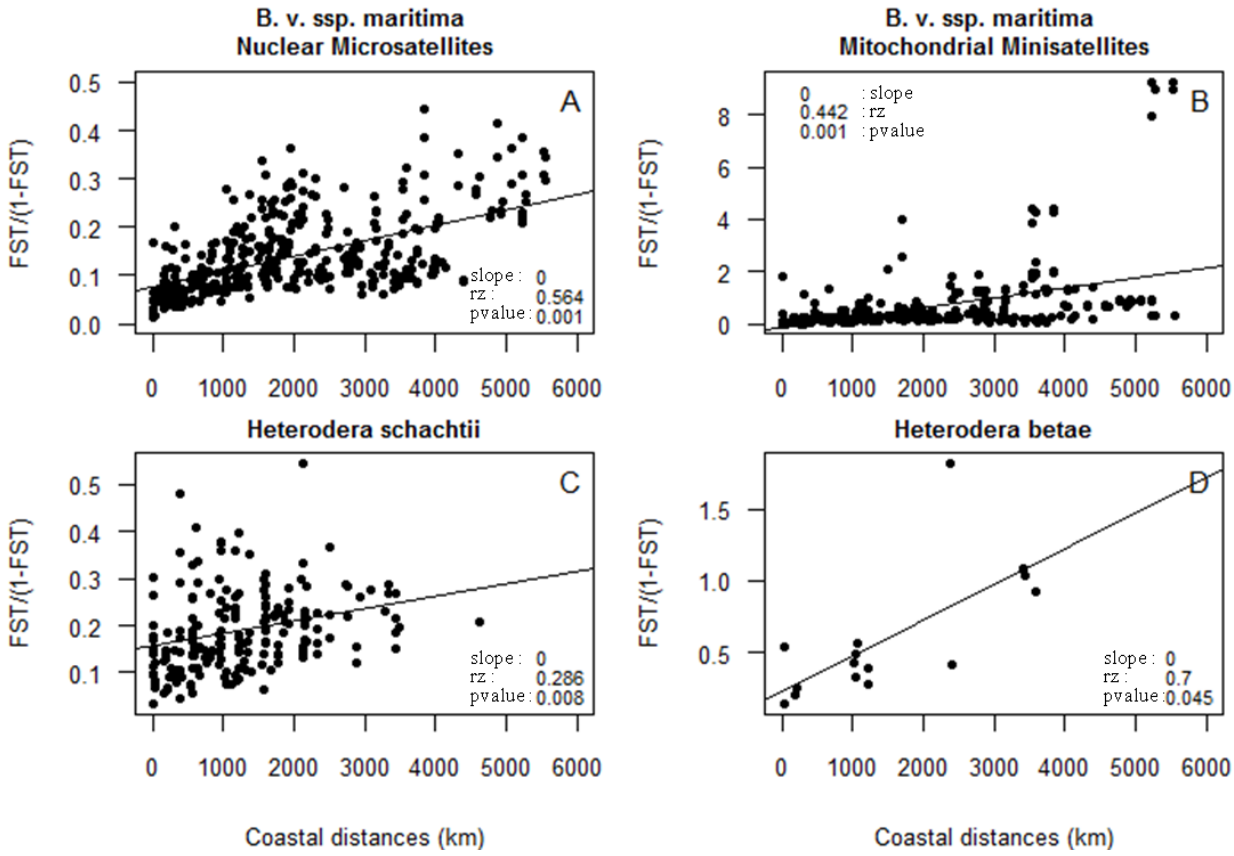

Figure S2: Isolation by distances over all populations in *Beta vulgaris* ssp. *maritima*, *Heterodera schachtii* and *Heterodera betae*. A: *Beta vulgaris* ssp. *maritima*, nuclear microsatellites; B: *Beta vulgaris* ssp. *maritima*, mitochondrial minisatellites; C: *Heterodera schachtii*; D: *Heterodera betae*. slope: slope of the linear regression model; rz : rz Mantel coefficient; pvalue : p-value of the Mantel test.

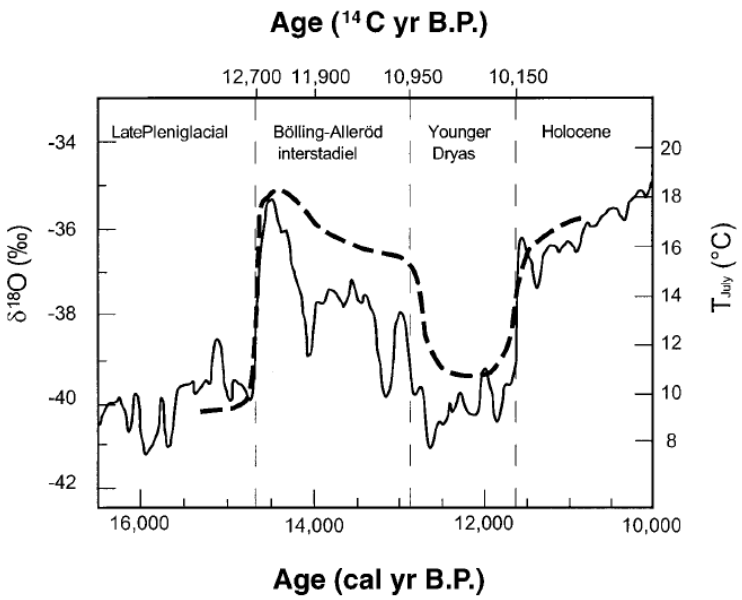

Figure S3: Late Weichselian and Holocene geochronology mean July temperatures for the British Isles based on beetle data (dashed line) and d18O values (after Isarin and Bohncke 1999).

2.4. What is new and what to do next

As for their wild host, our data supported that the two nematodes may have recolonized Europe from southern areas. This is clearly in contradiction with the current literature on *H. betae* (Steele and Whitehand 1984). Our studies further highlight the interest to combine data from the sea beet and the two nematodes to better understand the observed patterns of parasite genetic structure and to propose new hypotheses on parasites' histories. Additionally, the two studies give new information on the occurrence, the origin and the history of the two nematodes and one of their wild host-plant species. This information was currently almost inexistent for telluric crop pathogens though they may be interesting for the management of field pest populations. For example, cyst beet nematodes were more widely distributed along the Atlantic coastline than expected given their limited active dispersal capabilities (Wallace 1968). This observation and the potential impact of main marine currents in driving the dispersal of cyst nematodes strongly questioned the extent of passive dispersal of cyst nematodes and of the identification of potential dispersal vectors. The mismatch observed among *B. v.* ssp. *maritima* and *H. schachtii* suggested a non-shared history between the two species. From an agronomic point of view, this may prevent the presence of major resistance genes in the sea beet. Therefore, it brings into question the nature of resistance mechanisms found in wild host-plant populations and its consequence for the development of new resistant varieties of sugar beet. However, several conclusions suggested by these studies require additional supports.

2.4.1. Comparative phylogeography of the three species: a work still in progress

The comparison of evolutionary histories of the three species requires additional data. Microsatellite loci gave results which allowed defining a relevant scenario of postglacial recolonization of Europe by the sea beet. Nonetheless this pattern was not evident for nematode species, particularly for *H. betae*. This may be partly related to the small sample sizes in several locations, which were thus excluded from most of performed analyses. Small sample sizes are usually not a problem in phylogeographical studies (*e.g.* Kadereit et al. 2005) provided that a sufficient number of loci is used (Robinson 2014). Different markers may give higher statistical power to phylogeographic analysis and may help us to refine the evolutionary histories of the three species. As a result, increasing the number of molecular markers may improve the strength of our hypotheses.

We thus planned to incorporate a set of additional sequences from nuclear, mitochondrial and choloroplastic genomes to the current dataset to capture additional information (Appendix 1). Each genome has a specific evolution rate and is thus informative on different time scales. For example, microsatellites are fast evolving markers showing relatively recent evolutionary history, but mitochondrial and choloroplastic genomes are able to trace back past historical events further in time. We thus chose to sequence three chloroplastic and one mitochondrial gene in *B. v.* ssp. *maritima*. We also sequenced seven nuclear and two mitochondrial loci in nematodes. These data may help validate hypotheses on nematode histories. All these data will be first analyzed to investigate the spatial patterns of genetic structure in each species and refine post-glacial colonization scenarios. A careful description of genetic diversity is required before any statistical evaluation of scenarios by model comparison like Approximate Bayesian Computation (ABC) methods to define the most relevant models for our data to avoid incorrect inferences (Lombaert et al. 2014).

ABC is a statistical framework for model comparison and parameter estimations mainly based on summary statistics and extensive simulations which allow to not performed the exact likelihood calculations (Beaumont 2010; Bertorelle et al. 2010; Csilléry et al. 2010). More precisely, this approach estimates posterior probabilities of different models or parameters by evaluating the similarities between the observed values of some summary statistics and the values coming from a large number of simulated datasets. ABC methods were already successfully used to infer invasion routes and colonization history of two crop pests, *Diabrotica virgifera virgifera* and *Mycrocyclus ulei* (Miller et al. 2005; Barrès et al. 2012). ABC analyses may thus complement our current pictures of biogeographical patterns. During my PhD, ABC analyses were initiated during a short stay in the laboratory of Michael Hickerson (The City College of New York), which is collaborating on these features of the study. The complete picture of the European colonization of the two nematodes and their wild host is thus still in progress and will be developed further in the next few months.

2.4.2. *Heterodera betae* is still an open question

Adding an additional set of sequences to analyze the population genetic structure of *H. betae* may also give clues on key life-history traits, such as the breeding system or the ploidy level which are still poorly documented. In the current literature most studies investigating the biology of *H. betae* were made before its description as a full species by Wouts and coworkers in 2001. These studies actually considered an irregular biotype of *H. trifolii* instead of *H. betae* individuals, making difficult to draw any clear conclusions. For example, mitotic

parthenogenesis has early been suggested for this nematode. Nonetheless, specimens used in these studies were not definitively identified as *H. betae* individuals, resulting in uncertainties in the reproduction mode of this species (Steele and Whitehand 1984). The substantial levels of genetic differentiation observed among our surveyed *H. betae* populations supported a parthenogenetic reproduction mode. Levels of population genetic differentiation were indeed higher when compared to *H. schachtii* which is a sexual species with probably similar dispersal capabilities. Our results may thus give the first support for a parthenogenetic mode of reproduction in *H. betae*. Another uncertainty comes from the ploidy level of *H. betae*. As for the reproduction mode, cytological studies suggesting that *H. betae* may be tetraploid were performed far before the true description of the species (Triantaphyllou and Hirschmann 1978; Steele and Whitehand 1984). Moreover, microsatellite multilocus genotypes only rarely evidenced triploid or tetraploid profiles, preventing us to clearly determine whether these genotypes were the results of amplification artifacts or the results of additional copies of amplified loci. Therefore, we decided to remove irregular profiles and to analyze *H. betae* dataset using the same analyses as for *H. schachtii*. A rigorous estimation of *H. betae* ploidy level is obviously required before performing supplementary population genetic analyses.

Another area of uncertainty comes from a diversity of problems with the molecular characterization which led us to consider with caution the conclusions drawn by the analysis of genetic diversity of *H. betae*. Several *H. betae* populations were characterized by amplification failures at multiple microsatellite loci. This situation was already reported for cross-species amplified markers (Selkoe and Toonen 2006), and may induce a loss of statistical power in analyses on this species. However, genotyping with molecular markers do not allow the complete distinction between the two nematodes species. Indeed, we performed a Discriminant Analysis of Principal Components (DAPC), which is a suitable analysis to identify and describe genetic clusters. We wanted to examine the specificities of microsatellites multilocus profiles to each species by testing the presence of distinct genetic clusters corresponing for each nematode species (Jombart et al. 2010; Figure 13). The DAPC clearly allowed the distinction of most of *H. schachtii* and *H. betae* populations. However, in sympatric populations, where *H. betae* and *H. schachtii* co-occurred in the same host-plants, there is no distinction of the two species anymore as evidenced by the overlapping of some populations on the DAPC. This overlapping of several *H. betae* and *H. schachtii* individuals raise the question of the correct identification of *H. schachtii* specimens and of the boundaries of the *H. betae* species. Similarly, in field populations composed with *H. schachtii* and *H. betae* individuals, the genetic and morphological differences between the two species are

however more pronounced (data not shown). However, three individuals coming from a Finnish field population with both species showed an intermediary microsatellite profile containing both *H. betae* and *H. schachtii* characteristics, which may correspond to hybridization between the two species (data not shown). This observation was unexpected because of the difference in ploidy level between *H. betae* and *H. schachtii*: it raises the question of the species delimitation of *H. betae*.

These uncertainties may have numerous reasons, such as non-informative molecular markers, incorrect identification of nematodes, and shared history between the two species, as *H. betae* is assumed to have recently evolved from *H. schachtii* (Mulvey 1957, 1958, 1959; Triantaphyllou and Hirschmann 1964; Steele and Whitehand 1984; Subbotin et al. 2010). These uncertainties mainly evidenced the crucial necessity to clarify the biology and the definition of *H. betae* as well as its phylogenetic relationships with *H. schachtii* before going further into the investigation of population genetic structure in this species. Therefore, we chose to not pursue the work on *H. betae* which is not included in the fine-scale studies that are presented in the next section.

2.4.3. *Heterodera schachtii*: how explaining large-scale patterns of population genetic structure?

For both species, population genetic structure observed along the Atlantic coastline mainly evidenced the little influence of global evolutionary processes on spatial genetic structure. Coastal populations of the two species are also potentially affected by marine hydrodynamics which was unexpected for a strictly terrestrial species. Moreover, these results suggest the differences observed among the two nematodes species may be related to their different reproduction mode or different levels of gene flow with inland populations. The striking absence of genetic structuring in space observed in most of *H. schachtii* populations and the strong genetic differentiation observed at the population level point out the importance of the influence of local evolutionary processes in this species.

This conclusion was not particularly surprising for a parasite and was documented in various plant pathogen species (Capelle and Neema 2005; Tack et al. 2012). Nevertheless, the absence of structuring patterns strongly contrasts with a study on field *H. schachtii* populations which suggested this nematode may be able to disperse among fields that are 150 km distant of each other (Plantard and Porte 2004). Natural and field populations of *H. schachtii* populations may thus be differently inter-connected and human activities, which play a central role in the agro-ecosystems functioning, are potentially involved in this

discrepancy. Finally, dispersal capabilities appeared to be a key factor in understanding both natural and field population dynamics of *H. schachtii*. Dispersal capabilities determine the relative influence of gene flow and genetic drift on the evolution of genetic diversity within and among populations over space and time. The investigation of these two micro-evolutionary forces at small spatial scales may thus provide insights into the genetic structuring observed over a continental scale.

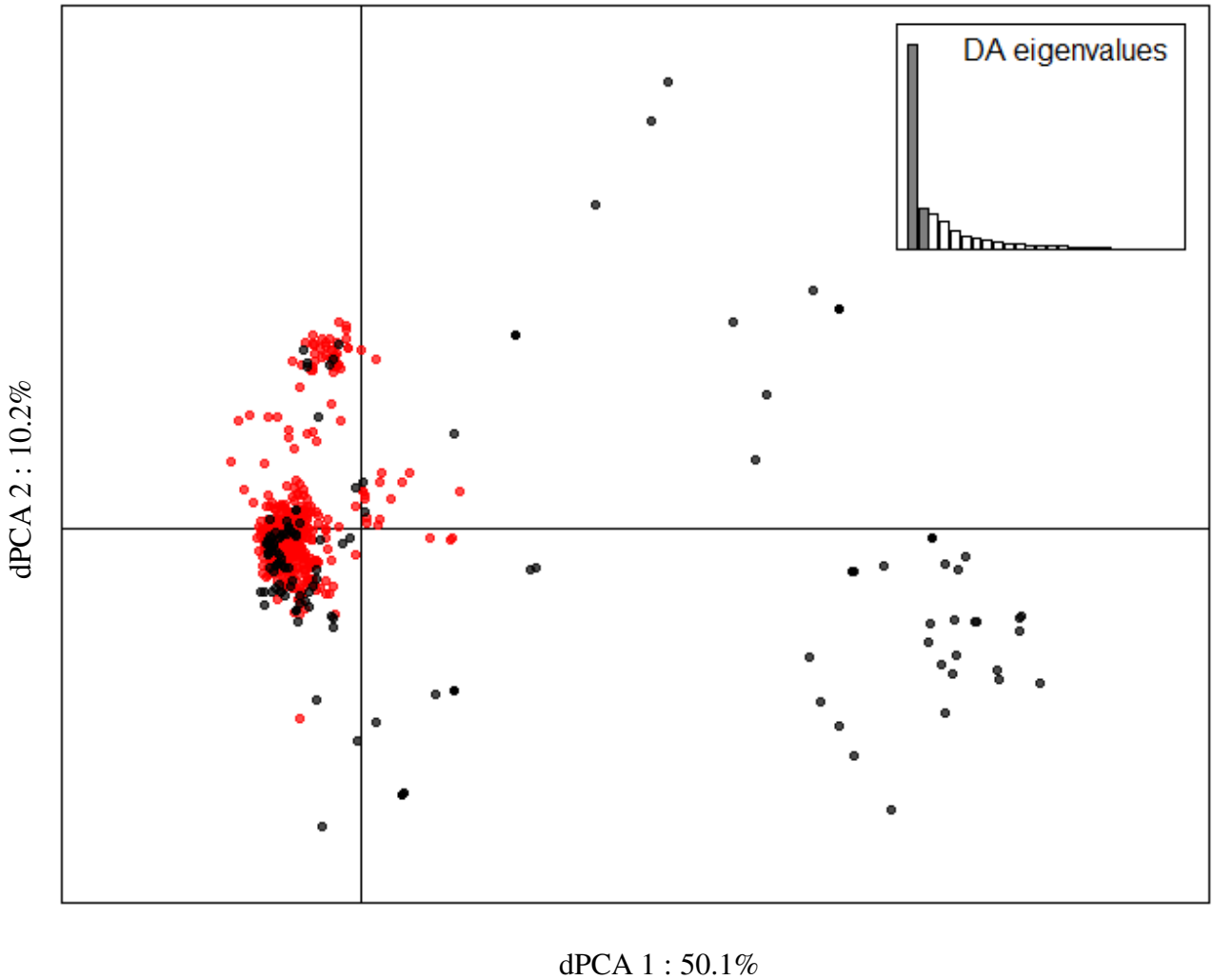

Figure 13: Genotypic discrimination of the two nematodes species. A discriminant of principal component analysis (DAPC, Jombart et al. 2010) was performed on the 25 *H. schachtii* (red) and 21 *H. betae* (black) wild populations included in the phylogeographical study. Points represent individuals. The analysis uses genotypic profiles obtained with 8 microsatellites and was performed with the Adegenet package of the software R version 3.1.1 (Jombart 2008a; R Development Core Team 2014). The first principal component axis a 50.1% of the total inertia and mainly discriminate the two species. In contrast, the second dPCA axis represents only 10.2% of the total inertia of the data and evidences the genetic variability among *H. betae* populations. In contrast, the 27 wild populations of *H. schachtii* overlap suggesting they are genetically homogeneous.

3. Population genetic structure of *Heterodera schachtii* at a local scale

3.1. Context, objectives and strategy

3.2. Heterozygote deficits in cyst plant parasitic nematodes: possible causes and consequences

3.3. Temporal sampling and variation in the genetic structure of naturally occurring populations of a plant-parasitic nematode.

1. Insights from the estimation of effective population sizes

3.4. Temporal sampling and variation in the genetic structure of naturally occurring populations of a plant-parasitic nematode.

2. Separating the relative effects of gene flow and genetic drift

3.5. Illuminating the evolutionary biology of *H. schachtii* in the wild and in agro-ecosystems

3.1.1. Fine-scale studies to understand patterns of genetic diversity in wild and field nematode populations

The first objective of fine-scale studies was to elucidate the interplay between micro-evolutionary processes in wild *H. schachtii* populations, which may give insights into the phylogeographical patterns observed along the Atlantic coastline in this species. Genetic structure results from the interaction between gene flow, which spreads alleles among populations, and genetic drift, which randomly reduces genetic variation. The understanding and the estimation of their respective influences are crucial to define nematode population boundaries and to predict their adaptive capabilities to a new host or the durability of control strategies. Levels and patterns of gene flow are related to intrinsic characteristics of pathogen species such as dispersal ability and reproductive mode, but also to extrinsic factors such as landscapes features and interspecific interactions (Kokko and López-Sepulcre 2006). Patterns of population genetic structure observed in wild nematode populations may thus result from equilibrium between all these components.

As for all pathogen species, human activities in agro-ecosystems correspond to an additional external factor able to modify this initial balance. Human-mediated interferences generally disturb the natural evolutionary dynamics of pathogen populations (Stukenbrock and McDonald 2008). This may result in nematode outbreaks in fields. Despite the accumulating evidence attesting of the influence of human activities on nematode population structure (Plantard and Porte 2004; Picard and Plantard 2006; Villate et al. 2010; Boucher et al. 2013; Gilabert and Wasmuth 2013; Alenda et al. 2014), there was no investigation on dynamics of wild nematode populations and how exactly human activities has changed the original population evolutionary dynamic. These questions should be examined in an area relatively free of human influence, like natural ecosystems and requires a sampling at the suitable spatial scales to allow the comparison of results with field populations. The fine-scale study of wild nematode populations may be thus also interesting to improve the understanding of the evolutionary dynamic of field nematode populations and to propose possible improvements for management strategies in sugar beet crops (van der Putten et al. 2006; Burdon and Thrall 2008).

3.1.2. Sampling in space, but also in time

To investigate issues developed above, a particular care should be given to the sampling design. Indeed, considering multiple spatial scales is required to take into account local and regional genetic structuring observed in *H. schachtii* among sites distant from a few hundred meters up to 150 km. It is also particularly suitable for the detection and the estimation of gene flow events among populations (Broquet and Petit 2009; Guillot et al. 2009). The inclusion of sites that are 150 km distant of each other shall also favor the comparison of results with existing data from nematode field populations, which were sampled on similar spatial scales (Plantard and Porte 2004).

Empirical studies have shown that genetic structure may be not stable over time (e.g. Honnay et al. 2009; Antoa et al. 2011). This suggests that it would be difficult to generalize our results if only one point estimation of genetic structure is considered. Similarly, while pairwise estimates of F_{ST}, a statistic widely used to investigate patterns of gene flow, are able to provide insights into the historical connection of populations, they cannot determine whether genetic connectedness results from ongoing migration or is the product of historical processes such as recent common ancestry (Holsinger and Weir 2009). Temporal sampling may help to distinguish between historical and contemporary signature (Broquet and Petit 2009). Temporal surveys also allow the evaluation of the influence of genetic drift through the estimation of effective population size. Effective population size corresponds to the number of individuals of an ideal population, where all individuals participate similarly to the next generations, and which experiences as much genetic drift as the real population (Wright 1931). There is a wealth of methods designed to estimate effective populations size (Barker 2011). However, only those based on temporal methods are compatible with biological characteristics of *H. schachtii*, especially a high level of inbreeding (Plantard and Porte 2004; Montarry et al. 2015). Several sampling sessions spaced in time were thus planned to reliably estimate the effective population size and to assess the stability of genetic structure over time.

Most of the work presented in this second section was based on the sampling of a set of sea beet individual plants collected within four beaches distant of 300 m to 150 km from each other. All the sampling procedure was performed twice during the PhD, in falls 2012 and 2013. My work was to develop the sampling design, to organize and perform sampling sessions, to help in the sample treatment (cyst extraction and genotyping) and to analyze the data. The two latter tasks were performed with the collaborations of two Master 2's students, Eric Olivier and Pierre-Loup Jan, whom I co-supervised during their Master's thesis.

3.1.3. How picturing the population genetic structure in *Heterodera schachtii*?

Little is known about the genetic structure of plant parasitic-nematode, and all data available come from studies on few nematode species mostly performed on field populations (Plantard and Porte 2004; Picard et al. 2006; Villate et al. 2010; Alenda et al. 2014; but see Mallez et al. 2014). These studies showed that cyst nematode populations are generally connected by long-distance passive dispersal events potentially mediated by human activities or abiotic factors such as water or wind. In the specific case of *H. schachtii*, very low level of genetic differentiation was observed among field populations separated by 150 km from one another, and most of the genetic variation was observed at a very local scale, within fields (Plantard and Porte 2004). Altogether, these observations suggest that *H. schachtii* populations may be potentially connected by long-distance episodes of gene flow and/or characterized by high effective population sizes. At a finer scale, the very small size of this nematode (<1mm) induces a physical limit of its active dispersal capabilities (Wallace 1968). Similarly, the high F_{IS} values reported in field populations of *H. schachtii* were considered as a result of mating among related individuals barely able to disperse (Plantard and Porte 2004). The current resulting model of of *H. schachtii* population functioning thus assumes that spatially-restricted dispersal of nematodes favors inbreeding within populations and passive long-dispersal events counteract the genetic differentiation among populations.

This model may be consistent with a regional pattern of genetic structure observed in the Saint-Malo gulf and in northern Spain populations. However, it does not fit well with the absence of genetic structuring observed among other wild populations along the shoreline. Moreover, this model has several weaknesses that need to be addressed, even for field populations. First, the investigation of gene flow resulting from passive and active dispersal is incomplete. Indeed, at large spatial scale, there is no information on the intensity of gene flow among fields. Their real impact on the evolution of genetic diversity thus remains unknown. At finer scale of investigation, high fixation index (F_{IS}) values may also result from methodological artifacts, such as sub-population structure or null alleles. These explanations deserve to be explored given that recent work reported that *H. schachtii* juveniles are able to move at least 30 cm to reach a sugar beet root in field conditions (Westphal 2013). This may thus limits inbreeding at the scale of the host-plant. Second, effective population size has never been estimated and the role played by genetic drift on nematode population genetic structure is unknown. Lack of relevant data in field populations is related to the high levels of

field infestation which suggests high population sizes difficult to measure. However, genetic drift is a major non-adaptive evolutionary process acting on pathogen evolution even in large populations (Huyse et al. 2005). It has to be investigated to fully understand the evolutionary dynamic of wild nematode populations. The work presented in this section aimed to give insights on the relative effect of gene flow and genetic drift and is structured in three parts:

1. The first paper corresponds to results obtained in a project developed by Josselin Montarry and Eric Petit and for which I was involved in data gathering. This study aimed at disentangling the relative impact of inbreeding and population sub-structuring in observed levels of heterozygote deficiencies. Data coming from two sampling sessions were used to investigate this question in wild populations. This study gave insights conditioning my PhD project. This work was recently accepted in *Molecular Ecology*.

2. The study of the second part of this section corresponds to the first article of a set of two companion papers studying the genetic structure of wild populations of *H. schachtii*. This article focused on the estimation of the effective population size of wild populations of *H. schachtii* using a temporal sampling. This work was performed in 2014 during the Master thesis of Pierre-Loup Jan has and is planned to be submitted to *Evolutionary Applications*.

3. The last part of this section corresponds to the second paper on genetic structure of wild populations *H. schachtii* that examines the level and the scale over which gene flow occur among nematode populations during two consecutive sampling years. This article will also be submitted to *Evolutionary Applications*.

3.2. Heterozygote deficits in cyst plant parasitic nematodes: possible causes and consequences

Josselin MONTARRY, Pierre-Loup JAN, Cécile GRACIANNE, Andrew D. J. OVERALL, Sylvie BARDOU-VALETTE, Eric OLIVIER, Sylvain FOURNET, Eric GRENIER and Eric J. PETIT

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ABSTRACT

Deviations of genotypic frequencies from Hardy-Weinberg equilibrium (HWE) expectations could reveal important aspects of the biology of populations. Deviations from HWE due to heterozygote deficits have been recorded for three plant parasitic nematode species. However, it has never been determined whether the observed deficits were due (i) to the presence of null alleles, (ii) to a high level of consanguinity and/or (iii) to a Wahlund effect. The aim of the present work was, while taking into the possible confounding effect of null alleles, to disentangle consanguinity and Wahlund effect in natural populations of those three economically important cyst nematodes using microsatellite markers: *Globodera pallida*, *G. tabacum* and *Heterodera schachtii*, pests of potato, tobacco and sugar-beet, respectively. The results show a consistent pattern of heterozygote deficiency in the three nematode species sampled at the spatial scale of the host plant. We demonstrate that the prevalence of null alleles is weak and that heterozygote deficits do not have a single origin. Our results suggested that it is restricted dispersal that leads to heterozygote deficits through both consanguinity and sub-structure, which effects can be linked to soil movement, cyst density, and the number of generations per year. We discuss potential implications for the durability of plant resistances that are used to protect crops against parasites in which mating between relatives occur. While consanguineous mating leads to homozygosity at all loci, including loci governing avirulence/virulence, which favors the expression of virulence when recessive, the Wahlund effect is expected to have no particular effect on the adaptation of nematodes to resistances.

Keywords: consanguinity, heterozygote deficit, microsatellite, nematode, null allele, Wahlund effect

Deviations of genotypic frequencies from Hardy-Weinberg equilibrium (HWE) expectations can theoretically reveal important aspects of the biology of populations. Heterozygote excess or deficits can be linked to the way individuals disperse between populations and mate within populations (Paxton et al. 1996; Goudet et al. 2002). For example, deficits are characteristic of selection against hybrids in hybrid zones (Alexandrino et al. 2005). They can also inform the researcher about their ignorance of population limits (Waples and Gaggiotti 2006) when for example the deficit is linked to various gene pools being mixed in a sample (Wahlund effect - Wahlund 1928). Experimental artefacts can also lead to deviations from Hardy-Weinberg equilibrium. Heterozygote deficits can arise when an allele is not amplified because the flanking region to which a primer sequence has been targeted bears a mutation (so-called null alleles, Callen et al.1993; Brookfield 1996; Chapuis and Estoup 2007), or when alleles drop-out because DNA template concentrations are low (Taberlet *et al.* 1996) (Taberlet et al. 1996). Heterozygote excess can appear when false alleles are called because of low DNA quality or quantity, inappropriate experimental procedures, or human errors (Pompanon et al. 2005). Contrary to allelic dropouts or false alleles that are mostly encountered in noninvasive genetic sampling, null alleles are frequent when employing cross-species amplification or in species harboring large effective sizes (Chapuis and Estoup 2007). It is hence difficult to distinguish whether the cause of heterozygote deficit is biological or experimental.

Picard et al. (2004), Plantard and Porte (2004), and more recently Alenda et al. (2014) have documented strong heterozygote deficits in three parasitic cyst nematodes, *Globodera pallida*, *Globodera tabacum,* and *Heterodera schachtii*, which attack potato, tobacco and sugar-beet, respectively. As soil-borne obligatory parasites of very small sizes (length is about 500 µm on average), the active dispersal of nematodes is limited to relatively short distances (Wallace 1968). This possibly favors mating between siblings, which could be responsible for the strong heterozygote deficit observed (Picard et al. 2004; Plantard and Porte 2004; Alenda et al. 2014). This is the hypothesis that has been presented in these cases and others (Gilabert and Wasmuth 2013), although alternative explanations exist (see above) and have not been explored. This is important in the case of such parasites because the methods that are used to control their populations are based on the deployment of resistant host plants. The overcoming of host resistance corresponds to the acquisition by the parasite of the capacity to infect and multiply in host genotypes resistant to other parasite genotypes, hereafter termed virulence (Vanderplank 1963, 1968). Exploring evolutionary processes responsible for

parasite adaptation to a new resistant host is crucial for the development and sustainable use of plant resistances. Plant-parasite interactions often conform to the widely accepted gene-for-gene model, which predicts that successful disease resistance is triggered only if a resistance gene product in the host plant recognizes, directly or indirectly, a specific avirulence gene product from the pathogen (Flor 1956; Flor 1971; Jones and Dangl 2006). The durability of such resistance genes depends on the ability of the parasites to express virulence, which, in pure genetic (Flor 1942; Janssen et al. 1991; Staskawicz et al. 1995), as well as in metapopulation genetic models (Cornell et al. 2003), is facilitated in a homozygous state. In contrast to sub-structure and null alleles, heterozygote deficit due to consanguinity reflects a true population scenario, *i.e.* inbreeding. Consequently, among the three hypotheses we formulate, consanguinity is the only one that represents true excess homozygosity and is thus relevant to the durability of resistance genes.

Overall and Nichols (2001) have described a likelihood-based method allowing the differentiation between excess homozygosity caused by consanguineous mating and excess homozygosity due to undetected population sub-structure through analysis of multilocus genotypes. The method is based on the fact that consanguinity and population subdivision can generate distinctive patterns of homozygosity in multilocus data, with individuals showing an excess homozygosity at each locus for the former, and individuals homozygous at different loci for the latter. Here, we used that method in order to calculate a likelihood surface for the genetic correlation due to population subdivision (θ) and the proportion of the population practicing consanguinity (C) (Overall & Nichols 2001) in three plant parasitic nematode species attacking different crops: the potato cyst nematode *G. pallida*, the tobacco cyst nematode *G. tabacum* and the beet cyst nematode *H. schachtii*. In addition, we used a combination of methods to check whether null alleles are prevalent in these species.

MATERIALS AND METHODS

Biology of cyst nematodes

Nematodes are a group of worms that include free-living species such as *Caenorhabditis elegans* as well as many parasitic species of animals and plants. Plant-parasitic nematodes are major parasites that cause considerable economic losses in agriculture: worldwide crop losses caused by nematodes have been estimated around $100 billion per year (Sasser and Freckman 1987). Cyst nematodes enter the plant roots as second stage juveniles (J2) and establish a specialized feeding structure, the syncytium (Jones and Northcote 1972), which is a severe nutrient sink for the plant. Sex is environmentally determined through the size of the

syncytium that is induced (Trudgill 1967; Grundler et al. 1991). Adult males leave the root in order to mate females. The females continue to feed and when egg development is completed, they die and form a cyst, enclosing hundreds of eggs, which constitute a survival stage that can stay viable for several years in soils.

G. pallida, *G. tabacum*, and *H. schachtii* are gonochoristic (*i.e.* with separate males and females) and diploid organisms with obligatory sexual reproduction. Contrary to *G. pallida* and *G. tabacum,* which are specialized on Solanaceous plants, the host range of *H. schachtii* is large, causing damage to other species belonging to plant families Chenopodiaceae, Cruciferae and Brassicaceae (Raski 1950). *G. pallida* and *G. tabacum* are considered native to South-America, and *H. schachtii* native to Europe (Cooke 1992; Grenier et al. 2011). *G. pallida* achieves only one generation per year, whereas *G. tabacum* and *H. schachtii* can achieve between two to five generations per year in some environmental conditions (Raski 1950; Adams et al. 1982; Johnson 1998).

Nematode populations sampling

Given the restricted active dispersal ability of plant parasitic nematodes (Wallace 1968), and because it is the smallest scale at which heterozygote deficits have already been reported (Picard et al. 2004), we chose to sample populations at the plant scale. Because the three species we sampled infest different crops, we had to deal with different local partners who have specific constraints when sampling, which led to slightly different sampling schemes at the field scale. Likewise, these three species have different biogeographic origins, which allowed us to sample various compartments (field vs. wild populations in *H. schachtii*; Europe vs. South America in *G. pallida*) when possible. Our aim here was not to investigate the particular influence of specific environmental factors on heterozygote deficits encountered in these species, but to investigate their origin in all conditions where these species can be sampled given our current knowledge of their distribution and biology.

For *G. pallida*, 19 populations were sampled at the plant scale (Table 1). Among those populations, four were sampled in a Peruvian potato field located in the region of Andahuaylas (GPS point: 13°38’11.47’’N and 73°27’31.19’’O) during summer 2002 (Picard *et al.* 2004), and for which DNA samples were maintained at -80°C ever since, whereas the other populations were sampled in Western France during summer 2012: ten coming respectively from five plants randomly chosen in two distinct potato fields located in the island of Noirmoutier, and five coming from five plants randomly chosen in a potato field

near Saint-Malo (Fig. S1, Supporting information). For each *G. pallida* population, between 20 and 40 larvae taken from distinct cysts were genotyped.

For *G. tabacum*, 13 populations were sampled in Southwestern France during summer 2012 from six distinct tobacco fields (Fig. S1, Supporting information), with a unique plant from four fields and four and five plants randomly chosen for the two other fields. For each *G. tabacum* populations, between 25 and 33 larvae from distinct cysts were genotyped (Table 2).

Forty-one *H. schachtii* populations were sampled in Northern France during summer and fall 2012 (Table 3). Among those populations, eight were sampled in two cultivated sugar-beet fields after randomly choosing four plants by field, whereas the other populations were sampled in the wild compartment from four distinct beaches with wild beet plants, *Beta maritima* (Fig. S1, Supporting information). For each *H. schachtii* population, between 16 and 29 larvae from distinct cysts were genotyped.

Microsatellite genotyping

Cysts of the three nematodes species were obtained from soil using a Kort elutriator. In order to obtain enough DNA from single larvae (*i.e.* one second-stage juvenile J2), an extraction procedure using sodium hydroxide and proteinase K was performed following Boucher et al. (2013). Species identity of these samples were checked by PCR-RFLP of the ITS region as previously described (Thiéry and Mugniéry 1996).

G. pallida populations were genotyped using 11 microsatellite markers (Gp106, Gp108, Gp109, Gp111, Gp112, Gp116, Gp117, Gp118, Gp122, Gp126 and Gp135 – Table S1, Supporting information) developed directly from available genomic sequences (Cotton et al. 2014). The program QDD (Meglécz *et al.* 2010) was used to select sequences and design primer pairs for sequences containing perfect microsatellite motifs with at least four repeats and flanking regions without tandem-repetition. Among those markers, we discarded small PCR products (< 100 nucleotides) and all tri-nucleotides motifs (the most likely to appear in coding regions because they do not cause any frameshift; Toth et al. 2000). Two multiplex panels were defined to reduce the time and cost required to genotype the 585 individuals at the 11 loci (Multiplex Manager Software - Holleley and Geerts 2009).

G. tabacum populations were genotyped using eight microsatellite markers (Gp108, Gp116, Gr56, Gr58, Gr67, Gr68, Gr82 and Gr85 – Table S1, Supporting information) initially developed for *G. pallida* and *Globodera rostochiensis* (Boucher et al. 2013) and selected for their ability to cross-amplify in *G. tabacum.* Two multiplex panels were defined to genotype the 383 individuals at the eight loci.

Table 1: Localization of the sampled potato fields, *G. pallida* population name, number of larvae genotyped (n), F_{IS}, proportion of loci showing significant heterozygote deficit, inter-loci variance of F_{IS}, allelic richness (R) and unbiased gene diversity (H). The right part of the table shows the joint maximum-likelihood of θ and C for each *G. pallida* population with a significant heterozygote deficit.

	localization	population name	n	F_{IS}		significant loci proportion	inter-loci variance	R (n=16)	H	θ	C
Globodera pallida	Andahuaylas (Peru)	215-33	40	0.041	ns	2 / 11	0.016	4.93	0.56	.	.
		215-34	36	0.118	*	3 / 11	0.031	4.85	0.61	0.11	0.00
		215-8	37	0.134	*	2 / 11	0.069	4.55	0.59	0.15	0.00
		215-9	33	0.171	*	4 / 11	0.035	4.67	0.55	0.14	0.00
	Noirmoutier field 1	N1-02	29	-0.051	ns	0 / 10	0.122	2.39	0.35	.	.
		N1-04	30	0.080	*	2 / 11	0.049	3.41	0.46	0.00	0.42
		N1-06	30	0.084	*	1 / 11	0.028	3.11	0.42	0.00	0.41
		N1-08	34	0.021	ns	1 / 11	0.021	2.82	0.39	.	.
		N1-10	31	0.009	ns	0 / 11	0.007	2.71	0.38	.	.
	Noirmoutier field 2	N2-01	20	-0.047	ns	0 / 7	0.300	2.00	0.29	.	.
		N2-02	34	0.011	ns	1 / 10	0.168	2.50	0.30	.	.
		N2-06	27	0.017	ns	0 / 9	0.172	2.26	0.31	.	.
		N2-08	26	0.036	ns	0 / 10	0.102	2.51	0.32	.	.
		N2-09	31	0.086	ns	0 / 9	0.165	2.41	0.32	.	.
	Saint-Malo	SM-01	26	0.243	*	4 / 11	0.027	3.05	0.53	0.11	0.51
		SM-03	31	0.142	*	2 / 11	0.037	3.10	0.50	0.00	0.47
		SM-05	29	0.094	*	2 / 11	0.056	3.36	0.50	0.00	0.43
		SM-08	29	0.108	*	1 / 11	0.014	3.51	0.52	0.00	0.44
		SM-10	32	0.113	*	1 / 11	0.016	3.19	0.50	0.00	0.46
		total	585	0.087							

Table 2: Localization of the sampled tobacco fields, *G. tabacum* population name, number of larvae genotyped (n), F_{IS}, proportion of loci showing significant heterozygote deficit, inter-loci variance of F_{IS}, allelic richness (R) and unbiased gene diversity (H). The right part of the table shows the joint maximum-likelihood of θ and C for each *G. tabacum* population with a significant heterozygote deficit.

	localization	population name	n	F_{IS}		significant loci proportion	inter-loci variance	R (n=16)	H	θ	C
Globodera tabacum	La Réole	Reo-01	30	0.156	*	1 / 7	0.038	2.19	0.38	0.16	0.00
		Reo-02	30	0.247	*	3 / 7	0.093	2.04	0.37	0.22	0.00
		Reo-03	32	0.131	*	3 / 8	0.187	2.12	0.34	0.12	0.00
		Reo-04	30	0.216	*	2 / 7	0.038	2.32	0.37	0.20	0.00
		Reo-05	31	0.240	*	2 / 8	0.062	2.37	0.38	0.03	0.77
	Calvignac	calvi	31	0.289	*	2 / 4	0.061	1.50	0.17	0.17	0.58
	Guinarthe	gui-01	27	0.701	*	1 / 2	0.500	1.19	0.02	0.74	0.51
		gui-02	26	1.000	*	1 / 1	0.000	1.12	0.01	0.90	1.00
		gui-03	29	1.000	*	2 / 2	0.000	1.20	0.02	0.90	1.00
		gui-05	32	0.551	*	2 / 4	0.339	1.36	0.03	0.62	0.60
	Salvagnac	26	25	0.842	*	1 / 2	0.416	1.21	0.06	0.83	0.52
	Larnagol D	LarD	33	0.044	ns	0 / 4	0.015	1.40	0.13	.	.
	Larnagol P	LarP	27	0.293	*	0 / 4	0.066	1.49	0.17	0.31	0.00
		total	383	0.233							

Table 3: Localization of the sampled sugar-beet fields (Thibie and Pomacle) and beaches (Granville North, Granville South, Montfarville and Saint Léonard), *H. schachtii* population name, number of larvae genotyped (n), F_{IS}, proportion of loci showing significant heterozygote deficit, inter-loci variance of F_{IS}, allelic richness (R) and unbiased gene diversity (H). The right part of the table shows the joint maximum-likelihood of θ and C for each *H. schachtii* population with a significant heterozygote deficit.

	localization	population name	n	F_{IS}		significant loci proportion	inter-loci variance	R (n=16)	H	θ	C
Heterodera schachtii	Thibie	HsP101	23	0.012	ns	0 / 8	0.074	2.78	0.46	.	.
		HsP102	25	0.174	*	3 / 7	0.101	3.05	0.47	0.03	0.62
		HsP103	25	0.106	*	2 / 8	0.080	2.98	0.46	0.12	0.00
		HsP104	16	0.063	ns	1 / 8	0.117	1.88	0.49	.	.
	Pomacle	HsP301	28	0.247	*	3 / 7	0.139	2.81	0.44	0.24	0.00
		HsP303	22	0.184	*	2 / 7	0.148	3.03	0.40	0.21	0.00
		HsP304	25	0.188	*	1 / 7	0.046	2.94	0.41	0.16	0.00
		HsP305	16	0.258	*	3 / 7	0.121	2.50	0.44	0.24	0.00
	Granville North	Fra.71N.P2.1	25	-0.162	ns	0 / 6	0.057	1.94	0.32	.	.
		Fra.71N.P2.2	25	0.056	ns	0 / 8	0.027	2.38	0.36	.	.
		Fra.71N.P2.3	25	0.021	ns	0 / 7	0.031	2.44	0.39	.	.
		Fra.71N.P2.4	23	0.021	ns	0 / 7	0.017	2.06	0.36	.	.
		Fra.71N.P2.5	22	-0.040	ns	0 / 7	0.037	2.51	0.41	.	.
		Fra.71N.P2.6	24	0.013	ns	0 / 8	0.079	2.13	0.38	.	.
		Fra.71N.P2.7	24	0.123	ns	1 / 7	0.142	2.03	0.34	.	.
		Fra.71N.P2.8	22	0.071	ns	1 / 8	0.060	2.63	0.37	.	.
		Fra.71N.P1.4	25	0.085	ns	0 / 8	0.041	2.65	0.41	.	.
		Fra.71N.P3.7	21	0.051	ns	0 / 8	0.021	2.15	0.40	.	.
		Fra.71N.P4.1	27	0.103	ns	1 / 7	0.151	2.33	0.33	.	.
		Fra.71N.P5.2	26	0.162	*	2 / 7	0.086	2.21	0.36	0.15	0.00
	Granville South	Fra.7_1.P5.1	22	0.101	ns	0 / 8	0.021	2.91	0.45	.	.
		Fra.7_1.P5.2	25	0.145	*	1 / 6	0.132	2.58	0.34	0.10	0.00
		Fra.7_1.P5.3	25	0.030	ns	0 / 7	0.007	1.98	0.29	.	.
		Fra.7_1.P5.4	20	0.014	ns	0 / 8	0.007	2.54	0.34	.	.
		Fra.7_1.P5.6	20	0.036	ns	2 / 8	0.220	2.44	0.38	.	.
		Fra.7_1.P5.7	25	0.008	ns	0 / 8	0.068	2.69	0.42	.	.
		Fra.7_1.P5.10	23	0.108	ns	2 / 8	0.130	2.76	0.37	.	.
		Fra.7_1.P2.5	24	-0.072	ns	1 / 8	0.189	2.39	0.35	.	.
		Fra.7_1.P4.1	20	0.136	ns	1 / 8	0.043	2.29	0.34	.	.
	Montfarville	Fra.8_4.P1.1	25	0.391	*	2 / 7	0.239	2.21	0.35	0.33	0.00
		Fra.8_4.P1.2	25	0.269	*	2 / 7	0.130	2.50	0.38	0.10	0.75
		Fra.8_4.P1.3	25	-0.307	ns	0 / 6	0.135	2.60	0.41	.	.
		Fra.8_4.P1.4	22	0.192	*	1 / 6	0.023	2.33	0.34	0.17	0.00
		Fra.8_4.P1.5	28	0.097	ns	1 / 6	0.063	2.65	0.38	.	.
		Fra.8_4.P2.9	23	0.165	*	1 / 6	0.099	1.99	0.35	0.14	0.00
		Fra.8_4.P3.1	26	-0.094	ns	1 / 6	0.092	2.57	0.41	.	.
	Saint Léonard	Fra.7_4.P1.1	21	-0.243	ns	0 / 6	0.020	2.55	0.40	.	.
		Fra.7_4.P1.2	24	-0.054	ns	0 / 6	0.017	2.34	0.33	.	.
		Fra.7_4.P1.7	24	-0.218	ns	0 / 7	0.023	2.08	0.29	.	.
		Fra.7_4.P1.8	24	0.096	ns	1 / 6	0.085	2.07	0.34	.	.
		Fra.7_4.P2.1	29	0.063	ns	1 / 7	0.060	2.34	0.32	.	.
		total	969	0.061							

H. schachtii populations were genotyped using eight microsatellite markers (Hs33, Hs36, Hs55, Hs56, Hs68, Hs84, Hs111 and Hs114 – Table S1, Supporting information). Those markers were developed according to the procedure developed by Malausa et al. (2011) using next-generation sequencing and library enrichment. Genomic DNA of several *H. schachtii* populations was extracted from entire cysts with the DNeasy® Blood and Tissue Kit (Qiagen) using the protocol for animal tissue. Construction, emulsion PCR and 454 GS-FLX titanium pyrosequencing of the microsatellite-enriched DNA libraries was carried out by Genoscreen (Lille, France). The program QDD (Meglécz et al. 2010) was used to select sequences and design primer pairs as described above for *G. pallida*. Three multiplex panels were defined to genotype the 969 individuals at the eight loci.

PCR was performed using a 384-well reaction module (BIO-RAD C1000) in a 5 µL volume containing 1X of Type-it Microsatellite PCR kit, 0.4 µM of primer mix and 1 µL of template DNA. Cycling conditions included an initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 57 °C (and 58°C for *H. schachtii*) for 90 s and extension at 72 °C for 30 s, followed by a final extension at 60 °C for 30 min. PCR products were then diluted to 1:25 for *G. pallida*, 1:40 for *G. tabacum* and 1:10 for *H. schachtii* in sterile water and 3 µL of this dilution were mixed with 0.05 µL of GeneScan 500 LIZ Size Standard (Applied Biosystems) and 5 µL of formamide (Applied Biosystems). Analyses of PCR products were conducted on ABI Prism® 3130xl sequencer (Applied Biosystems).

Allele sizes were determined by the automatic calling and binning module of GeneMapper v4.1 (Applied Biosystems) with manual examination of irregular results. To minimize the rate of genotyping errors, a second round of PCR and electrophoresis was performed for 10% (190 randomly drawn larvae among the 1,937 that were genotyped) of the global number of individuals, considering the three species altogether.

Genetic diversity and heterozygosity estimation

Genetic diversity was estimated through allelic richness (R) and unbiased gene diversity (H) (Nei 1978). Allelic richness R corresponds to the mean number of alleles per locus and gene diversity H corresponds to the average probability across loci to draw at random different alleles in the same population. R was estimated on a reduced sample of 16 individuals using the rarefaction method implemented in Populations 1.2.32 (Langella 2000). H and F_{IS} were computed using GENETIX 4.05.2 (Belkhir et al. 1996). The statistical significance of F_{IS} values

for each population was tested using the allelic permutation method (1000 permutations) implemented in GENETIX. The proportion of loci showing a significant heterozygote deficit and the inter-locus variance were calculated using the outputs of GENETIX.

Estimation of null allele frequency

In order to test for the presence and to estimate the frequency of null alleles in our data sets, two independent methods were used. First, the relationship between the percentage of missing data for each locus and F_{IS} was examined (Beaumont et al. 2001) with the Spearman's rank correlation coefficient rho using the statistical software R, version 2.15.0 (R Development Core Team, 2012). A positive relationship between F_{IS} and missing data would indicate that amplification failure is due to individuals bearing a null allele at the homozygous state. Second, we used the likelihood-based method of Chybicki and Burczyk (2009), implemented in the INEst program, that simultaneously estimates null allele frequencies and inbreeding coefficients. Finally, data sets simulated with EasyPop v2.0.1 (Balloux 2001) were used in order to assess the effect of null alleles on θ and C estimates. We simulated a set of 20 populations made of 20 diploid individuals (10 females and 10 males mating randomly) with a mutation rate of 0.0005 at 10 distinct loci. Each locus had 99 possible allelic states, the same probability to mutate to any allelic state, and free recombination between loci was allowed. The migration model used for all simulations was an island model with a migration rate of 0.01 for both males and females. This simulation scheme was replicated ten times for simulations that spanned enough time (1,000 generations) to reach equilibrium. One population was sampled in each of the ten replicates, and used to infer C and θ, which were expected to equal zero. Null alleles were then added for alleles showing frequencies between zero and 30% by steps of approximately 2.5%, and we looked at the difference between the estimated and expected C and θ values. Likewise, we used five of our populations (showing less than 3.5% of null alleles, estimated using INEst) to add null alleles with frequencies that reached up to 35% in order to explore their influence on the estimation of C and θ on real data.

θ and C estimation

In order to distinguish consanguinity and Wahlund effect, we used the method of Overall and Nichols (2001). The method requires knowledge of the reproductive biology and ecology of species in order to define the degree of relatedness between individuals implied in consanguineous mating. Because larvae coming from a cyst are sisters, we chose a degree of

relatedness of 1/4, which corresponds to the probability that the offspring of siblings will inherit two identical-by-descent genes at any one locus.

To evaluate the power of the method, we simulated a total of sixteen scenarios that mixed consanguinity and the Wahlund effect with allele frequencies of each of three populations we sampled. These populations were chosen because they exhibited high (the 215-33 *G. pallid*a population), intermediate (the Fra.7_1.P5.7 *H. schachtii* population), or low (the LarD *G. tabacum* population) gene diversity and no significant heterozygote deficit (Tables 1, 2 and 3). The sixteen scenarios corresponded to four C values (0, 0.1, 0.3 and 0.5) and four θ values (0, 0.1, 0.3 and 0.5). The starting point for each simulation was the actual population's allele frequencies. Population sub-structure was simulated by manually adjusting the allele frequencies until the desired θ was obtained between two sub-populations (one with original allele frequencies and one with adjusted) according to $= \sum_{alleles}\left(p - \bar{p}\right)^2 / \bar{p}\left(1 - \bar{p}\right)$. From the resulting allele frequency distributions, arrays of all possible single locus genotypes were generated in proportion to their expected frequencies as described in Overall and Nichols (2001). Multilocus genotypes were generated by randomly drawing from these arrays. Population sizes were held at N = 100. For example, for the scenario C = 0.1, θ = 0.5, the two arrays of allele frequencies differing by θ = 0.5 were each used to generate 50 multilocus genotypes, 5 of which were in Hardy-Weinberg equilibrium, 45 of which were inbred with F_{IS} = 0.25. These were then combined to give a population of N = 100. This procedure was repeated five times for each population scenario and the resultant likelihoods combined.

The method was then applied to the 10, 12 and 12 populations showing significant heterozygote deficits in the plant parasitic nematode *G. pallida*, *G. tabacum* and *H. schachtii*, respectively. The output matrices of likelihood values were used to identify the most likely parameter combination (over a grid of 10,000 combinations of θ and C values) for explaining the heterozygote deficit observed, and to draw graphs of the likelihood surface for each nematode population using the software R.

RESULTS

Heterozygote deficits and genotypic diversity

The newly developed markers confirmed the results of Picard *et al.* (2004) for the four Peruvian populations of *G. pallida* ($0.04 < F_{IS} < 0.17$, Table 1). Regarding French populations, the five populations from Saint-Malo showed significantly positive F_{IS} ($0.09 < F_{IS} < 0.24$, Table 1), whereas only two populations from Noirmoutier, among the 10 tested,

showed a significantly positive F_{IS}. Gene diversity H was higher for populations from Peru (H varied between 0.55 and 0.61, Table 1) and Saint-Malo (H varied between 0.50 and 0.53, Table 1) than for populations from Noirmoutier (H varied between 0.29 and 0.46, Table 1). Accordingly, the allelic richness R was the highest in Peru (R varied between 4.55 and 4.93, Table 1), intermediate in Saint-Malo (R varied between 3.05 and 3.51, Table 1), and the lowest in Noirmoutier (R varied between 2.00 and 3.41, Table 1).

All but one of the *G. tabacum* populations (LarD with a $F_{IS} = 0.04$) showed a significantly positive F_{IS} ($0.13 < F_{IS} < 1.00$, Table 2). Gene diversity H ranged from 0.01 to 0.38 (Table 2) and allelic richness from 1.12 to 2.37 (Table 2) revealing a low genotypic diversity for that species.

Six *H. schachtii* populations from cultivated fields, among the eight tested, showed a significant heterozygote deficit ($0.01 < F_{IS} < 0.26$, Table 3). However, for *H. schachtii* populations from the wild compartment, only six populations, among the 33 tested, showed a significantly positive F_{IS} ($-0.31 < F_{IS} < 0.39$, Table 3). In terms of genotypic diversity, gene diversity and allelic richness were higher in the cultivated compartment ($0.40 < H < 0.49$ and $1.88 < R < 3.05$, Table 3) than in the wild compartment ($0.29 < H < 0.45$ and $1.94 < R < 2.91$, Table 3), as seen mainly from the H statistic.

Disentangling θ and C by a simulation approach

In all situations, the 95% confidence envelopes of estimated θ and C values contained the simulated values (Fig. S2, Supporting information), except in the 215-33 *G. pallida* population when $\theta = 0.5$ and $C \leq 0.1$. It is striking, however, that, for the range of gene diversities observed in our nematode populations, more information can be retrieved on sub-structure than on consanguinity levels. Unless the maximum likelihood is at C = 0, C is often over-estimated and confidence intervals for this parameter almost always encompass the whole range of possible values. In contrast, θ is always close to its true value or slightly under-estimated, and point estimates which confidence intervals do not comprise $\theta = 0$ are clearly indicative of a Wahlund effect.

Frequency of null alleles and their effect on θ and C estimations

Results provided by INEst showed low frequencies of null alleles: 0.67%, 0.00% and 3.83% for *G. pallida*, *G. tabacum* and *H. schachtii*, respectively. A locus by locus analysis showed that two markers had potentially null alleles in *G. pallida* (2.92% for Gp135 and 4.49% for Gp112) and that four markers had potentially null alleles in *H. schachtii* (0.47% for Hs36,

8.32% for Hs56, 9.88% for Hs84 and 11.95% for Hs68) with frequencies that did not exceed 12%. Moreover, the relationship between the percentage of missing data for each locus and F_{IS} was never significant ($P > 0.05$). The simulations showed that the estimation of θ was not influenced by the addition of null allele in the data sets. Variation of C became important (*i.e.* ± 0.1) only if the null allele frequency was above 20% (Fig. 1). These results were confirmed using allelic frequencies of five of our real populations, which showed little variation in estimated C and θ values, at least as long as null allele frequencies were in the range of what we observed in the populations sampled (Fig. S3, Supporting information). Altogether, those results showed that loci used here were only weakly affected by null alleles, or that estimated frequencies of null alleles have no consequence on the estimation of θ and C.

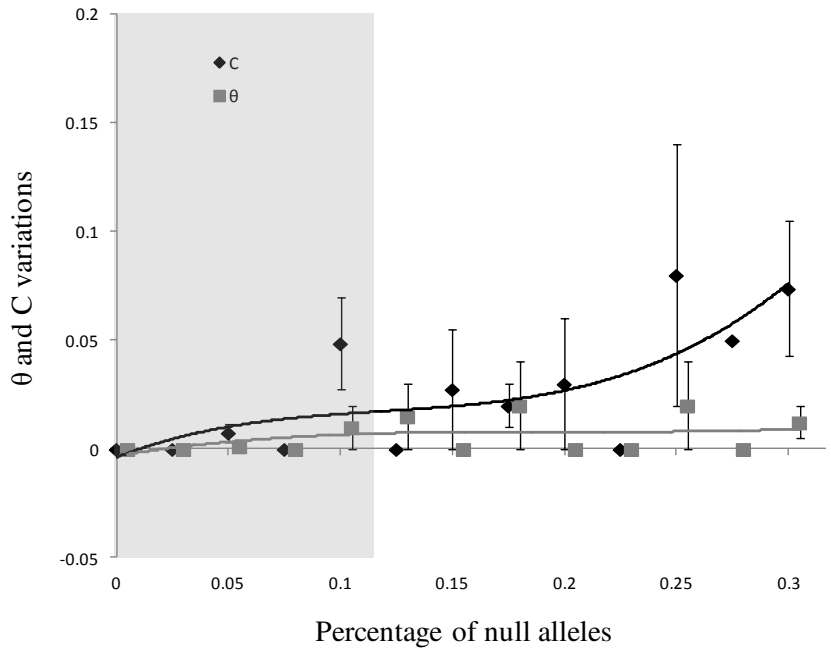


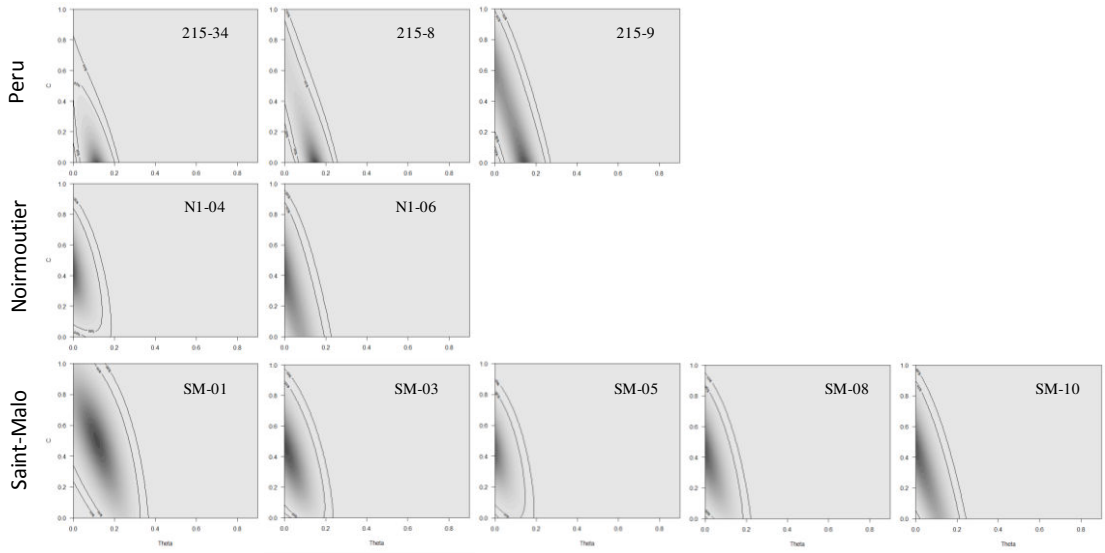
Fig. 1 Variations of the genetic correlation due to population subdivision (θ) and the proportion of the population practicing consanguinity (C) (± standard error) according to the percentage of null alleles (between 0 and 30%) from simulated populations. The hatched area represents the zone corresponding to our empirical data (*i.e.* a maximum of 12% of null alleles, as estimated by INEst).

θ and C in nematode populations

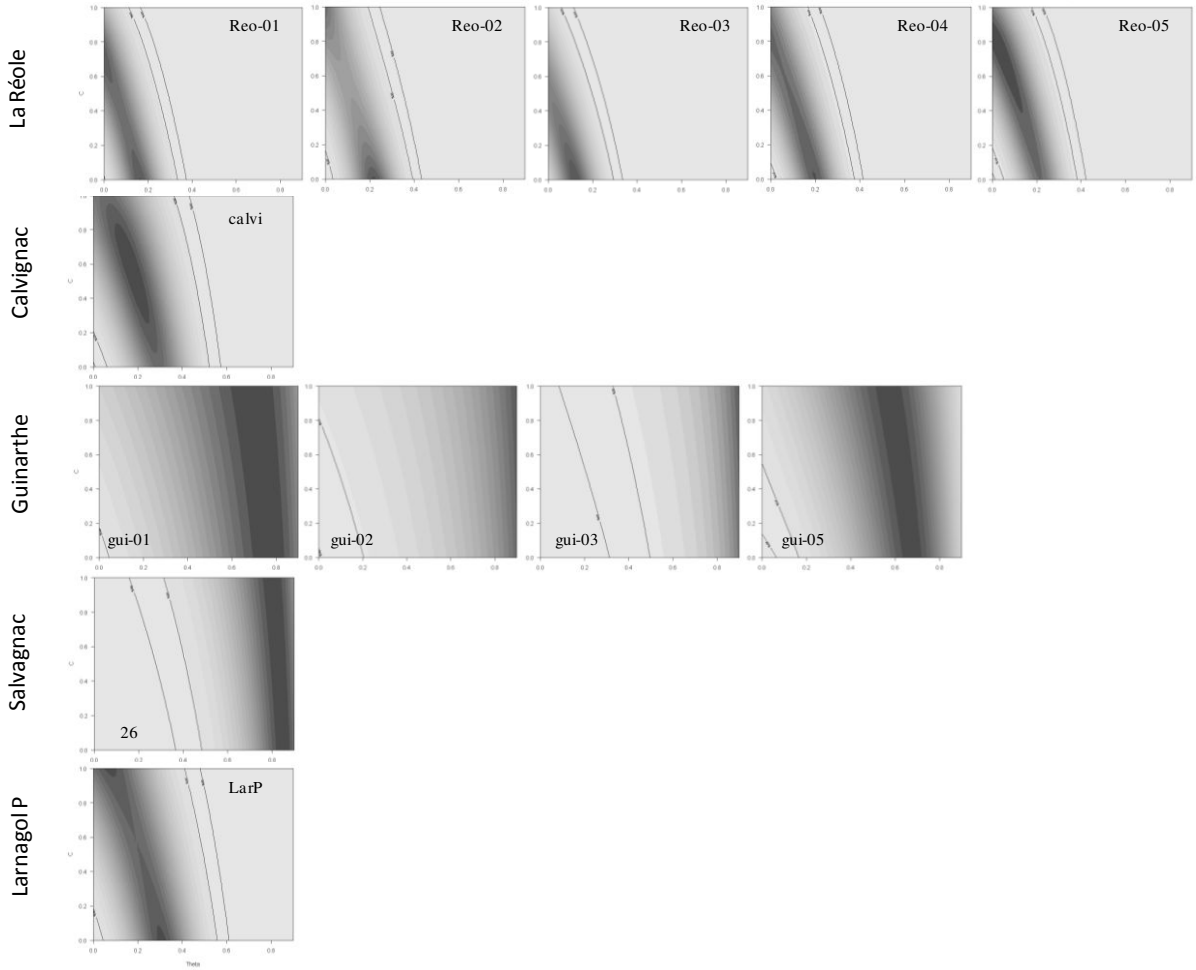
Among the ten *G. pallida* populations showing a heterozygote deficit, the deviation from HWE was attributed to genetic structure at a sub-population scale (a Wahlund effect) only for the three Peruvian populations (Fig. 2A and Table 1), whereas it was mainly attributed to consanguineous mating for French populations (Fig. 2A and Table 1) with a proportion of the population practicing consanguinity varying between 41% and 51% (Table 1). For one French

population (SM-01), the most likely parameter combination for explaining the heterozygote deficit included both sub-structure and consanguinity.

(A)



(B)



(C)

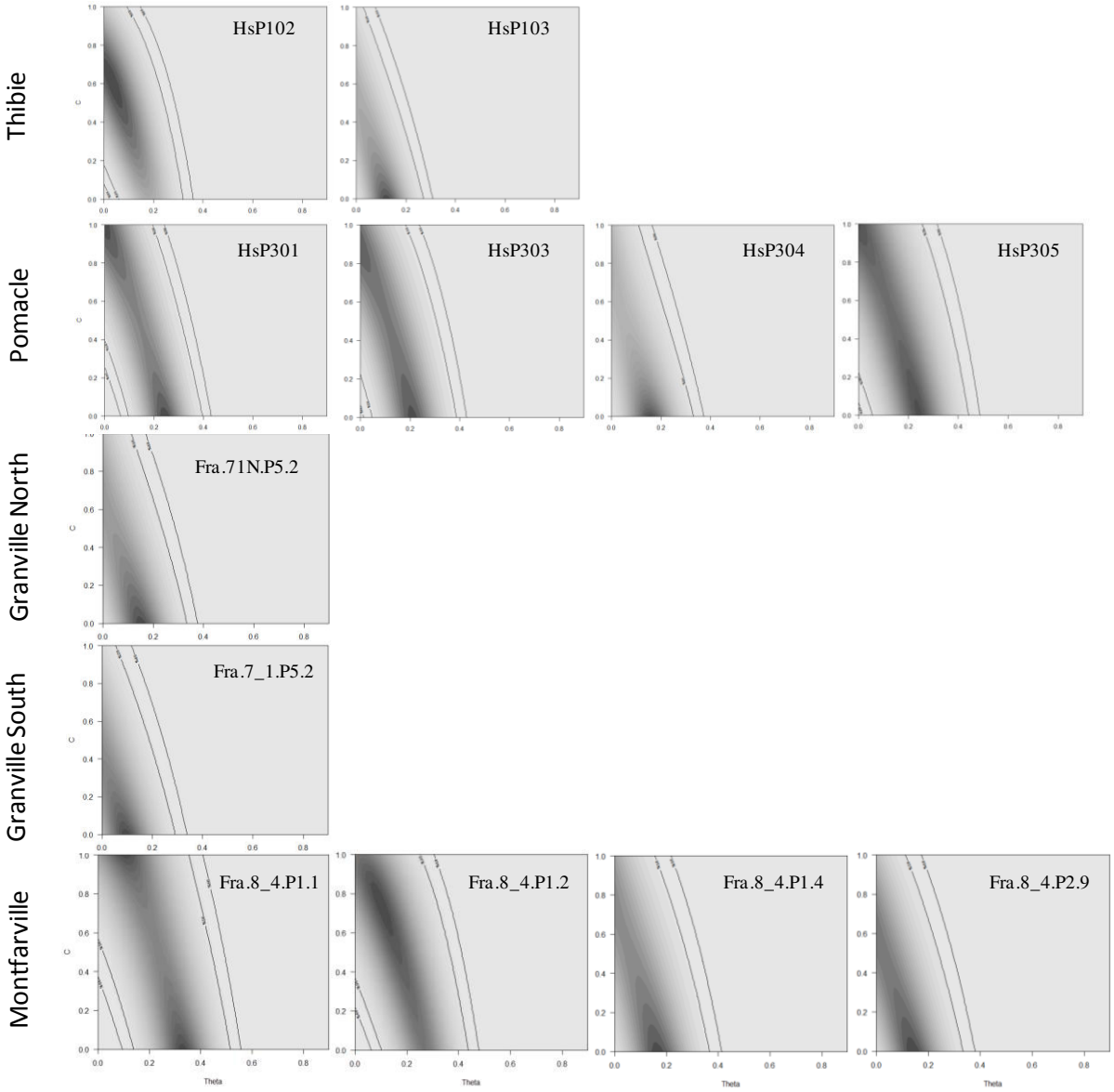


Fig. 2 Likelihood surfaces showing estimated θ and C values with 95 and 99% confidence envelopes (internal and external envelopes of the highest likelihoods, visualized as grey shades, respectively) for (A) the 10 *G. pallida* populations, (B) the 12 *G. tabacum* populations and (C) the 12 *H. schachtii* populations showing significant heterozygote deficits, respectively. θ and C are represented on the x-axis and y-axis, respectively.

For all *G. tabacum* populations, the most likely parameter combination included a high level of sub-structure (Fig. 2B and Table 2). There was no evidence of consanguinity for four of the five populations from La Réole and the last population (Reo-05) showed a higher θ and a lower C when we run the method with a degree of relatedness of 1/8 (θ = 0.15 and C = 0.63). Such a degree of relatedness could correspond to unions between half-sibs. The population from Calvignac showed also both consanguinity and sub-structure for explaining the heterozygote deficit. The LarP surface differs from the others (Fig. 2B), with two maxima

giving quite different interpretation of the data (θ = 0.08 and C = 1.00 or θ = 0.31 and C = 0.00). This is to be expected from this method, which is only able to disentangle C from θ whenever C < 100% (Overall & Nichols 2001). With alternative values of the degree of relatedness (1/8 and 1/16), the maximum-likelihood value favors the sub-structure scenario (not shown). Finally, the method did not disentangle θ and C for *G. tabacum* populations from Guinarthe and Salvagnac showing $F_{IS} > 0.5$ (Fig. 2B and Table 2).

In all *H. schachtii* field populations but HsP102, the heterozygote deficit was attributed to sub-structure (Fig. 2C and Table 3). The heterozygote deficit in *H. schachtii* populations from the wild compartment was also attributed to sub-structure, except for the population Fra.8_4.P1.2 (Fig. 2C and Table 3).

Altogether, those results showed that heterozygote deficits have not a single origin whatever comparisons are made between species or between populations. Indeed, we found evidence for both consanguineous mating and the Wahlund effect to be associated with observed heterozygote deficits.

DISCUSSION

We report a consistent pattern of heterozygote deficiency in three species of plant parasitic nematodes. Though it has been shown that parasitic nematodes can exhibit null alleles (Grillo et al. 2007; Silvestre et al. 2009; Brouat et al. 2011), we demonstrate that their prevalence is weak in our focal species. We furthermore show by simulation that the level of null allele frequency we detect is unlikely to bias our estimates of consanguinity and of the Wahlund effect. Because the probability of homozygosity is a function of allele frequencies, the method of Overall and Nichols (2001) requires the use of highly polymorphic markers (Selkoe and Toonen 2006). Molecular markers developed for the three nematode species were polymorphic, however those used to type *G. tabacum* populations were less variable than those used to type *G. pallida* and *H. schachtii* populations. That difference could be due to the fact that the eight microsatellite markers used in this species were issued from another species. Several studies have indeed reported a loss of informativeness in cross-amplifying markers between species due to mutations within the microsatellite arrays (FitzSimmons et al. 1995; Fredholm and Wintero 1995; Selkoe and Toonen 2006). A consequence was that it was more difficult to disentangle consanguinity from the Wahlund effect in *G. tabacum* when compared to the other two species.

We detected heterozygote deficits by sampling at the most restricted spatial scale, that is, at the level of a single individual host, as is commonly practiced in parasitic species that

frequently exhibit a distribution aggregated according to host individual (e.g. Thiele et al. 2008; Guzinski et al. 2009; Dharmarajan et al. 2010; Dharmarajan et al. 2011). Facing a common pattern in three species that differ in host, host range, distribution, number of generations/year, etc., we expected to find a common process that could explain our results. But, like in other parasitic (Rougeron et al. 2009; Dharmarajan et al. 2011) and non-parasitic (Castric et al. 2002) species, it seems that heterozygote deficits have not a single origin. This conclusion holds whatever comparisons are made between species or between populations within species (our examples, Castric et al. 2002; Thiele et al. 2008). The common feature between these different case studies is that restricted dispersal lies behind both main biological processes that favor heterozygote deficits: Wahlund effect (this study; Thiele et al. 2008; Rougeron et al. 2009; Dharmarajan et al. 2011) and mating between relatives (this study; Castric et al. 2002; Guzinski *et al.* 2009).

In our studied species, we found evidence for both consanguineous mating and the Wahlund effect to be associated with observed heterozygote deficits. Although C could be over-estimated (Fig. S2, Supporting information) and confidence intervals of this parameter are large, this is not the case for θ, at least in *G. pallida* and *H. schachtii*, and point estimates of both variables do vary between populations. We explored these variations in point estimates of θ and C to examine whether they are consistent with the idea that it is restricted dispersal that leads to heterozygote deficits through both sub-structure at the plant scale and consanguinity. First, soil movement should erase any sign of the Wahlund effect through the mixing of cysts. Field populations of beet and potato parasitic nematodes sampled in Europe are mixed every year through ploughing and tuber or root harvesting. The other populations live in soils that are more stable: the region of Andahuaylas (Peru) is a hilly region where growers do not use agricultural machines in their potato fields; tobacco fields are less ploughed than potato or beet fields, and the harvesting steps do not lead to soil movement; the sand of beaches where we sampled *H. schachtii* only moves during strong storms. Comparing θ values between these two groups of populations, we indeed find that evidence for the Wahlund effect is less pronounced in populations that go through regular soil movement (θ = 0.09±0.03; mean±standard error) than in populations that are in more stable grounds (θ = 0.31±0.06).

Second, the proportion of consanguineous mating should decrease with increasing nematode density, because of increased mating opportunities. In *G. pallida*, the only species for which we have density estimates, the proportion of nematode populations showing a significant heterozygote deficit that is attributed to consanguineous mating was higher in the

field near Saint-Malo (5/5, *i.e.* 100%) than in fields of Noirmoutier (2/10, *i.e.* 20%). Indeed, Fournet et al. (2013) reported 3 larvae *versus* 82 larvae per g of soil for these fields, respectively.

Finally, we note that *G. pallida* achieves only one generation per year whereas *G. tabacum* and *H. schachtii* are able to achieve several generations per year (Raski 1950; Adams et al. 1982; Johnson 1998). We think that this could explain why the heterozygote deficit in nematode populations found in tobacco and beet fields is mainly attributed to sub-structure, meaning that the Wahlund effect can build up within a single crop season in these multivoltine species.

Altogether, these results point to a plausible scenario for heterozygote deficits in cyst forming phytoparasitic nematodes. After a new nematode population is founded (through any mechanism of passive dispersal that allows individual cysts to meet suitable hosts), low densities associated with restricted active dispersal capabilities lead to consanguineous mating that result in heterozygote deficits that are mainly attributed to consanguinity. For field populations that live on plants that are harvested after each nematode generation (*G. pallida*), this signal is easily erased through soil movements. If inoculates contain large enough nematode populations (Noirmoutier), this does not allow heterozygote deficits to arise through sib-mating. In multivoltines species, the succession of different generations associated with low active dispersal abilities allows the accumulation of allele frequency differences between sub-populations at the plant scale during a single crop season, leading to Wahlund effects. This signal can be eventually erased after harvesting and tillage.

The fact that variations in θ and C values between the investigated species and populations are consistent with the biology of cyst nematodes supports the view that our results are sound and interpretable in terms of restricted dispersal. We must acknowledge, however, that our sampling scheme was not set up to test whether soil movement, cyst density, or the number of generation per year, influence the frequency of consanguineous mating or the buildup of a Wahlund effect. This clearly will require further investigations before definitive conclusions on the relative importance of these two processes are reached. The scenario we propose is testable through both field experiments and population genetic modeling. This scenario for instance predicts that restricted dispersal should lead to heterozygote deficits through consanguineous mating in all three species: this could be tested by sampling populations of the different species after only one generation on the host. A second prediction that could be tested through appropriate sampling schemes is that the Wahlund effect increases through time in mutlivoltine species. We already have some support

for this prediction. Among the populations of *H. schachtii* that we sampled in the wild compartment in 2012, some were resampled one year later in the course of a companion project on effective population sizes (PL Jan, C Gracianne, S Fournet and EJ Petit, data not shown). We used this opportunity to look at the temporal evolution of the heterozygote deficit and its causes in sixteen populations that were sampled in both years. We observed an increase of F_{IS} in all but one population, and this increase was linked to an increase of the Wahlund effect (permutation test for paired values, 10,000 permutations, $P = 0.005$) but not to an increase in the proportion of individuals that originate from consanguineous mating (permutation test for paired values, 10,000 permutations, $P = 0.130$). Finally, population genetic modeling through individual based simulations could further help understanding under which conditions of density and dispersal limitation heterozygote deficits can build up through consanguinity and the Wahlund effect, and whether the two processes can follow each other in multivoltine species.

What remains, though, is that we could identify several *G. pallida* populations, sampled in potato fields, in which all the heterozygote deficit was attributed to consanguineous mating (N1-04, N1-06, SM-03, SM-05, SM-08 and SM-10, see Fig. 2A), a result that is not sensitive to our choice of the relatedness coefficient (1/4) between mating partners (data not shown). This is important in terms of transfer to agro-ecosystems, and in particular for the management of plant resistances. Because the control of plant parasitic nematodes through application of toxic chemical nematicides has been reduced in the European Union because of environmental concern, growth of resistant cultivars has become the preferred method for nematode control. While consanguineous mating leads to homozygosity at all loci, including loci governing avirulence/virulence, which favors the expression of virulence when recessive (Flor 1942; Janssen et al. 1991; Cornell et al. 2003), heterozygote deficits due to the Wahlund effect are expected to have no particular effect on the adaptation of nematodes to resistance genes. Our results imply that sexually reproducing parasites in which consanguinity occurs should get around resistance genes more efficiently than randomly mating ones, thus lowering durability, a process that is not included in the conceptual model proposed by McDonald & Linde (2002). This opens new research areas to identify agricultural practices that improve the durability of cyst nematode resistance genes.

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Vilaine, France), R. Chivet (Institut Technique de la Betterave, France) and B. Cailleteau (Imperial Tobacco, France) for their help in soil sampling. We also acknowledge four anonymous reviewers for their useful comments.

DATA ACCESSIBILITY

Three files containing the genotypic data (Genepop format) for each species (*Globodera pallida*, *Globodera tabacum* and *Heterodera schachtii*) have been deposited onto DRYAD (doi:10.5061/dryad.04m3v).

AUTHOR CONTRIBUTIONS

SBV, CG, SF, EO, ADJO and JM performed the experiments and simulations according to a protocol elaborated jointly by JM, SF, EG and EJP. JM, PLJ and EJP analyzed the data. JM and EJP wrote the text and prepared the figures. All authors edited the paper and have approved the current version.

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

Table S1: Microsatellite markers developed for the three cyst nematode species, *Globodera pallida*, *Globodera tabacum* and *Heterodera schachtii*. Microsatellite motif, PCR product size and primers (forward and reverse) were indicated for each marker.

	Locus name	Repeat motif	PCR product size	forward primer	reverse primer
G. pallida	**Gp106**	ATTTT	185-196	TCTGTTCAGCGCACTTATGG	ATTTGATCGTTCCCTCGTTG
	Gp108	AATC	189-290	TAACGGCTATCAGCCCAATC	TCGGCCAAAACGTAAAACTC
	Gp109	ACGG	122-143	TCTCGCAGAAGGGAAAAGAA	TAAAAGACGGAAGAACGGGA
	Gp111	TCGG	107-115	TCCATTTGTTTTGGGGACAT	CCGTGTCCGATAAATTCCTG
	Gp112	AATG	211-255	GTTTTAAGCAGACAAGGCCG	ATCTCATAGCAATTTGCCCG
	Gp116	CGTC	131-166	ATTCATTCGCAATGTTTCCC	TGGAAATGTGAGAAAGGGCT
	Gp117	GCCC	112-132	GTCTATTGGCGGCACGTATT	TTCCAAATCCGCCATAATTG
	Gp118	TCCG	130-188	ACCGATGAAGAACATCGTCC	TCGTTCCGTCTTCGTAATCC
	Gp122	CATT	134-154	AGAGGGTGCCTTTGCTTCTT	ATTGAGTGCCAATAATCCGC
	Gp126	GATT	193-223	GTTATTGTGGCGGATGGAAT	GTACTGTATGATGCCGGGCT
	Gp135	GA	145-159	GCGAAATGAACGGTCGTAGT	ATTACATTGCCCAAATCGGA
G. tabacum	**Gp108**	AATC	263-378	TAACGGCTATCAGCCCAATC	TCGGCCAAAACGTAAAACTC
	Gp116	CGTC	126-131	ATTCATTCGCAATGTTTCCC	TGGAAATGTGAGAAAGGGCT
	Gr56	GT	303-309	TACAGATGTGCCTGAATGGC	CTTCCTAAAGCAACAACGGC
	Gr58	GA	255-257	GTTTTGGAAAGGCCTTGGAT	TGAATGAGCTTATCACCGCC
	Gr67	GT	166-172	ACCTGAACGTCGTCATTTCC	TTTTCTTACCCGAATGGCAC
	Gr68	TG	176-183	TGCAAAAGACAATCCATGAAA	GAACGTGTCTGTCTGAACGC
	Gr82	GA	137-139	CGTCTGCATTTTGTCGTGTT	GTTCCGGCCAAATCCGTC
	Gr85	CA	120-126	CCAAAAATTGATTGGCATCC	AATATCGCGTTGTTCCCAAG
H. schachtii	**Hs33**	TC	375-444	TCGATCAGTCTCGAGGTCAA	AAATACATTTCGCTCGGGTG
	HS36	TC	289-292	AATTTCGTGTGAAAATGGGG	TTGAATGCCTCCAAAAATCC
	Hs55	CT	146-156	ACTGATCACTTCTGACGGGG	CCGACACAACAACACTCGAC
	HS56	GA	147-150	CGTCCCATTACGAAAATGTC	GTCTCTCTCTTCTCTTTGCTTC
	Hs68	AG	106-120	TGAGGAGAAAGAGGGAGAGGA	TCTTCCCTTCCACTCTCCAA
	Hs84	GT	228-247	CACTGGTGGCCTTTCAAAAT	CATAAGCCCTGAGGGGATG
	Hs111	GT	112-133	ATCAATTGACCAATCGACGG	AAATTTACTCCAGATTTTATTGTCACAT
	Hs114	TC	121-140	ATTCACAAAAAGCCGCAAAT	CACATTTGTGCGCAGTATCA

Fig. S1: Map of France showing the localization where the different cyst nematode populations have been collected (*Globodera pallida*, *Globodera tabacum* and *Heterodera schachtii*).

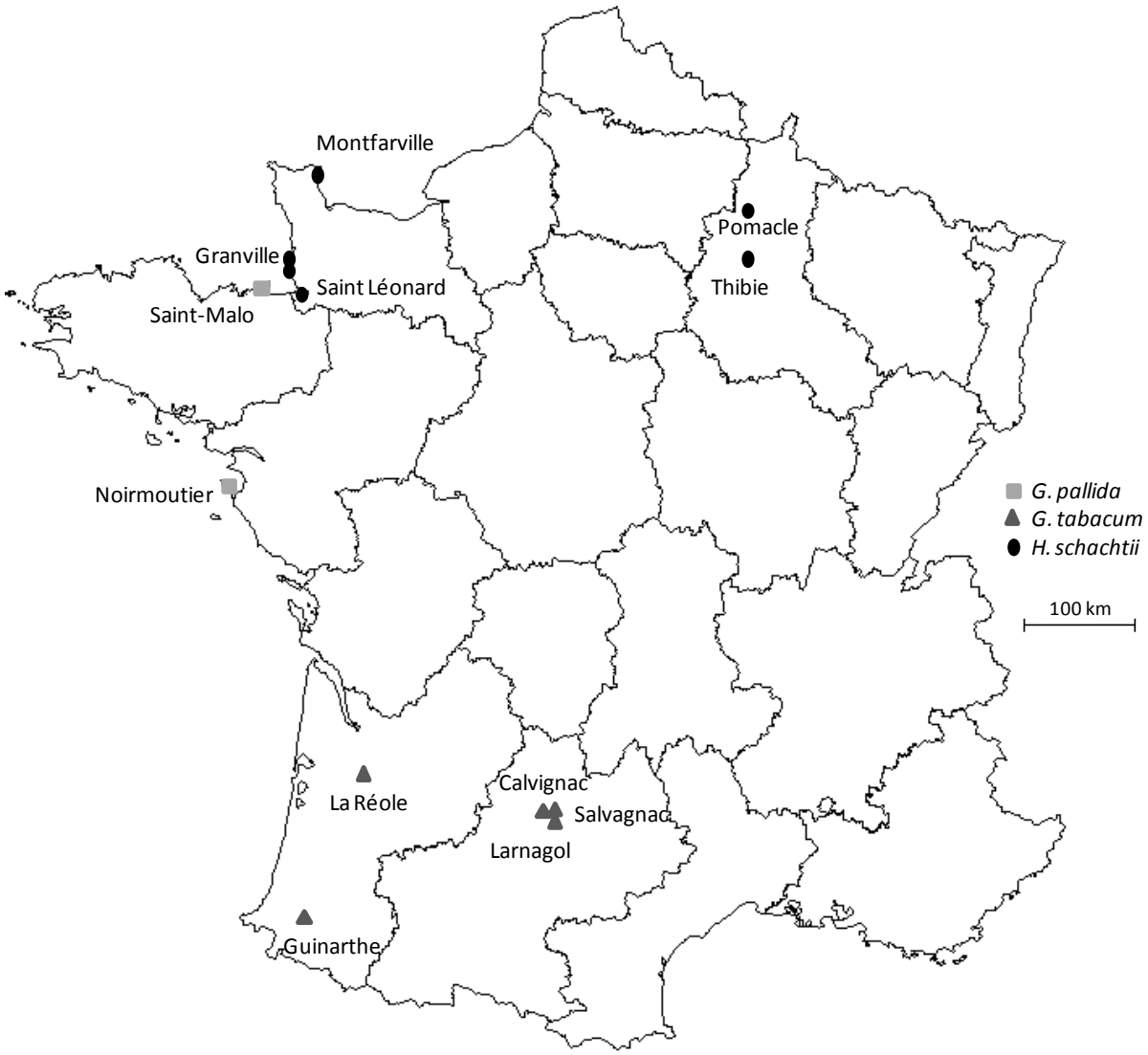


Fig. S2: Consensus likelihood surfaces for five simulations for each population scenario, showing estimated θ and C values (black dots) with 95 and 99% confidence envelopes (internal and external envelopes of the highest likelihoods, visualized as grey shades, respectively) for 16 scenarios of C and θ values (showed as thin strait lines). (A) Simulations run with allele frequencies of the 215-33 *G. pallid*a population; (B) Simulations run with allele frequencies of the Fra.7_1.P5.7 *H. schachtii* population; (C) Simulations run with allele frequencies of the LarD *G. tabacum* population.

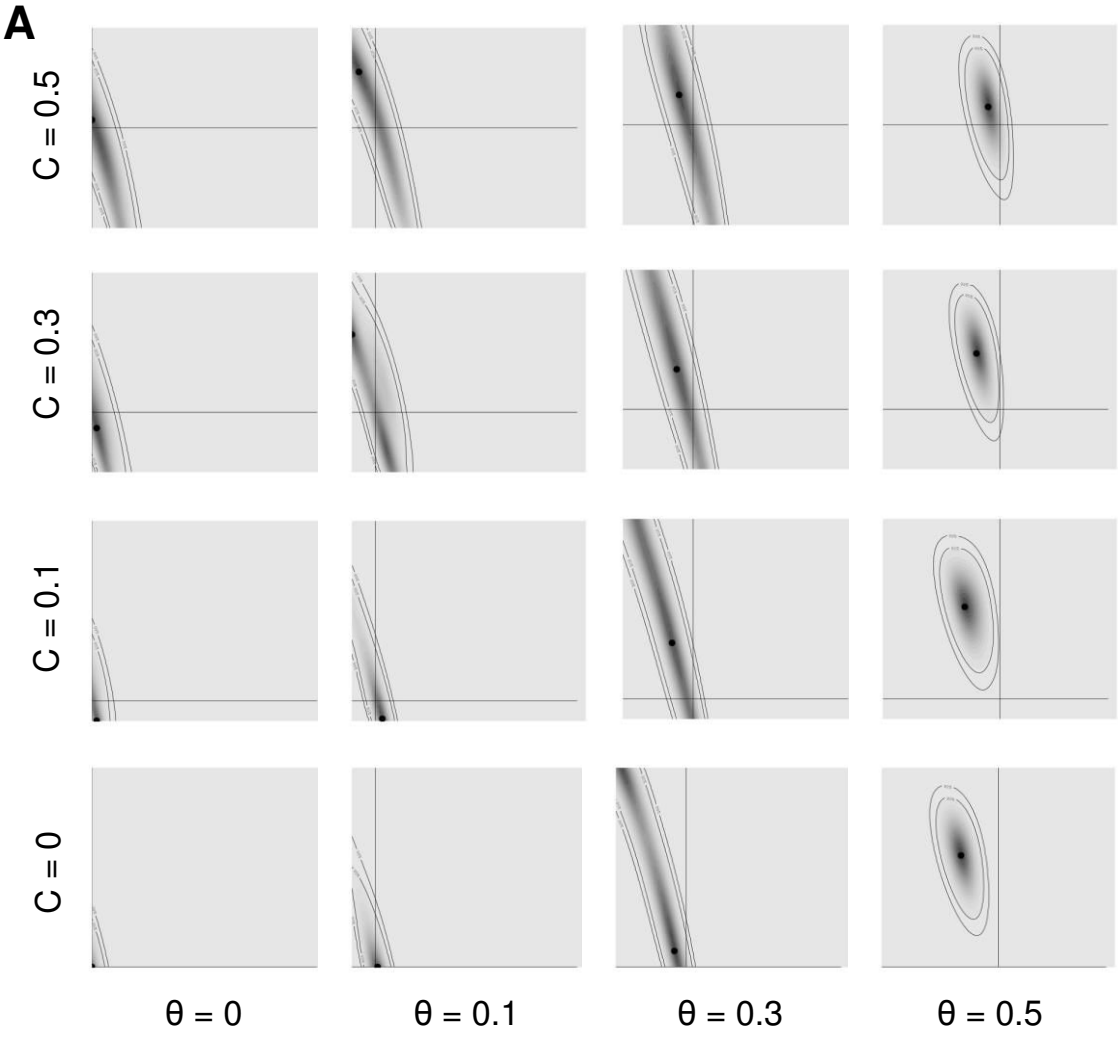
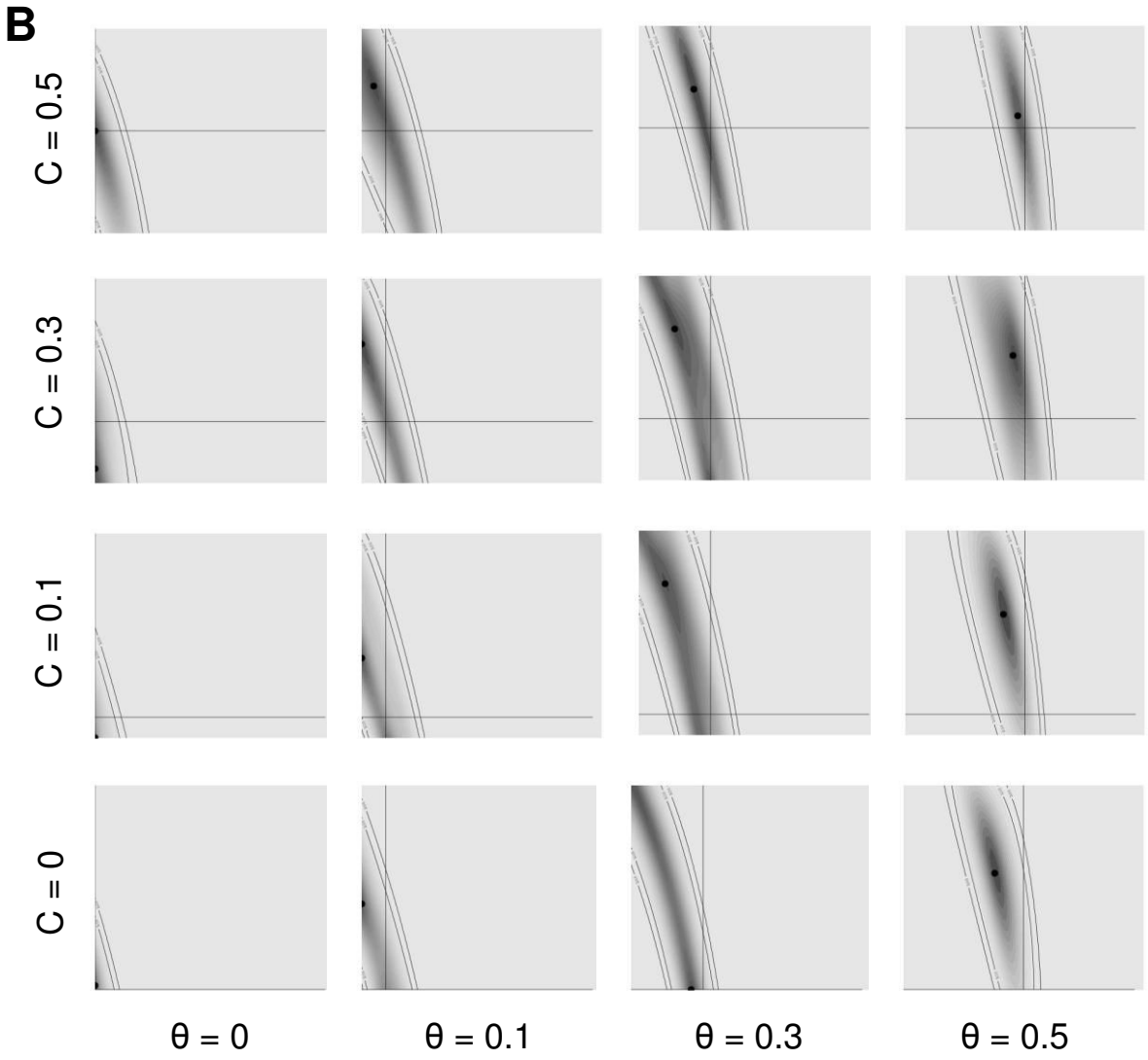


Fig. S2 (continued)



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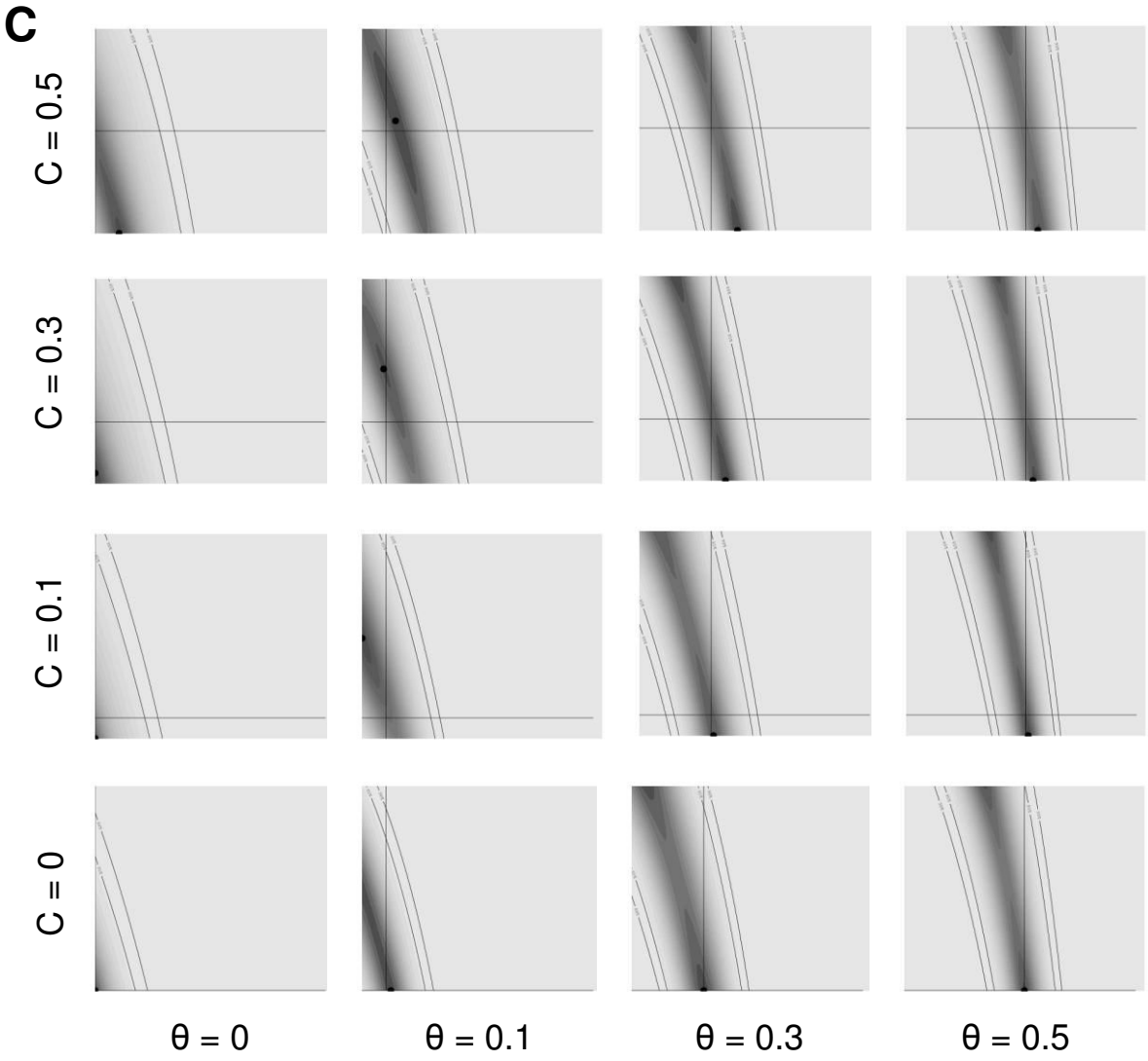
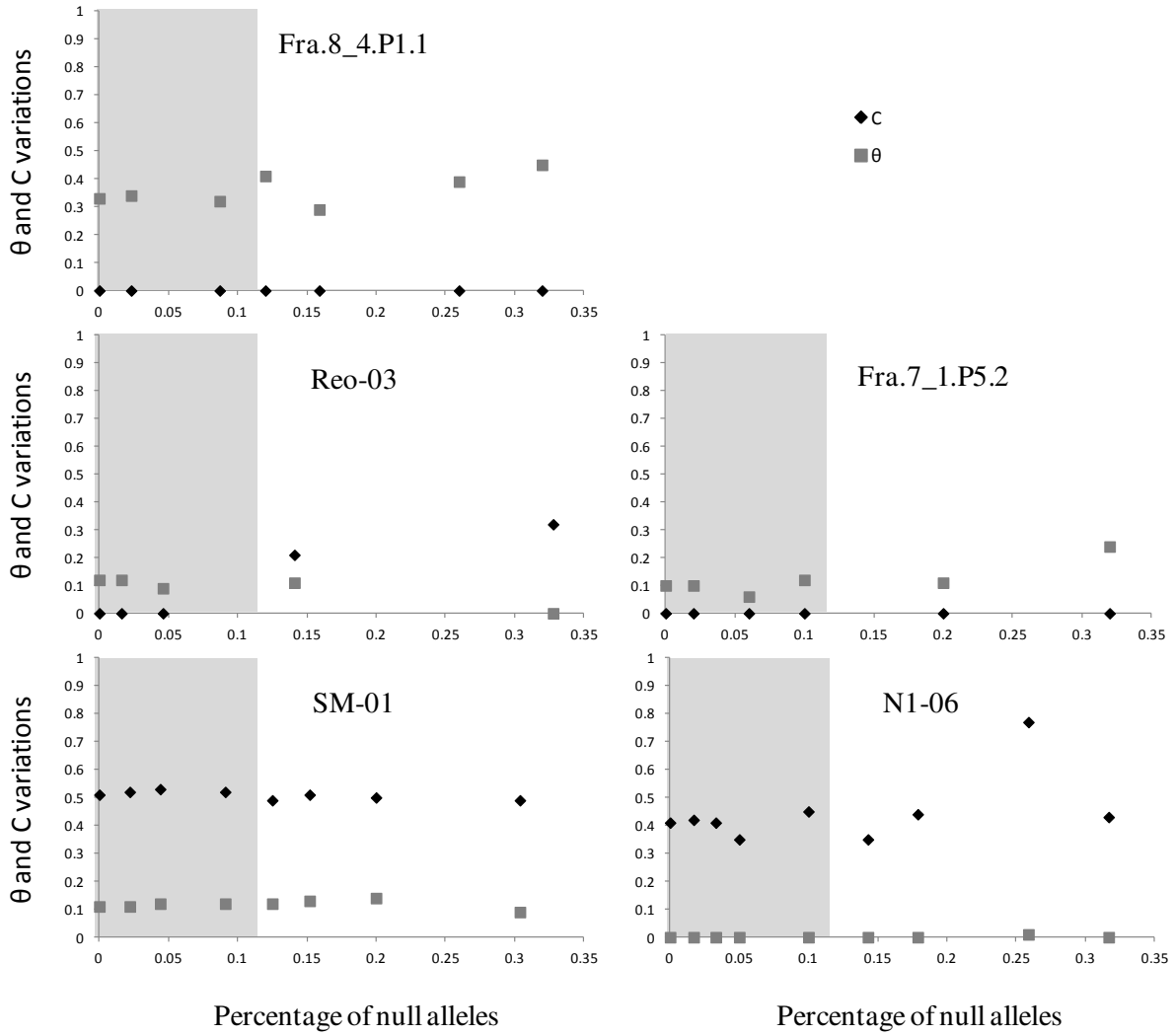


Fig. S3: Variations of the genetic correlation due to population subdivision (θ) and the proportion of the population practicing consanguinity (C) according to the percentage of null alleles (between 0 and 35%) using allelic frequencies of five nematode populations. The shaded area represents the zone corresponding to our real empirical data.



3.3. Temporal sampling and variation in the genetic structure of naturally occurring populations of a plant-parasitic nematode. 1 - Insights from the estimation of effective population sizes

Pierre-Loup JAN, **Cécile GRACIANNE**, Sylvain FOURNET, Eric OLIVIER, Jean-François ARNAUD, Catherine PORTE, Sylvie BARDOU-VALETTE, Marie-Christine DENIS, Eric J. PETIT

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ABSTRACT

Sustainability of modern agriculture relies on strategies that can control the ability of pathogens to overcome chemical or genetic resistance through natural selection. This evolutionary potential, which depends on effective population size (N_e), is largely affected by human activities. In this context, wild pathogen populations can provide valuable information for assessing the long-term risk associated with crop pests. In this study, we estimated the effective population size of the beet cyst nematode, *Heterodera schachtii*, by sampling 34 populations infecting the sea beet *Beta vulgaris* spp. *maritima* twice within a one-year period. Only 20 populations produced enough generations to analyze the variation in allele frequencies, with the remaining populations showing a high mortality rate of the host plant after only one year. The 20 analyzed populations showed surprisingly low effective population sizes, with most having N_e close to 85 individuals. We attribute these low values to the variation in population size through time, high inbreeding, and unbalanced sex-ratios. Our results suggest that *H. schachtii* has low evolutionary potential in natural environments. Pest control strategies in which crop populations mimic wild populations may help prevent parasite adaptation to host resistance.

Keywords: Heterodera schachtii, *Beta vulgaris* spp. *maritima*, effective population size, temporal sampling, wild nematode populations.

INTRODUCTION

Modern agriculture must develop integrated strategies that can control epidemics with reduced pesticide use (Pimentel 2005; Berny 2007; Meissle et al. 2010). These strategies involve reconciling agronomical, economical and sociological constraints with biological realities. One particular challenge ahead is to build strategies that account for the evolutionary potential of pests, which is a determining factor of long-term pathogen-related risks (McDonald and Linde 2002; Barrett et al. 2008). The use of chemical or genetic resistance commonly employed for parasite control may not be sustainable if the parasites have the evolutionary potential to overcome these resistance mechanisms via natural selection (see Mundt 2014 for a recent review and Fournet et al. 2013 for an example based on a cyst nematode). This potential can be partly estimated by the effective population size (N_e), a key evolutionary parameter that determines the strength of genetic drift within populations. Effective population size depends on demographic factors — such as census population size, dispersal ability or the mating system — and is a fundamental factor in the estimation of the risk associated with resistance durability in parasite populations (McDonald and Linde 2002; Barrett et al. 2008; Charlesworth 2009). Small effective population sizes imply that genetic drift predominates and genetic diversity is reduced, making populations less prone to respond to natural selection. Thus, populations with small effective sizes are likely to have reduced capacity for adaptation and, in the special case of pathogens, a low probability of overcoming chemical or host resistance.

Crop pathogens or parasites can also be found in wild hosts (Barrès et al. 2008; Barrett et al. 2009; Gracianne et al. 2014), particularly because wild hosts are the initial host species before their domestication or because parasites have shift from wild host to some cultivated plants (Stukenbrock and McDonald 2008). Once in the cultivated compartment, the geographical distribution, host associations, and disease dynamics of pests are mainly influenced by human activities (Morgan et al. 2012; Bousset and Chèvre 2013). Knowledge on their genetic structure in natural areas free of human influence is thus a valuable approach for understanding the evolutionary histories of populations but also for calibrating the evolutionary potential of populations, *i.e.* the ability of populations to evolve under natural selection.

The taxonomic group of nematodes contains more than 4,100 plant parasitic species causing damages estimated at $US80 billion per year (Hugot et al. 2001; Jones et al. 2013). Control of these parasites mainly includes crop rotation and the use of genetically resistant

crops (Van der Putten et al. 2006; Nicol et al. 2009); however, the control of plant-parasitic nematodes would greatly benefit from knowledge on spatial and evolutionary patterns that arise in wild ecosystems (Van der Putten et al. 2006). Nonetheless, to date, no studies have investigated the effective population sizes of these parasites. We thus set out to assess the effective population size in a plant-parasitic nematode, *Heterodera schachtii*. This cyst nematode infests the roots of the sugar beet (*Beta vulgaris* spp. *vulgaris*) and is one of the most damaging pathogens of sugar beet crops (Zhang et al. 2008). Genetic resistance of sugar beet against this nematode is considered as the most promising control method, and has already proven effective (Thureau et al. 2010). In the fields, population genetic structure of cyst nematodes is thought to be largely influenced by human activities (Alenda et al. 2014). The wild sea beet (*Beta vulgaris* spp. *maritima*), the wild ancestor of all cultivated beets, is also one of the host plants for *H. schachtii* in natural ecosystems (Maas and Heijbroek 1982). Our study focused on wild populations of *H. schachtii* to assess effective population sizes of wild populations free of human-related disturbance.

There are a wealth of methods for estimating contemporary effective population size (Wang 2005; Palstra and Ruzzante 2008; Luikart et al. 2010). They can be divided into two basic categories. First, single-sample estimators extrapolate N_e from parameters such as linkage disequilibrium and heterozygote excess, or summary statistics included in an approximate Bayesian computation (Pudovkin et al. 1996; Tallmon et al. 2008; Waples and Do 2008). Second, temporal methods consider the variation in allele frequencies between temporally spaced samples as the impact of genetic drift, which can be translated into N_e estimates. Both types of methods have their advantages and drawbacks, but the biological characteristics of *H. schachtii* may especially affect the results of single-sample estimators, namely the potentiality for inbreeding and fine-scaled sub-structuring of populations (Plantard and Porte 2004; Montarry et al. 2015). Regular inbreeding prevents the extrapolation of effective size from the excess of heterozygotes (Zdhanova and Pudovkin 2008) or linkage disequilibrium (Waples and Do 2010) and population sub-structuring is known to downward bias single-sample estimators (Holleley et al. 2014). Therefore, to avoid these potential biases we estimated N_e of *H. schachtii* populations by using temporal methods.

To estimate the effective size of wild populations of *H. schachtii* using temporal methods, we sampled 34 populations parasitizing wild sea beets in Normandy, France twice, with a sampling interval of one year. Beyond the basic understanding of genetic structure, the N_e of these populations may give insights into the understanding of the evolutionary dynamics of *H. schachtii* and help assess the long-term risks of overcoming host resistance in the field.

Biology of *Heterodera schachtii*

H. schachtii is a cyst nematode that infests the roots of wild sea beets and cultivated sugar beets. Active dispersal abilities of *H. schachtii* are considered to be extremely limited — about a few centimeters — due to their small size and their inability to move in anything but fluids (Plantard and Porte 2004). Thus, one nematode population is defined at the scale of a single, individual plant of *B. vulgaris* spp. *maritima*. Cyst nematodes are characterized by the solidification of the female cuticle after reproduction with one or several males (Triantaphyllou and Esbenshade 1990). Females then become a cyst that contains the eggs of the next generation. Eggs can be dormant for a long time, especially when cysts do not receive the proper stimuli, and hatching time can be extremely variable among cysts (Zheng and Ferris 1991). The development of larvae involves distinct stages, with the second-stage juvenile (J2) being the only larval stage that is free-living and mobile. The genetic diversity and population structure of *H. schachtii* has been studied in sugar beet fields and substantial departures from Hardy-Weinberg (HW) expectations suggest extensive inbreeding due to mating between relatives (Plantard and Porte 2004). A recent study conducted both on field and wild populations suggested that HW departures may also be explained by genetic sub-structuring due to Wahlund effects at the scale of the host plant (Montarry et al. 2015).

Biological material and sampling design

Soil samples were collected around roots of *B. vulgaris* spp. *maritima* plants on four different beaches in Normandy, France. The beaches, namely Montfarville (1°14’31” W, 49°38’46” N), Granville Nord (1°34’10” W, 48°48’22” N), Granville Sud (1°34’5” W, 48°48’12” N) and Saint Léonard (1°26’42” W, 48°39’17” N), were separated by distances ranging from 300 m to 150 km (Fig. 1). This geographical sampling range is of the same order of magnitude as previous investigations on this nematode conducted in sugar beet fields (Plantard and Porte, 2004). Populations of the host plant, the sea beet, are often composed of individuals clustered in geographically and genetically distinct patches (De Cauwer et al. 2010). Thirty-four sea beet plants, with a maximum of 10 plants on each beach, were sampled. The temporal method requires two distinct sampling sessions, ideally separated by at least five generations (Waples and Yokota 2007). In the first sampling (November 2012), sampled plants were marked with numbered plastic tags for easy identification during the second sampling session (November 2013). We recorded whether sampled plants were dead or alive at the second sampling session. Cysts of *H. schachtii* were extracted from soil samples using homemade sieves (250

µm and 800 µm) and manual examination of filtrates. Cysts were stored at 4°C in moistened sand before molecular characterization.

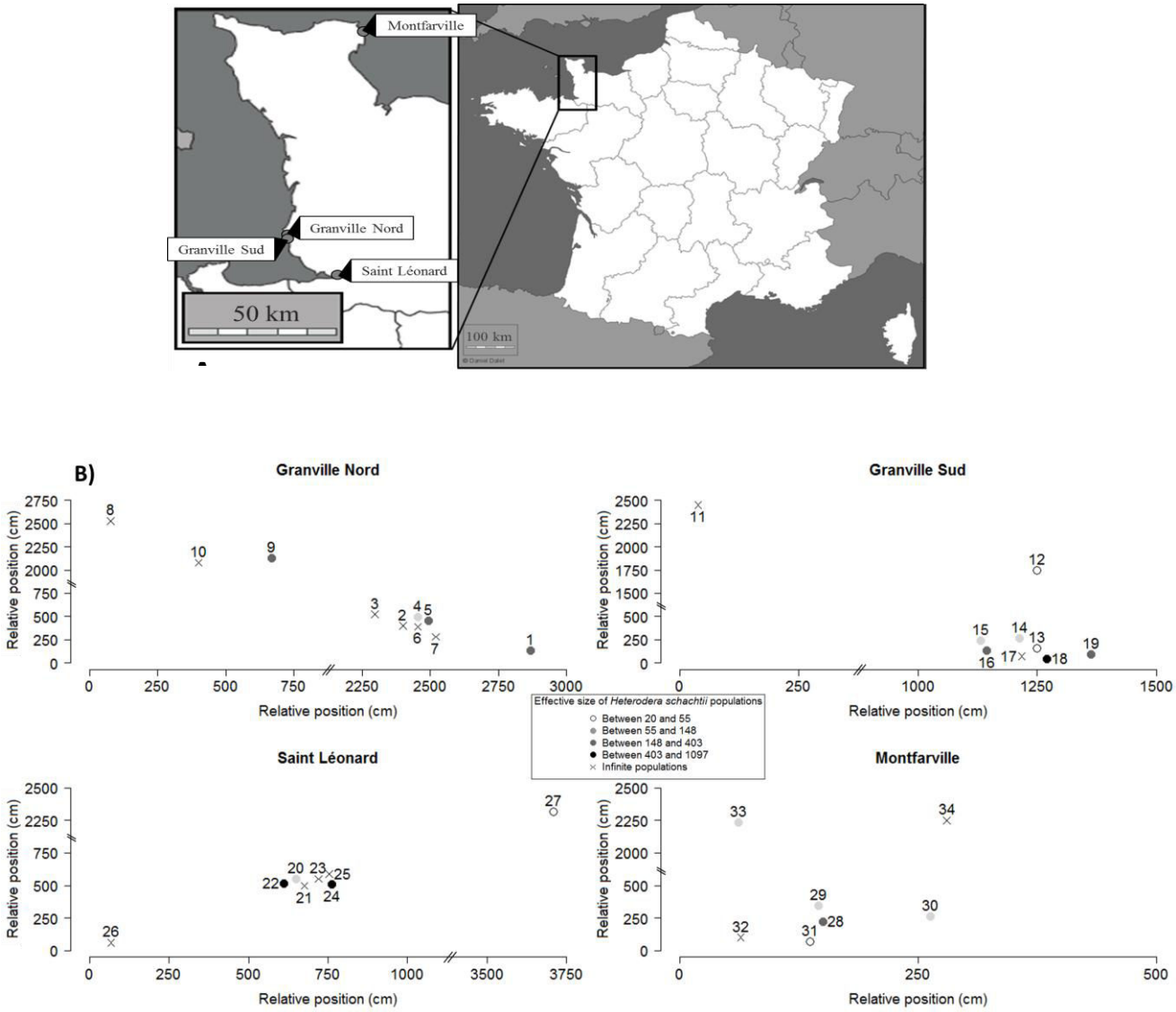

Figure 1: Beach locations (a) and relative locations of sampled populations (host plants) on each beach (b). Symbols show effective sizes estimated with the pseudo-likelihood method (see text). Effective population size classes (and the corresponding gray scale) were taken from Fig. 2.

Molecular characterization and genotyping

For each sampled nematode population, 40 second-stage juvenile larvae were used to perform DNA extraction and each larva was extracted from a different and randomly chosen cyst, to avoid family structure biases caused by sibling relationships. A soil sample can contain cysts from different *Heterodera* species; therefore, molecular characterization based on the restriction profiles of ITS sequence was used for species identification. By multiplying the ratio of *H. schachtii* among the 40 larvae with the number of cysts we sampled, we estimated

the number of *H. schachtii* cysts contained in our samples. DNA extraction, PCR amplification of ITS sequence and digestion of PCR products were performed as described in Gracianne et al. (2014).

We ultimately genotyped 761 and 854 *H. schachtii* individuals in 2012 and 2013, respectively, using eight microsatellite loci, named Hs33, Hs36, Hs55, Hs56, Hs68, Hs84, Hs111 and Hs114 and described in Montarry et al. (2015). Microsatellite PCR products were analyzed on an ABI Prism® 3130xl sequencer (Applied Biosystems) and allele sizes were identified using the automatic calling and binning procedure of GeneMapper v4.1 (Applied Biosystems) and completed by a manual examination of irregular results. Samples with dubious genotypes were reamplified.

Characterization of basic genetic parameters

Single and multilocus departures from Hardy-Weinberg (HW) equilibrium were tested for all populations on each beach using permutation tests (10,000 permutations) adjusted for multiple tests with Bonferroni corrections, as implemented in the software Fstat version 2.9.3 (Goudet 1995). This procedure is based on the estimation of F_{IS} for each population and its statistical significance. Genetic diversity of nematode populations was evaluated by estimating expected heterozygosity (H_e) and allelic richness (Ar) using Fstat. Allelic richness was estimated using the rarefaction method described in El Mousadik and Petit (1996).

Number of generation produced between the two sampling sessions

The temporal method requires knowledge of the number of generations produced between sampling periods. To date, there is no data on the generation time of *H. schachtii* in wild populations. However, *H. schachtii* produces about four generations a year in cultivated fields (Subbotin et al. 2010). Sugar beets can be parasitized during approximately seven months in cultivated fields before being removed during the harvest in autumn, whereas sea beets are perennial plants in our surveyed geographical areas (Hautekèete et al. 2002; De Cauwer et al. 2010, 2012). Thus, we assumed that wild populations of *H. schachtii* produce at least four generations in one year, and consider it the minimum number of generations produced per year. The generation time of cyst nematodes also strongly depends on temperature (Griffin 1988), as measured by Kakaire et al. (2012) under controlled laboratory conditions. We modeled their results on minimum generation time at different temperatures with a non-linear regression model based on the least squares method and a Gaussian curve using the software R (R Development Core Team 2011, version 2.12.2). This model was then used along with monthly temperature data provided by two weather stations (Météo-France data), one located

near Granville and Saint Léonard, and the other one located near Montfarville, to estimate the maximum number of generations produced by *H. schachtii* between the two sampling sessions. In subsequent analyses, we used these minimum and maximum numbers of generations as well as their median value to evaluate the impact of this demographic parameter on effective population size estimations.

Effective population size estimation

Two methods were applied to estimate the effective population sizes of *H. schachtii*. We used the moment-based method developed by Jorde and Ryman (2007) implemented in the software NeEstimator v2 (Do et al. 2014). Alleles with frequencies below 0.05 were excluded to avoid any bias caused by rare alleles, as described in (Do et al. 2014). We also used the pseudo-likelihood method of Wang (2001) implemented in the software MNLE 1.0 (Wang and Whitlock 2003). Both methods are complementary: the moment-based method yields unbiased but rather imprecise N_e estimates, while the pseudo-likelihood method gives N_e estimates that can be slightly upwardly biased, but that are more precise (Jorde and Ryman 2007; Do et al. 2014). If the variation in allele frequencies between temporal samples is too low compared with sampling error, the moment-based method yields an infinite estimate of effective population size. Given differences in computing techniques, the pseudo-likelihood estimate reaches in such cases the upper limit of possible values (here fixed at 35,000 individuals), which is then considered infinite as well.

To check whether the two kinds of N_e estimators gave similar and biologically meaningful results, the two set of results were compared for significant differences using a Wilcoxon signed-rank test on nematode populations characterized by “finite” effective population size results.

Both methods assume that no migration or selection occurs and that the variation in allele frequencies between temporal samples is only due to genetic drift and sampling errors. To verify this hypothesis, we performed an exact test of homogeneity in allele frequencies using the software Genepop v4.2 (Raymond and Rousset 1995). Exact tests can detect nematode populations that show significant changes in allele frequencies between two temporal samples. Populations with significant changes were selected as candidates to test for the presence of non-random variation in allele frequencies using the generalized test described in Waples (1989). The generalized test was performed *a posteriori* because it requires an estimation of effective population size. To this end, we used the pseudo-likelihood estimation

of N_e, thought to be more precise, assuming the median number of generations between the temporal samples to perform this test.

Waples and Wilcoxon tests were computed with R (R Development Core Team 2011, version 2.12.2).

RESULTS

Number of generations produced between the two sampling sessions

The relationship between temperature and the minimum generation time of *H. schachtii* was modeled by a Gaussian curve (Fig. S1). The resulting maximum number of generations produced each month ranged from 0.2 to 1.2 (Fig. S2). Overall, adding up all the generations over the whole year resulted in 9.9 generations for Montfarville beach and 10.6 for the three remaining surveyed beaches. Wild *H. schachtii* populations may thus have produced up to ten generations between two sampling sessions. The number of generations that elapsed between the two sampling sessions was therefore set to the minimum (four generations), maximum (ten generations) and their median values (seven generations) in subsequent analyses.

Estimations of effective population sizes

Characterization of the microsatellite loci and populations used for effective population size estimation are presented in Table 1 and 2, respectively. When considering the median value of seven generations, effective population sizes were extremely variable among populations, with 14 populations showing an infinite effective population size for at least one estimator (Table 3). The other 20 populations showed finite effective population size estimates with both methods. These populations will be called "finite populations" as opposed to the 14 remaining "infinite populations". Based on a seven-generation span among temporal samples, mean effective sizes of finite populations were equal to 218 and 209 individuals for the moment-based and the pseudo-likelihood estimates, respectively. The distribution of the effective size of finite populations fit a log-normal distribution (Fig. 2) and the modes of these distributions were 80 for the moment-based estimator and 91 for the pseudo-likelihood estimator. As expected (Jorde and Ryman 2007), the moment-based method had greater variance (80,826) than the pseudo-likelihood method (52,167). However these methods did not give significantly different estimates (Wilcoxon signed rank test, p=0.67) for finite populations. Effective population sizes obtained when considering a ten-generation span were twice as large as results with a four-generation span (Fig. 3). Given the small sizes observed,

the number of generations generally had a low influence on most of estimates of effective population size.

Impact of non-random forces

The exact homogeneity tests detected significant changes in allele frequencies between the two sampling sessions in nine populations. Of these, only population 2 exhibited a significant action of non-random forces such as selection or migration (Waples test, $p<0.05$, Table S1).

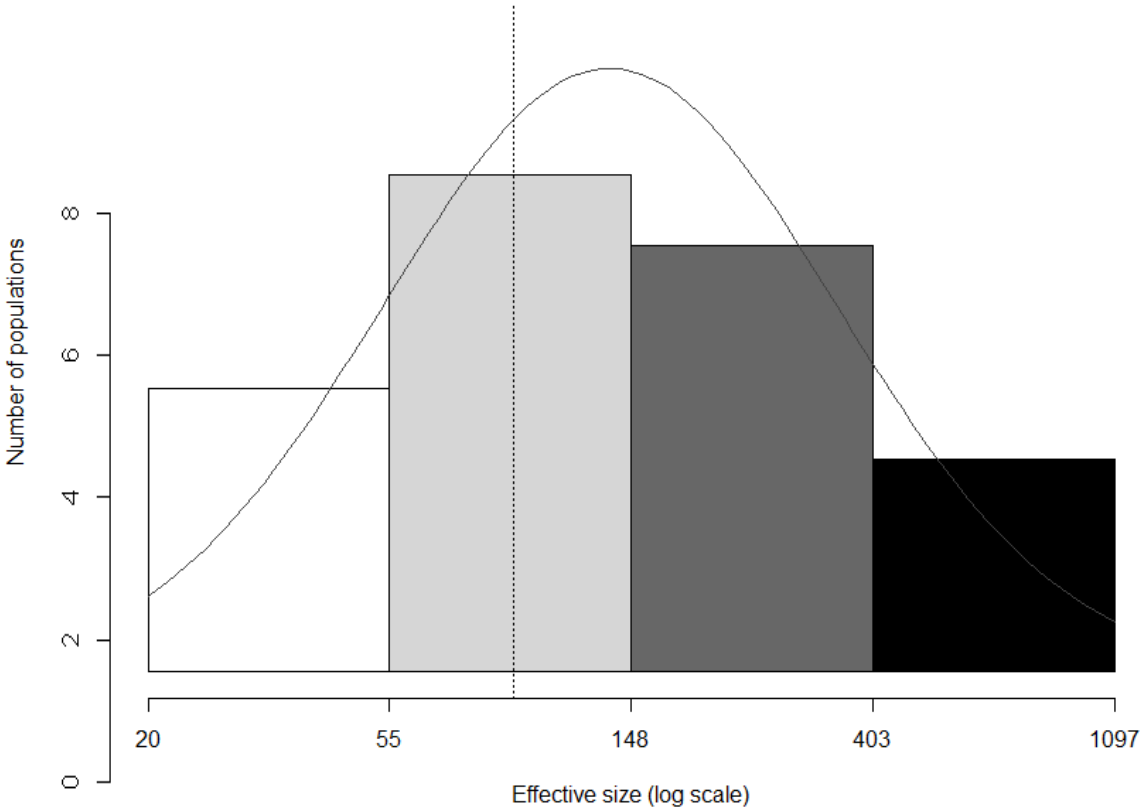

Figure 2: Effective sizes of finite populations of *Heterodera schachtii* (log scale). Effective sizes were estimated with the pseudo-likelihood method (see text). The dashed line indicates the mode of this distribution. The gray curve corresponds to a theoretical log-normal distribution.

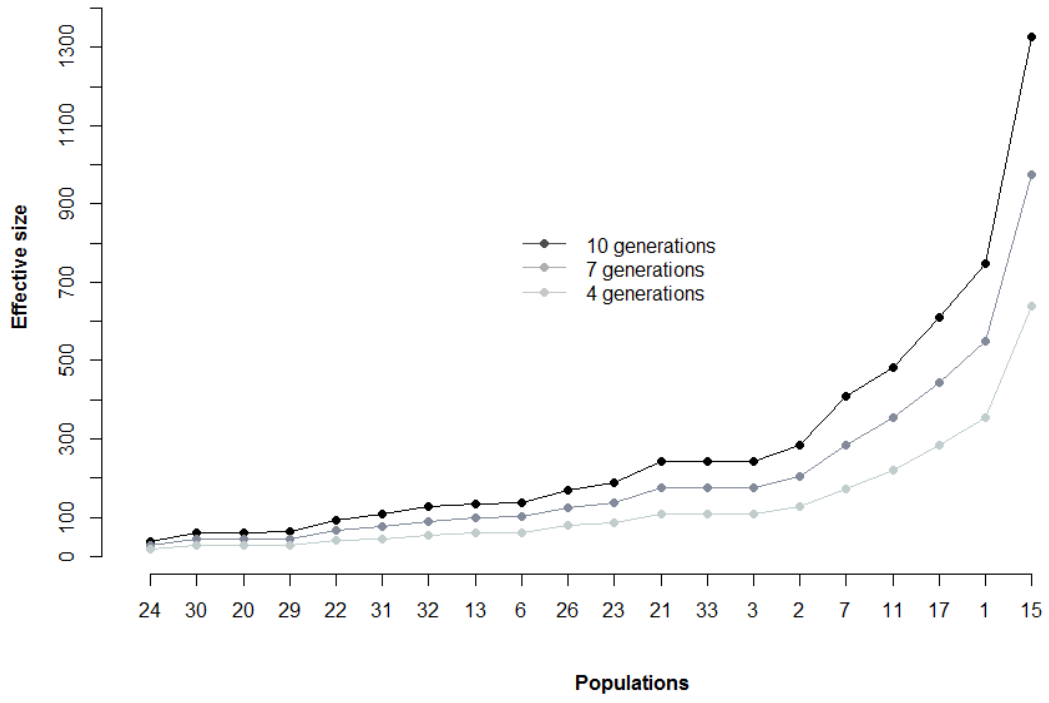

Figure 3: Influence of the number of generations on the effective sizes of finite *Heterodera schachtii* population. Effective sizes were estimated with the pseudo-likelihood method (see text). Populations are ordered from the lowest to the highest effective size.

DISCUSSION

The aim of this study was to estimate the effective population size of *H. schachtii* in wild ecosystems. Despite the occurrence of 14 populations showing infinite N_e estimates for at least one of the estimators used, the 20 other effective population sizes ranged between 20 and 1,300 individuals, with most of them having an N_e close to 80-90 individuals.

Requirements and reliability of effective population size estimates by temporal sampling

Most of single-sample estimators are biased in cases of strongly assortative mating or population sub-structuring (Zdhanova and Pudovkin 2008; Waples and Do 2009; Holleley et al. 2014). We therefore used temporal approaches to estimate effective population sizes. However, temporal methods also have underlying assumptions that can greatly affect the reliability of effective size estimates if they are violated. First, this method requires that temporal samples be separated by several generations. Because the generation time of *H. schachtii* in the wild is poorly documented, it was necessary to approximate this parameter using data from field nematode populations (Subbotin et al. 2010) and previous experiments (Kakaire et al. 2012). We determined that seven generations, plus or minus three, elapsed between samples. Second, temporal methods assume discrete generations, which did not

Table 1. Characterization of the polymorphic microsatellite loci employed on *Heterodera schachtii* at four study sites and two sampling sessions (2012 and 2013): Number of alleles (Nall), number of individuals typed (n) and expected heterozygosity (He).

Locus	Granville Nord					Granville Sud					Saint Léonard					Montfarville				
	Nall	n		He		Nall	n		He		Nall	n		He		Nall	n		He	
		2012	2013	2012	2013		2012	2013	2012	2013		2012	2013	2012	2013		2012	2013	2012	2013
Hs55	3	241	280	0.470	0.465	3	189	244	0.348	0.351	3	166	154	0.423	0.415	1	164	172	0.000	0.000
Hs68	4	240	280	0.481	0.476	4	189	242	0.485	0.509	5	165	156	0.492	0.502	6	140	141	0.539	0.560
Hs33	4	240	269	0.168	0.136	4	184	229	0.135	0.109	1	157	144	0.000	0.000	1	101	94	0.000	0.000
Hs36	2	198	211	0.269	0.198	2	141	167	0.299	0.295	2	146	117	0.130	0.068	2	151	147	0.204	0.134
Hs56	3	241	274	0.038	0.078	2	189	242	0.132	0.111	2	166	155	0.245	0.210	2	165	164	0.453	0.451
Hs84	7	239	270	0.452	0.473	5	181	240	0.474	0.468	5	165	154	0.410	0.347	6	151	161	0.635	0.582
Hs111	5	241	281	0.519	0.555	6	189	240	0.525	0.514	5	166	156	0.458	0.430	3	165	172	0.543	0.531
Hs114	5	241	280	0.578	0.582	6	189	242	0.573	0.542	5	166	156	0.403	0.428	4	165	171	0.594	0.538
Mean	4.1	235.1	268.1	0.372	0.370	4	181.4	230.8	0.371	0.362	3.5	162.1	149.0	0.32	0.300	3.1	150.3	152.8	0.371	0.350

Table 2. Characteristics of *Heterodera schachtii* populations: number of individuals typed (n), allelic richness (Ar), expected heterozygosity (He), and deviation from HW proportions (F_{IS}). Significant deviations are presented in bold. Population codes are identical to codes in Gracianne et al. (unpublished data).

Site	Population	Code	n		Ar		He		F_{IS}	
			2012	2013	2012	2013	2012	2013	2012	2013
Granville Nord	1	Fra.71N.P1.4	25	18	2.09	2.04	0.409	0.390	0.085	0.124
	2	Fra.71N.P2.1	25	23	1.78	1.96	0.324	0.361	**-0.162**	**0.143**
	3	Fra.71N.P2.2	25	16	1.93	1.94	0.361	0.346	0.056	**0.284**
	4	Fra.71N.P2.3	25	30	2.06	2.06	0.390	0.384	0.021	0.094
	5	Fra.71N.P2.4	23	27	1.90	2.07	0.358	0.396	0.021	**0.137**
	6	Fra.71N.P2.5	22	27	2.08	2.04	0.412	0.380	-0.04	**0.189**
	7	Fra.71N.P2.8	22	35	2.07	2.05	0.371	0.380	0.071	**0.151**
	8	Fra.71N.P3.7	21	35	2.01	2.00	0.398	0.388	0.051	**0.192**
	9	Fra.71N.P4.1	27	38	1.89	1.83	0.331	0.306	0.103	**0.170**
	10	Fra.71N.P5.2	26	32	1.92	1.93	0.361	0.361	**0.162**	**0.171**
Granville Sud	11	Fra.7_1.P3.7	19	19	2.02	2.02	0.421	0.415	**0.144**	0.129
	12	Fra.7_1.P4.1	20	22	1.85	1.89	0.339	0.333	0.136	**0.185**
	13	Fra.7_1.P5.1	22	25	2.29	2.06	0.445	0.407	0.101	**0.222**
	14	Fra.7_1.P5.2	25	25	1.94	1.86	0.341	0.312	**0.145**	0.092
	15	Fra.7_1.P5.3	25	32	1.79	1.80	0.294	0.312	0.030	-0.076
	16	Fra.7_1.P5.4	20	30	1.91	1.93	0.344	0.355	0.014	**0.191**
	17	Fra.7_1.P5.6	20	23	2.11	2.05	0.381	0.374	0.036	**0.281**
	18	Fra.7_1.P5.7	25	32	2.22	2.08	0.418	0.376	0.008	**0.099**
	19	Fra.7_1.P5.8	13	37	2.01	2.08	0.349	0.367	0.080	0.041
Saint Léonard	20	Fra.7_4.P1.1	21	25	2.06	1.82	0.398	0.316	**-0.243**	**0.201**
	21	Fra.7_4.P1.2	24	19	1.86	1.89	0.325	0.344	-0.054	**0.362**
	22	Fra.7_4.P1.3	13	11	1.87	1.73	0.335	0.276	0.025	0.141
	23	Fra.7_4.P1.7	24	22	1.82	1.91	0.292	0.365	**-0.218**	-0.077
	24	Fra.7_4.P1.8	24	17	1.84	1.70	0.339	0.293	0.096	0.125
	25	Fra.7_4.P1.9	19	28	1.97	1.87	0.368	0.328	0.070	0.058
	26	Fra.7_4.P2.1	29	17	1.86	1.71	0.318	0.296	0.063	0.138
	27	Fra.7_4.P3.5	12	17	1.53	1.43	0.188	0.172	-0.140	-0.112
Montfarville	28	Fra.8_4.P1.1	25	21	1.96	1.92	0.354	0.339	**0.391**	**0.199**
	29	Fra.8_4.P1.2	25	22	2.10	1.87	0.377	0.314	**0.269**	**0.294**
	30	Fra.8_4.P1.3	25	24	2.12	1.98	0.408	0.351	**-0.307**	**0.128**
	31	Fra.8_4.P1.4	22	33	2.00	1.92	0.343	0.315	**0.192**	**0.235**
	32	Fra.8_4.P1.5	28	26	2.14	2.04	0.385	0.371	0.097	**0.262**
	33	Fra.8_4.P2.7	17	24	2.04	1.96	0.375	0.374	**0.211**	**0.205**
	34	Fra.8_4.P2.9	23	22	2.08	2.12	0.345	0.368	**0.165**	**0.360**

Table 3. Effective population sizes of *Heterodera schachtii* when considering a seven-generation span using two estimation methods (see text). * indicate populations with an infinite estimate of effective size.

Beach	Population	Plant status in 2013	Estimated effective size (95% confidence interval)	
			Moment based (Jorde and Ryman 2007)	Pseudo-likelihood (Wang 2001)
Granville Nord	1	alive	329 (127 - 626)	548 (48 - ∞)
	2	alive	46 (15 - 94)	203 (59 - ∞)
	3	alive	37 (12 - 77)	175 (40 - ∞)
	4*	dead	∞ (∞ - ∞)	∞ (147 - ∞)
	5*	dead	∞ (∞ - ∞)	∞ (145 - ∞)
	6	dead	48 (19 - 89)	98 (35 - 1430)
	7	dead	1224 (492 - 2283)	284 (57 - ∞)
	8*	dead	∞ (∞ - ∞)	∞ (114 - ∞)
	9*	dead	∞ (∞ - ∞)	∞ (125 - ∞)
	10*	dead	∞ (∞ - ∞)	∞ (121 - ∞)
Granville Sud	11	dead	113 (39 - 226)	353 (40 - ∞)
	12*	alive	239 (78 - 489)	∞ (97 - ∞)
	13	dead	650 (280 - 1171)	102 (36 - 1495)
	14*	dead	∞ (∞ - ∞)	∞ (90 - ∞)
	15	dead	190 (57 - 401)	975 (92 - ∞)
	16*	dead	∞ (∞ - ∞)	553 (59 - ∞)
	17	dead	111 (44 - 207)	444 (59 - ∞)
	18*	dead	∞ (∞ - ∞)	417 (60 - ∞)
	19*	dead	∞ (∞ - ∞)	∞ (112 - ∞)
Saint Léonard	20	dead	39 (15 - 74)	43 (19 - 146)
	21	alive	162 (59 - 315)	175 (36 - ∞)
	22	alive	256 (70 - 560)	67 (13 - ∞)
	23	alive	65 (19 - 136)	136 (29 - ∞)
	24	alive	21 (6 - 44)	27 (10 - 90)
	25*	dead	∞ (∞ - ∞)	∞ (100 - ∞)
	26	alive	373 (129 - 743)	124 (29 - ∞)
	27*	alive	∞ (∞ - ∞)	264 (12 - ∞)
Montfarville	28*	alive	∞ (∞ - ∞)	1527 (56 - ∞)
	29	alive	40 (16 - 75)	44 (20 - 139)
	30	alive	51 (21 - 94)	43 (22 - 108)
	31	alive	140 (51 - 273)	76 (27 - 628)
	32	alive	342 (132 - 651)	90 (29 - 6905)
	33	dead	133 (49 - 259)	176 (32 - ∞)
	34*	dead	160345 (58793 - 311870)	∞ (73 - ∞)

necessarily hold in our case study. However, the determined number of generations between sampling sessions was high enough to reduce the influence of overlapping generations as shown by Waples and Yokota (2007), although there is likely a small upward bias for populations with only four or five generations. In the case of *H. schachtii*, however, an additional difficulty is that cysts sampled in 2013 may potentially come from the same generation as cysts sampled in 2012, because cysts can survive in the soil for many years in the case of unfavorable environmental conditions. We do not know whether environmental conditions influenced the dynamics of cyst hatching between the two sampling sessions. Therefore, few generations may have elapsed between the two sampling sessions, leading to insufficient genetic variation for estimating effective population size. This may explain the frequent occurrence of infinite population effective sizes. In fact, 79% of the host plants tagged and characterized by infinite nematode population sizes died between the two sampling sessions (Tab. 3). However, 40% of finite-size populations were also associated with dead host plants, suggesting a more complex situation. For example, survival of underground plant parts or recent death of the host plant may have allowed nematode populations to produce enough generations to correctly estimate effective population sizes. The analysis of temporal variation in mean multilocus F_{IS} estimates can give information on the likelihood that new generations constituted 2013 samples. The life cycle of *H. schachtii* and the low dispersal ability of larvae result in inbreeding and/or sub-structuring (Wahlund effect), which lead to an increase in heterozygote deficiencies over time (Plantard and Porte 2004; Montarry et al. 2015). However, this increase in heterozygote deficiencies is of course only possible in populations that produce new generations. As shown (see Fig. 4), an increase was indeed observed for populations with finite effective sizes, but not in populations with infinite effective sizes (paired permutation test; 10,000 permutations; $p<0.001$ and $p=0.12$, respectively). Furthermore, the number of cysts observed in our samples (corrected by the proportion of *H. schachtii* cysts relative to other species) was remarkably similar between finite and infinite populations (permutation test; 10,000 permutations; $p=0.60$), with a mean number of cysts of 158.2 ± 19.8 (s.e.m.) and 175 ± 23.8, respectively, refuting the hypothesis that infinite population size results from huge census sizes. In addition, infinite estimates of effective population size may also be caused by an overestimation of the number of generations between samples, which reduces the effect of genetic drift. However, all infinite N_e estimates remained infinite when we decreased the number of generations from ten to four. Overall, our findings suggest that the occurrence of infinite population sizes estimates correspond to nematode populations lacking significant variation in temporal allele

frequencies, probably because new nematode generations were not produced between the two sampling sessions.

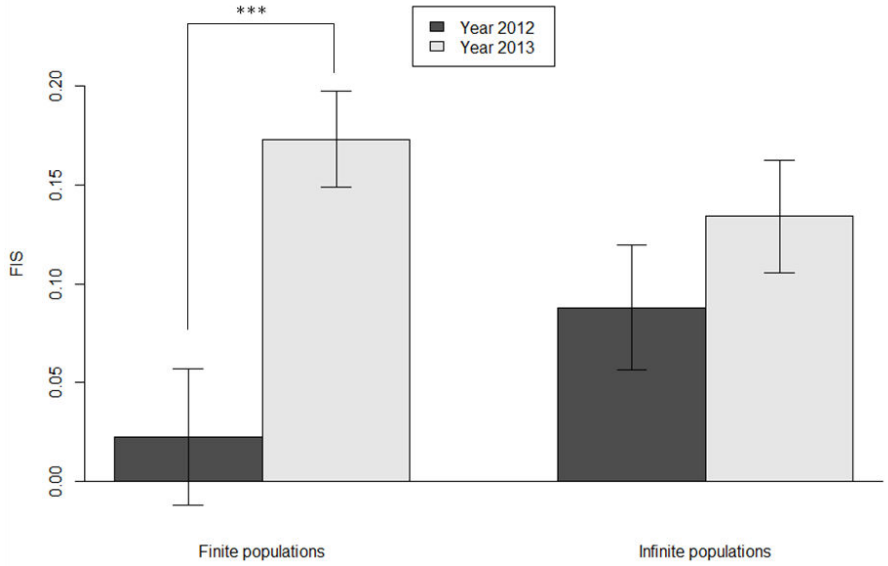

Figure 4: Variation of mean FIS of finite and infinite populations over time. Error bars represent standard error. ***: p<0.001.

Temporal methods assume no influence of other micro-evolutionary forces, such as migration or selection. The relevance of this assumption in our dataset was tested by applying a generalized test developed by Waples (1989): only population 2 exhibited a significant impact of non-random forces. The effective population size estimated by the pseudo-likelihood method, used to compute this test, was much higher than that of the moment-based method, for which the Waples test was non-significant (data not shown). Thus, it is possible that this population was not affected by non-random evolutionary forces, and N_e was over-estimated for this population. Therefore, we considered that our populations were not strongly affected by migration or selection during the one-year interval between the two samples and that the assumptions of the temporal method held for the finite-size populations, thereby giving reliable estimates.

Population density in soil samples was around 40 cysts of *H. schachtii* per 100 g of soil. Assuming that each cyst contains between 500 and 600 eggs (Subbotin 2010), the census size N of the populations we sampled was, at least, 10,000 individuals, while both estimators yielded effective populations sizes that were less than 1,300 individuals, with a majority of effective population sizes of around 85 individuals. Thus, the N_e/N ratio was less than 1% for most populations, which is extremely low. Low effective sizes of wild populations are common, but the N_e/N ratios in these populations are generally closer to 10% (Frankham,

1995; Palstra and Ruzzante, 2008). It is unlikely that we underestimated the effective population sizes because both N_e estimators gave very similar results. The moment-based estimator has been demonstrated to be essentially unbiased and the pseudo-likelihood estimator is slightly upwardly biased in specific cases (Jorde and Ryman 2007). Our low estimates of effective population sizes are thus conservative.

Biological insights gleaned from the estimation of effective population sizes for cyst nematodes

The effective population size is affected by many population features, three of which may dramatically influence effective sizes in *H. schachtii*. First, the factor with the highest impact on effective population size is the variation in population size through time (Frankham 1995; Charlesworth 2009). In the case of *H. schachtii*, the death of the host and the recolonization of a patch by host plants may eventually lead to variation in population census sizes. In Normandy, *B. maritima* lives seven to nine years on average (Hautekèete et al. 2002), and a significant rate of host death is unexpected in a one-year period. However, the high number of dead plants observed in the second sampling session supports the hypothesis of host instability. The death of these plants can be attributed to disturbance events such as storms (De Cauwer et al. 2012), but another possibility is that *B. maritima* populations contain numerous annual individuals along with bisannual and perennial individuals (Biancardi et al. 2012). Moreover, the distribution of effective size appears to fit a log-normal distribution (Fig. 2), which is common in populations living in a fluctuating environment (Lewontin and Cohen 1969). These facts suggest that *H. schachtii* lives in an environment in which local conditions often fluctuate, directly affecting the census size of the population, and possibly explaining the low effective size of their populations.

Second, low effective population sizes can also be explained by certain life-history traits in *H. schachtii*. Its populations are characterized by high levels of inbreeding (Plantard and Porte 2004), which increases the correlation between maternal and paternal alleles, and thus increases the impact of genetic drift and reduces effective population sizes (Charlesworth 2009). Third, the sex-ratio in *H. schachtii* can be extremely unbalanced because sex determination depends on environmental factors such as host resistance, which can greatly increase the male/female ratio in a population (Muller 1985). The wild sea beet has been recognized as a resistant host plant species for *H. schachtii* since a long time (see Panella and Lewellen 2007). Wild populations of *H. schachtii* may thus have very unbalanced sex-ratios, as well as high variance among individuals in reproductive success. Unequal sex-ratios and

variance in offspring number are two major factors that reduce N_e below the census size of the population (Charlesworth 2009). However, the actual resistance of the wild sea beets sampled in this study is poorly known and its possible impact on cyst nematode sex-ratios requires further investigation.

Implications for the management of field cyst nematode populations

This study demonstrated that populations of *H. schachtii* have effective population sizes of around 85 individuals. For comparison, estimates of the effective population size of the free-living nematode *Caenorhabditis elegans* range (i) from 200 to 9,600 individuals with AFLP markers (Barrierre and Felix 2005); (ii) from 50 to 10,200 with microsatellite markers (Sivasundar and Hey 2005); and (iii) around 80,000 with full-length sequences (Cutter 2006). The census sizes of most of *C. elegans* populations are of the order of 10,000 individuals or less (Félix and Duveau 2012). The effective size of an animal-parasitic nematode, *Trichostrongylus axei*, is estimated to be of the order of 10 million at the metapopulation scale (Archie and Ezenwa 2011). Comparison of these data with ours have to be made with caution, because we only estimated effective size at the host scale; however, macroparasite models predict an even lower N_e/N ratio when considering the whole metapopulation, mainly due to the subdivision of breeders between hosts (Criscione and Blouin 2005).

Thus, effective population sizes found in *H. schachtii* may be considered as surprisingly low compared with other nematodes. However, these results are consistent with the work of Barrett et al. (2008), which predicted a low effective population size for macroparasite populations subject to inbreeding, restricted dispersal, and developing on short-lived hosts like *H. schachtii* populations (*T. axei*, however, maintains high rates of gene flow and great genetic diversity). Low effective sizes have been observed in other plant pathogens such as fungi (Bayon et al. 2009) and viruses (Sentandreu et al. 2005, Fabre et al. 2012) although not in all (Zhan and McDonald 2004; Gurung et al. 2013; and see McDonald and Linde 2002 for a review).

Low effective population sizes imply that genetic drift strongly affects wild populations of *H. schachtii*, calling into question its capacity to evolve under changing environments. Wild plant-parasitic nematode complexes can be subject to long co-evolutionary histories, promoting the rise of new genetic resistance in the host plant, but also the occurrence of more virulent nematode populations (Cook 2004; Van der Putten et al. 2006). In regard to *H. schachtii* and its wild sea beet host, wild cyst nematode populations have the potential of being a dangerous source of virulent nematodes for crops, given the wide passive dispersal

abilities of cyst nematodes (Picard et al. 2004). However, the low effective population sizes suggest that wild *H. schachtii* populations have low evolutionary potential and make this scenario unlikely.

The use of genetic resistance in crop plants is considered as the most promising control method for *H. schachtii* in fields, given the negative environmental impact of nematicides and the economic costs of physical methods (Thureau et al. 2010). If actual effective field population sizes of *H. schachtii* are similar to those estimated in this study, then the genetic resistance used in sugar beet fields may be sustainable for a long time (Van der Putten et al. 2006). However, field populations of *H. schachtii* are expected to differ from wild populations (Plantard and Porte 2004; Porte et al. 1999). Increased passive dispersal and population density, in particular, can increase the effective size of field population compared with a population that is not subject to agricultural practices. Because our results have shown that *H. schachtii* populations are vulnerable to genetic drift, one clever use of this feature could prevent the parasite from overcoming plant resistance. Crop rotation is an efficient way to modify population census size of *H. schachtii* through time, by introducing large variation in host presence, similar to that experienced in wild populations. This method is however difficult to develop for controlling *H. schachtii* because it is a generalist parasite that can live off of most *Brassicaceae* plants. Furthermore, crop rotation does not suit intensive agricultural practices (Van der Putten et al. 2006; Thureau et al. 2010). Another way to influence effective population size would be to promote the host resistance affecting the sex-ratio of *H. schachtii* (Muller 1985). Small effective population sizes in the fields would be greatly beneficial for a long-term use of plant resistance to control *H. schachtii* populations.

Further comparative studies are needed to identify which factors are responsible for the low effective size of wild *H. schachtii* populations. For instance, fine-scaled sub-structuring or demographic fluctuations must be further documented to improve our understanding of the evolutionary dynamics and history of *H. schachtii*. As demonstrated here, low effective population sizes give insights regarding potential control methods for *H. schachtii* in sugar beet fields to preserve the effectiveness of resistant varieties over the long term. We recommend future studies on the estimation of effective population sizes of crop pests, in both natural environments and agrosystems to identify the factors that best control crop pathogen populations.

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DATA ARCHIVING STATEMENT

Data for this study are available at: to be completed after manuscript is accepted for publication.

Table S1. Waples' generalized test results for populations with significant temporal changes in allele frequencies (exact homogeneity test). *: significant, n.s.: non-significant.

Population	Effective size	Waples's test
2	203	*
6	98	n.s
13	102	n.s
20	43	n.s
24	27	n.s
29	44	n.s
30	43	n.s
31	76	n.s
32	90	n.s

Figure S1: Generation time of *Heterodera schachtii* as a function of temperature. The solid line corresponds to the data from Kakaire et al. (2012) and the dotted line to the non-linear regression model.

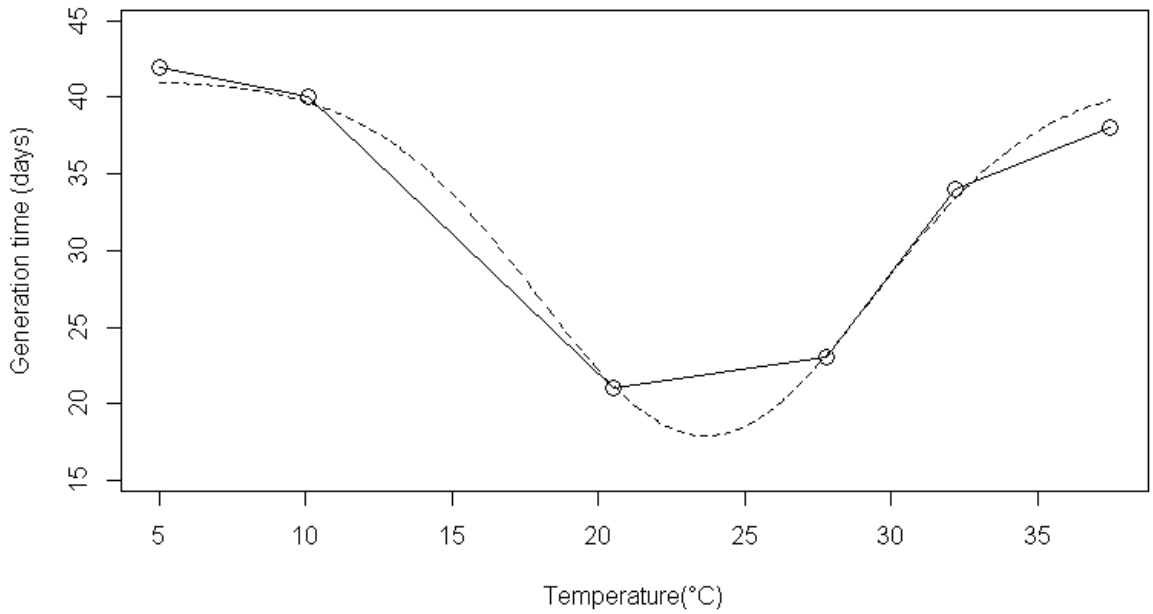
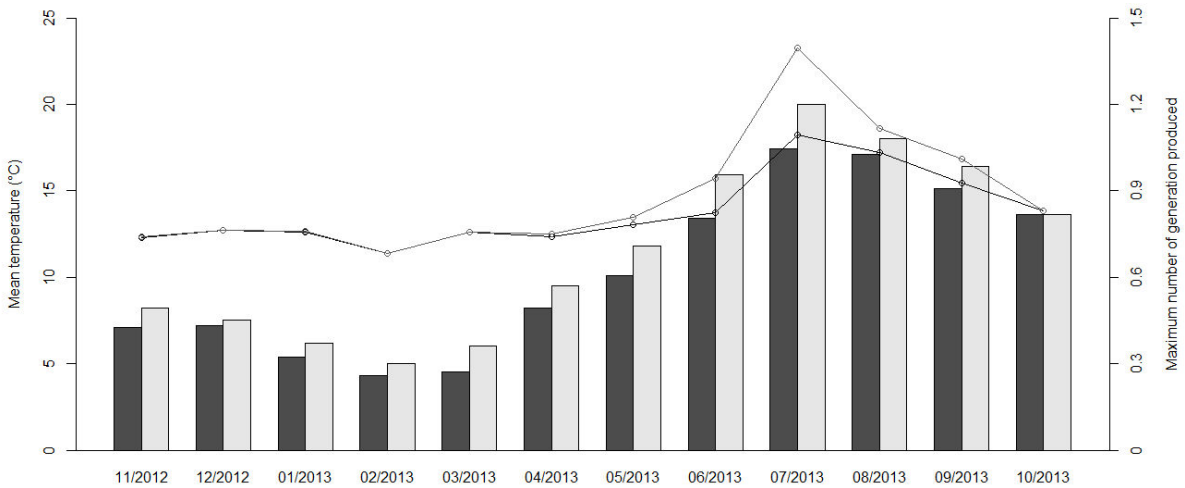


Figure S2: Mean temperature (bar plots) and maximum number of generations of *Heterodera schachtii* produced every month between the two sampling sessions (lines). Black: Montfarville; Gray: Granville Nord, Granville Sud and Saint-Léonard.



3.4. Temporal sampling and variation in the genetic structure of naturally occurring populations of a plant-parasitic nematode. 2 - Separating the relative effects of gene flow and genetic drift

Cécile GRACIANNE, Pierre-Loup JAN, Sylvain FOURNET, Eric OLIVIER, Jean-François ARNAUD, Catherine PORTE, Sylvie BARDOU-VALETTE, Marie-Christine DENIS, Eric J. PETIT

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ABSTRACT

Studying wild pathogen populations in natural ecosystems offers the opportunity to better understand the evolutionary dynamics of biotic diseases in crops and to enhance pest control strategies. We used simulations and genetic markers to investigate the spatial and temporal population genetic structure of wild populations of the beet cyst nematode *Heterodera schachtii* on a wild host plant species, the sea beet (*Beta vulgaris* spp. *maritima*), the wild ancestor of cultivated beets. Our analysis of the variation of eight microsatellite loci across four study sites showed that (i) wild *H. schachtii* populations displayed fine-scaled genetic structure with no evidence of substantial levels of gene flow beyond the scale of the host plant, and comparisons with simulations indicate that (ii) genetic drift substantially affected the residual signals of isolation-by-distance processes, leading to departures from migration-drift equilibrium. In contrast to what can be suspected for (crop) field populations, this showed that wild cyst nematodes have very low dispersal capabilities and are strongly disconnected from each other. Our results provide some key elements for designing pest control strategies, such as decreasing passive dispersal events and limiting the spread of virulence in field nematode populations.

Keywords: Heterodera schachtii, wild nematode populations, assignment tests, migration-drift equilibrium, gene flow.

Agrosystems are highly homogenous artificial environments that are particularly amenable to the emergence and development of pathogens (Stukenbrock and McDonald 2008). In such artificial, disturbed habitats, gene flow is a crucial parameter that determines the adaptive value of pathogen populations and the risk they represent for crops, especially through the evolution of virulence and resistance breakdown (McDonald and Linde 2002). This is particularly true for pathogens with high dispersal abilities that have alternative wild hosts outside the cropping area even in the absence of crops (Burdon and Thrall 2008). There is increasing evidence that crop pathogens can develop on wild host species, related or unrelated to the usual cultivated host (*e.g.* Lebeda et al. 2008; Monteil et al. 2013; Rouxel et al. 2014). Therefore, wild populations of pathogens may act as reservoirs of genetic diversity and initiate local crop epidemics (Burdon and Thrall 2008; Leroy et al. 2014). In this respect, a few studies on wild pathogen populations have investigated patterns of gene flow between wild and cultivated (see examples in Stukenbrock and McDonald 2008). However, there are no published reports on the patterns of gene flow among wild pathogen populations, which are nonetheless an important determinant of pathogen population structure and thus of its potential role as a virulence reservoir.

Studying wild pathogen populations offers the opportunity to better understand the evolutionary dynamics of crop pathogen populations, and can provide clues on the influence of human activities on the genetic structure of pathogen populations in agrosystems (Lebarbenchon et al. 2008b; Morgan et al. 2012). Although advocated, this approach has received very little attention thus far, with only one study on readily identifiable zoopathogens (see Morrison and Hoglund 2005). Soilborne diseases are difficult to diagnose, even in crop plants, because most symptoms occur underground or are very similar to those induced by abiotic factors on the aboveground organs of plants (Raaijmakers et al. 2009). Detection of these diseases in wild hosts is thus particularly challenging, but may have a potentially crucial role in improving crop management strategies.

Among soilborne pathogens, plant-parasitic nematodes are microscopic organisms and major crop pests of agrosystems that can cause severe economic losses annually (Jones et al. 2013). Interestingly, plant nematodes occur naturally in diverse ecosystems such as coastal sand dunes, grassland or coniferous forests (van der Putten et al. 2006). However, there are few detailed investigations of their population genetic structure, and even scarcer information on their spatial genetic structure in the wild (van der Putten et al. 2006; Gilabert and Wasmuth 2013). All available data come from two studies on the pinewood nematode, *Bursaphelenchus xylophilus*, and four crop field surveys on the dagger nematode *Xiphinema index* and three cyst nematodes *Globodera pallida*, *Globodera tabacum*, and *Heterodera schachtii* (Plantard and

Porte 2004; Picard and Plantard 2006; Villate et al. 2010; Mallez et al. 2013; Alenda et al. 2014; Mallez et al. 2014). The latter three plant-parasitic nematodes cause severe damage to vineyards and potato fields in Europe, tobacco fields and sugar beet fields worldwide, respectively. *B. xylophilus* damages pine forests around the world and differs from nematode species in its life history, requiring an intermediary insect species to disperse from one pine tree to another. The population genetic structure of this nematode indicates that *B. xylophilus* populations can be differentiated among individual trees at a very small spatial scale, according to the dispersal of the insect vector (Mallez et al. 2013). In contrast, cyst nematodes are soilborne endoparasitic nematodes with direct life history cycles, in which only two free-living developmental stages (males and juveniles) can disperse actively in the soil. Due to their small size (<1 mm), nematodes can travel over short distances only (Wallace 1968; Norton and Niblack 1991). Interestingly, populations of *G. pallida* and *H. schachtii* exhibit low genetic differentiation among cultivated fields located 50 km and 150 km apart respectively suggesting the occurrence of gene flow over large spatial scales. This pattern was attributed to large nematode population sizes in cultivated fields and passive dispersal of cysts in agricultural areas via natural factors and human activities (Picard et al. 2006; Villate et al. 2010; Alenda et al. 2014). However, there have been no investigations on wild populations of crop plant-parasitic nematodes to date, and the question remains as to whether plant-parasitic nematodes also passively disperse over large distances in wild ecosystems.

In this study, we examined the genetic structure of wild populations of a cyst nematode, *H. schachtii*, using both empirical and simulated datasets. Sugar beet (*Beta vulgaris* spp. *vulgaris*) is the usual cultivated host of *H. schachtii* which can also develop on wild relative of the sugar beet, the sea beet *Beta vulgaris* spp. *maritima* (Subbotin et al. 2010). Sea beets are common along the European Atlantic and Mediterranean coastlines (Hautekèete et al. 2002; De Cauwer et al. 2010). Wild populations of *H. schachtii* have been found on wild sea beet populations located along the Atlantic coastline from Spain to Denmark (Gracianne et al. 2014). This wide geographical occurrence provides the opportunity to explore the patterns of gene flow over different spatial scales in the wild. Specifically, (i) we investigated the levels of genetic differentiation in local wild populations of *H. schachtii* to examine what hierarchical scale should be considered to define a populations in the wild, (ii) we examined the spatial genetic structure of nematode populations to identify signatures of dispersal events and the scale over which they occur, and (iii) we separated the respective effects of gene flow and genetic drift in observed population genetic structure. We hypothesized that genetic drift has a strong influence on genetic structure due to small effective sizes of wild populations of *H. schachtii* (Jan et al. unpublished data). Nonetheless, the extent to which gene flow may potentially counteract the

influence of genetic drift depends on its intensity and its geographical range. Thus, to distinguish equilibrium from non-equilibrium dynamics, we tested the stability of local population structure using a temporal sampling design, and carried out extensive simulations to investigate the influence of genetic drift and to evaluate the reliability of assignments tests under different sampling schemes of populations structured through an isolation-by-distance model. Finally, we discuss our results in the light of how population genetics data from wild pathogen populations can be helpful for designing disease control strategies devoted to overcoming resistance in field nematode populations.

MATERIAL AND METHOD

Soil samples were collected around roots of *Beta vulgaris* spp. *maritima* plants on four different beaches in Normandy, France. Distances separating the four beaches (Montfarville, Granville Nord, Granville Sud, and Saint Léonard) ranged from 300 m to 150 km (for more details, see Jan et al. unpublished data). This geographical sampling range is of the same order of magnitude as previous investigations on *H. schachtii* conducted in sugar beet fields (Plantard and Porte, 2004). Populations of the host plant, the sea beet, are often composed of individuals clustered in geographically and genetically distinct patches (De Cauwer et al. 2010). Thus, a maximum of 10 sea beet plants were sampled in three to five arbitrarily defined patches of 3 m in diameter randomly distributed on each beach. In fall 2012, 120 plants were sampled and marked with plastic tags. Active dispersal of *H. schachtii* individuals occurs over very short spatial distances (Westphal 2013). Thus, the soil sampled around the roots of one plant was considered as being one nematode population for subsequent analyses. Thousands of cysts were found in all soil samples. To reduce the amount of biological material, molecular analyses were performed, for each beach, on all nematode populations coming from one sea beet patch and on one nematode population coming from the other patches of sea beets. We genotyped 40 nematode populations from this 2012 sampling. In fall 2013, only 34 of those 40 populations were resampled because six host plants disappeared between the two sampling dates. Cysts of *H. schachtii* were extracted from soil samples using homemade sieves (250 µm and 800 µm) and manual examination of filtrates. Cysts were stored at 4°C in moistened sand until molecular characterization.

Molecular characterization and genotyping

As a soil sample can contain cysts from different *Heterodera* species, we used restriction profiles of the ITS sequence for species identification. DNA extraction, PCR amplification of ITS sequence and digestion of PCR products were performed as described in Amiri et al. (2002). In all, 1754 *H. schachtii* individuals were identified and successfully genotyped at eight

microsatellite loci, named Hs33, Hs36, Hs55, Hs56, Hs68, Hs84, Hs111 and Hs114 and described in Montarry et al. (2015). Microsatellites PCR products were analyzed on an ABI Prism® 3130xl sequencer (Applied Biosystems). Allele sizes were identified using the automatic calling and binning procedure of GeneMapper v4.1 (Applied Biosystems) and completed by a manual examination of irregular results. Samples with dubious genotypes were reamplified.

Data analysis

Jan et al. (unpublished data) estimated the effective population sizes of *H. schachtii* on the 34 populations of nematodes found in the two sampling years. Of these populations, 14 had infinite estimates of effective population sizes, suggesting that some nematode populations were not able to produce enough generations to detect substantial genetic variation between the two sampling sessions. Therefore, to avoid potential pseudo-replication in our population dataset, we performed our analysis on the whole 2012 dataset, but only on populations with finite estimates of effective population size in 2013 (see Table 1).

Characterization of basic genetic parameters

Single and multilocus departures from Hardy-Weinberg (HW) equilibrium were tested by estimating F_{IS} values for all populations on each beach. Statistical significance of F_{IS} values was assessed using 10,000 permutation of alleles among individuals, adjusted for multiple tests with Bonferroni corrections, as implemented in the software Fstat version 2.9.3 (Goudet 1995). Similarly, linkage disequilibrium among loci was also assessed using permutation tests adjusted with Bonferroni corrections implemented in Fstat. Genetic diversity of nematode populations was evaluated through the estimation of expected heterozygosity (H_e) and allelic richness (Ar) using Fstat. Allelic richness was estimated using the rarefaction method as described in El Mousadik and Petit (1996).

Levels of genetic differentiation

Three hierarchical levels of population structure were considered to explain the partitioning of genetic differentiation: the level of the host plant, considered as hosting one nematode population, as described above; the level of the patch, defined as a geographical clustering of host plants within a beach; and the level of the beach, comprising all the set of host plants surveyed for nematode population sampling. We used the R package Hierfstat (Goudet 2005) to estimate variance components of each hierarchical level and to test their statistical significance, as described in De Meeûs and Goudet (2007). To test for isolation by distance (IBD), *i.e.* a gradual increase of genetic differentiation with increasing geographical distance among populations, we estimated pairwise population F_{ST} and performed regression analyses as

described in Rousset (1997) using the R package Adegenet (Jombart 2008b). Significance of the relationship between the two variables was tested using classical Mantel tests (Smouse et al. 1986). The same analyses were performed on both (2012 and 2013) datasets.

Migrant detection: empirical data

At migration-drift equilibrium, a pattern of IBD depicted through variation in genetic differentiation among populations reflects spatially restricted gene flow. However, such population structure can be a residual signal of ancestral gene flow that no longer occurs or can be the result of diverse non-equilibrium situations such as colonization (Barker 2013), secondary contact between allopatric populations (Petrou et al. 2013), or population expansion (Awad et al. 2014). Thus, to distinguish equilibrium from non-equilibrium situations in IBD patterns, we used assignment tests to detect real-time (*i.e.* first-generation) migrants, which are assumed to reflect current gene flow (Manel et al. 2005; Broquet and Petit 2009). Detection of first-generation migrants and population assignment of individuals were conducted using Bayesian criteria based on the computation of the probability of observing a given genotype in each population (see Rannala and Mountain 1997 for details). This approach is generally used when all population sources are not known and, in Geneclass 2 (Piry et al. 2004), it was applied to define the statistical criteria that estimate the likelihood that an individual originates from a given population. Detection of first-generation migrants was performed by computing the likelihood of the individual genotype within the population where the individual was sampled. For each individual, the probabilities of belonging to each sampled population was estimated by simulating 10,000 multilocus genotypes using a Monte-Carlo resampling procedure, as described in Paetkau et al. (2004). Individuals with a probability lower than 0.01 of occurring in the sampled population were considered as potential migrants. This threshold corresponds to the minimal tolerable type I error expected from assignment tests using this procedure (Paetkau et al. 2004). Assignments of individuals to a population were based on computation of individual exclusion probabilities. In this case, the population exhibiting the highest membership probability was considered as the population of origin of the individual. Individuals assigned to another population from which they were sampled were considered as migrants. To ensure that the number of detected migrants in each distance class was independent of the number of pairwise populations occurring in each distance class, we weighted the frequency of migrants by the number of times a particular class of distance was observed for both the empirical and simulated datasets (see below).

In contrast to simulated datasets, with known spatial positions, it is difficult to define which populations of *H. schachtii* are adjacent in natural ecosystems. For instance, roots can bring two populations into contact, although these populations may seem clearly spatially

separated according to the host plant position. Moreover, we did not sample all sea beet plants occurring in patches, and the two closest populations of the empirical dataset may be actually separated by one or several unsampled nematode populations. However, simulations allowed the estimation of proportion of migrants observed among truly adjacent populations (see below). We thus used this estimate to define a potential number of real migrants for each beach.

Assignment tests assumed Hardy-Weinberg equilibrium. In 2012, one locus in Saint Léonard, two loci in Montfarville and in Granville Nord, and three loci in Granville Sud exhibited significant departure from HWE (see below). In 2013, three loci in Montfarville and Granville Sud, four loci in Saint Léonard and seven loci in Granville Nord showed significant departures from HWE. To assess the influence of loci departing from HWE on results, assignment tests were performed with and without these loci for populations sampled in 2012. Results did not show any difference in distribution of detected migrants over distance classes (data not shown). As a result, we considered results from assignment tests considering all loci in 2012 and 2013 in subsequent analyses. All analyses computed on empirical datasets were performed separately for the different beaches.

Migrant detection: simulations

Reliability and performance of assignment tests are well documented for populations connected in an island model (Berry et al. 2004; Paetkau et al. 2004; Waples and Gaggiotti 2006). However, there is no information on statistical power of assignment tests for populations under IBD models. To improve our understanding of observed results in situations where IBD processes are suspected, we simulated datasets using genetic features mirroring our empirical population genetic data. To do so, we simulated a grid of 11 x 11 (121) populations each composed of 400 individuals with equal sex-ratio using the software Easypop version 2.0.1 (Balloux 2001). Assuming random mating, we used a two-dimensional stepping-stone migration model because it reflects the spatial positions of host sea beet plants, and it entails that migration events only occur among adjacent host plants, in accordance with the restricted dispersal capabilities in *H. schachtii*. We set a migration rate of 0.025 for both males and females, and a mutation rate of 0.0005 at five independent loci to mimic worst conditions in empirical datasets (*i.e.* the smallest number of loci at equilibrium in 2012). Mutations occurred following a K-allele model with eight possible allelic states, which corresponded to the maximal number of alleles observed in our set of microsatellite loci. Each simulation spanned enough time to reach migration-drift equilibrium (2000 generations). We randomly sampled 20 individuals in nine simulated populations, the same number as in the empirical dataset, from each of the 100 replicates of the simulation procedure. Sampled populations were located in the center of the simulated grid to avoid potential border effects. Two sampling schemes were applied. In the first

one, we sampled a square of 3 x 3 populations, including neighbors that exchange migrants in the stepping-stone migration model. The second scheme was based on a set of 100 simulations in which we sampled nine populations separated from each other by non-sampled populations. Under a strict stepping-stone model of population structure, we expected to detect migrants between adjacent populations only, and none for all other increasing distance classes, other than type I errors. Thus, in the first sampling scheme, we expected to detect migrants for the smallest distance class and none for the other distance classes, and in the second sampling scheme, none for all distance classes other than, again, type I errors. Additionally, assignment tests should detect more migrants in datasets of the first sampling scheme than in simulations where only non-adjacent populations were sampled. Statistical detection of migrants was performed as for empirical data. Handling and analysis of all datasets were performed using R version 3.1.1 (R Development Core Team 2012).

Impact of genetic drift

In the case of no gene flow, genetic drift is the only evolutionary force that may significantly affect variation in allelic frequencies between samples taken a few generations apart. Natural selection is expected to have negligible effect on the variation of neutral markers such as microsatellites. Moreover, in a companion paper (Jan et al., unpublished data), we estimated that populations of *H. schachtii* have small effective sizes and that the number of generations produced between the two sampling sessions is in the range of four to ten. Therefore, mutation cannot have a great influence on allelic frequencies during this short evolutionary period. Overall, due to small population sizes, genetic drift may have a strong influence on gene dynamics; particularly if the level of gene flow is low (see below). If genetic drift is the only evolutionary force that acts on population structure, IBD slopes should decrease and mean and variance in genetic differentiation should increase over time. If such variation is indeed observed, observed IBD patterns will actually correspond to a residual signal of gene flow that no longer occurs or to another non-equilibrium process.

To test for non-migration-drift equilibrium, we simulated the evolution of allele frequencies obtained from our data after several generations under pure genetic drift using allele frequencies of populations sampled in 2012, which we sampled with replacement to build the next generation using R. The number of generations elapsing between the two sampling sessions was assumed to range from four to ten. We thus repeated the sampling step four, seven, and ten times to simulate the random action of genetic drift that could have affected these populations between the 2012 sampling and the 2013 sampling. We used minimal and maximal values of effective population sizes estimated in Jan et al. (unpublished data) with the pseudo-likelihood method (Wang 2001) to set effective population sizes in the simulations. The whole procedure

was repeated 1000 times to generate distributions of IBD slopes, means and variances of pairwise F_{ST} values that were compared with estimates observed in 2013. All simulations, data handling, and testing were performed with R.

RESULTS

Genetic data

We did not detect any significant linkage disequilibrium and loci were consequently considered as independent. Multilocus genotypic data showed contrasting levels of genetic diversity among sampled beaches (Table 1). Forty-one alleles were observed (ranging from 1 to 7 per locus) and expected heterozygosity (H_e) ranged from 0 to 0.582. The overall average of F_{IS} was significant in 2012 (F_{IS}=0.041) and in 2013 (F_{IS}=0.153). Some loci showed significant departures from HW equilibrium, which represented 12% and 15% of the 320 and 272 single-locus tests performed among loci over all populations in 2012 and 2013, respectively. Among these tests, 8% and 15% (4% and 0%) of F_{IS} values were significant (positive or negative) in 2012 and 2013, respectively. These values were not associated with a particular locus or population, suggesting spurious effects of genetic drift and/or population genetic sub-structuring. As a result, 30% and 65% of populations exhibited significant multilocus F_{IS} values ranging from -0.307 to 0.391 and from 0.099 to 0.362 in 2012 and 2013, respectively (Table 1). Some (20% in 2012 and 65% in 2013) were significantly positive, suggesting heterozygote deficiencies (Table 1).

Population differentiation and isolation by distance

Only 1.5% and 1.8% of the genetic variance could be attributed to differences between host plant patches in 2012 and 2013, respectively. Population differentiation explained by host plant patch membership was low, or virtually absent, and marginally significant only for the 2012 dataset. In contrast, populations were significantly different among beaches and host plants in 2012 ($F_{Beaches/Total}$=0.120; $F_{Plants/Beaches}$=0.049; all at P<0.001) and in 2013 ($F_{Beaches/Total}$=0.146; $F_{Plants/Beaches}$=0.051; all at P=0.001).

There was a significant IBD signature over the whole dataset (P<0.01 in 2012 and 2013; Fig. 1A). Within single-beaches, we observed contrasting situations with either non-significant (Granville Nord and Montfarville for both sampling sessions; see Fig. 1B and 1E) or significant IBD patterns (Granville Sud and Saint Léonard for populations sampled in 2012 only; see Fig. 1C and 1D).

Table 1: Genetic diversity and summary statistics for nematode populations sampled in 2012 and 2013. Significant values of F_{IS} are highlighted in bold. n: number of genotypes; Ar: allelic richness; He: expected heterozygosity.

Site	Plant code	n		Ar		Hs		F_{IS}	
		2012	2013	2012	2013	2012	2013	2012	2013
Granville Nord	Fra.71N.P1.4	25	18	2.09	2.04	0.409	0.390	0.085	0.124
	Fra.71N.P2.1	25	23	1.78	1.96	0.324	0.361	**-0.162**	**0.143**
	Fra.71N.P2.2	25	16	1.93	1.94	0.361	0.346	0.056	**0.284**
	Fra.71N.P2.3	25	30	2.06	2.06	0.390	0.384	0.021	0.094
	Fra.71N.P2.4	23	27	1.90	2.07	0.358	0.396	0.021	**0.137**
	Fra.71N.P2.5	22	27	2.08	2.04	0.412	0.380	-0.04	**0.189**
	Fra.71N.P2.6	24	-	1.87	-	0.379	-	0.013	-
	Fra.71N.P2.7	24	-	1.91	-	0.344	-	0.123	-
	Fra.71N.P2.8	22	35	2.07	2.05	0.371	0.380	0.071	**0.151**
	Fra.71N.P3.7	21	35	2.01	2.00	0.398	0.388	0.051	**0.192**
	Fra.71N.P4.1	27	38	1.89	1.83	0.331	0.306	0.103	**0.170**
	Fra.71N.P5.2	26	32	1.92	1.93	0.361	0.361	**0.162**	**0.171**
Granville Sud	Fra.7_1.P2.5	24	-	1.90	-	0.345	-	-0.072	-
	Fra.7_1.P3.7	19	19	2.02	2.02	0.421	0.415	**0.144**	0.129
	Fra.7_1.P4.1	20	22	1.85	1.89	0.339	0.333	0.136	**0.185**
	Fra.7_1.P5.1	22	25	2.29	2.06	0.445	0.407	0.101	**0.222**
	Fra.7_1.P5.2	25	25	1.94	1.86	0.341	0.312	**0.145**	0.092
	Fra.7_1.P5.3	25	32	1.79	1.80	0.294	0.312	0.030	-0.076
	Fra.7_1.P5.4	20	30	1.91	1.93	0.344	0.355	0.014	**0.191**
	Fra.7_1.P5.6	20	23	2.11	2.05	0.381	0.374	0.036	**0.281**
	Fra.7_1.P5.7	25	32	2.22	2.08	0.418	0.376	0.008	**0.099**
	Fra.7_1.P5.8	13	37	2.01	2.08	0.349	0.367	0.080	0.041
	Fra.7_1.P5.10	23	-	2.09	-	0.365	-	0.108	-
Saint Léonard	Fra.7_4.P1.1	21	25	2.06	1.82	0.398	0.316	**-0.243**	**0.201**
	Fra.7_4.P1.2	24	19	1.86	1.89	0.325	0.344	-0.054	**0.362**
	Fra.7_4.P1.3	13	11	1.87	1.73	0.335	0.276	0.025	0.141
	Fra.7_4.P1.7	24	22	1.82	1.91	0.292	0.365	**-0.218**	-0.077
	Fra.7_4.P1.8	24	17	1.84	1.70	0.339	0.293	0.096	0.125
	Fra.7_4.P1.9	19	28	1.97	1.87	0.368	0.328	0.070	0.058
	Fra.7_4.P1.10	18	-	1.91	-	0.352	-	0.015	-
	Fra.7_4.P2.1	29	17	1.86	1.71	0.318	0.296	0.063	0.138
	Fra.7_4.P3.5	12	17	1.53	1.43	0.188	0.172	-0.140	-0.112
Montfarville	Fra.8_4.P1.1	25	21	1.96	1.92	0.354	0.339	**0.391**	**0.199**
	Fra.8_4.P1.2	25	22	2.10	1.87	0.377	0.314	**0.269**	**0.294**
	Fra.8_4.P1.3	25	24	2.12	1.98	0.408	0.351	**-0.307**	**0.128**
	Fra.8_4.P1.4	22	33	2.00	1.92	0.343	0.315	**0.192**	**0.235**
	Fra.8_4.P1.5	28	26	2.14	2.04	0.385	0.371	0.097	**0.262**
	Fra.8_4.P2.7	17	24	2.04	1.96	0.375	0.3Fra.7_4.	**0.211**	**0.205**
	Fra.8_4.P2.9	23	22	2.08	2.12	0.345	0.368	**0.165**	**0.360**
	Fra.8_4.P3.1	26	-	2.17	-	0.409	-	-0.094	-

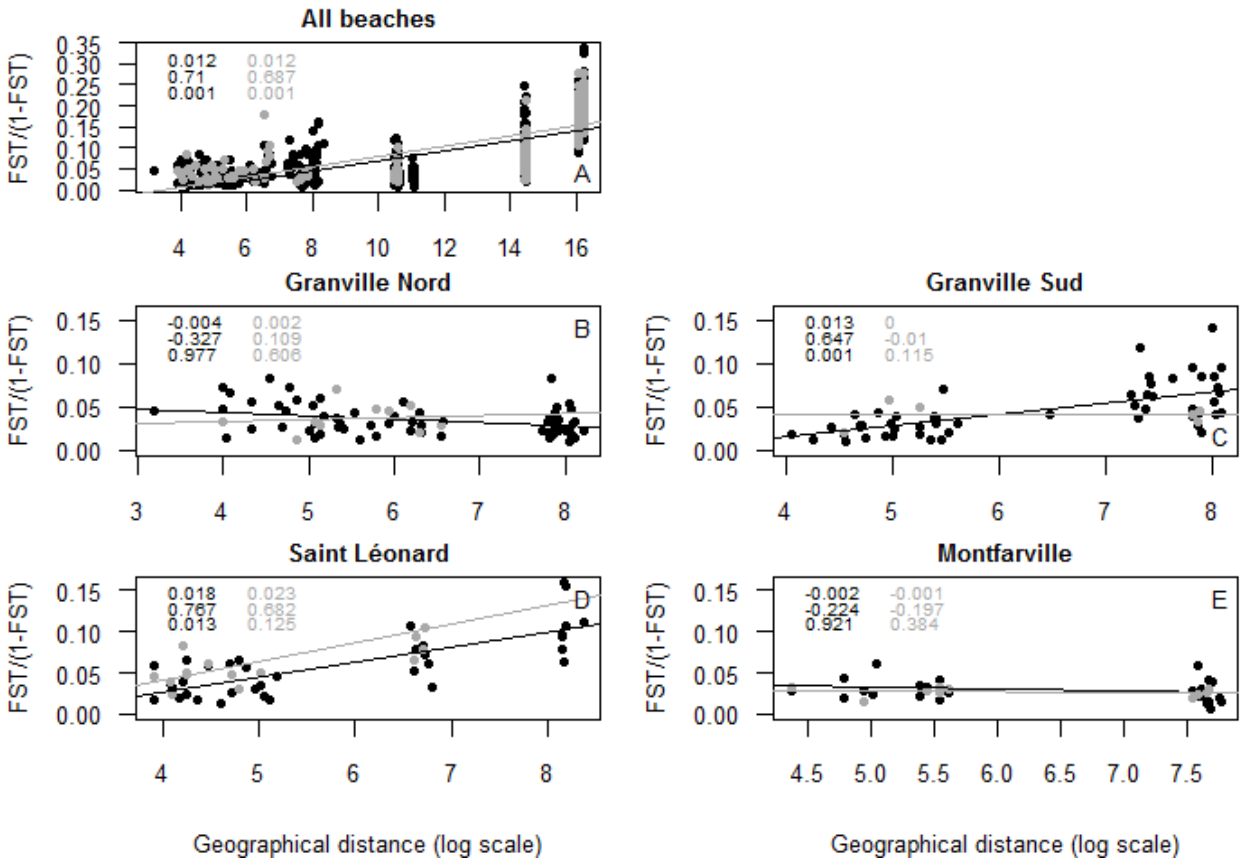


Fig. 1: Patterns of isolation by distance (IBD). Black and gray dots and lines correspond to 2012 and 2013 data, respectively. Displayed scores correspond, in descending order, to slopes, the *rz* Mantel correlation coefficient and p-value of Mantel tests for 2012 (black) and 2013 (gray). Regression lines of genetic differentiation with respect to distance (log scale) for all populations on all beaches (A), for populations at Granville Nord (B); for populations at Granville Sud (C); populations at Saint Léonard (D), for populations at Montfarville (E).

Detection of migrants

We detected 26 potential migrants in 2012 and 16 in 2013 over the whole empirical dataset. A similar proportion of migrants was observed for all distance classes on all beaches and frequencies of migrants were always in the same range as frequencies of simulated datasets (Fig. 2A). Many more migrants were detected with datasets obtained from simulations of migration-drift equilibrium IBD patterns. When sampling comprised adjacent populations, 257 migrants were equivalently detected in all distances classes, with only 35% of migrants being detected in the smallest distance class. In datasets with non-adjacent populations only, 282 migrants were detected and were also distributed over all distance classes. There was no overrepresentation of migrants in the smallest distance class for datasets with adjacent populations, and assignment tests detected a similar proportion of migrants whether strictly adjacent populations were sampled or not. Therefore, most detected migrants were false-

positives (65% and 100% of detected migrants in datasets with adjacent and non-adjacent populations, respectively), because no exchange of individuals can occur between non-adjacent populations in a strict stepping-stone model of migration (Fig. 2B). We thus considered that a maximum of 35% of detected migrants were potential migrants in the empirical datasets. Applying this percentage to the number of migrants detected on each beach, one migrant may be considered as a real migrant in Granville Nord and Granville Sud in 2012, with no migrants detected in other beaches, nor in all beaches sampled in 2013.

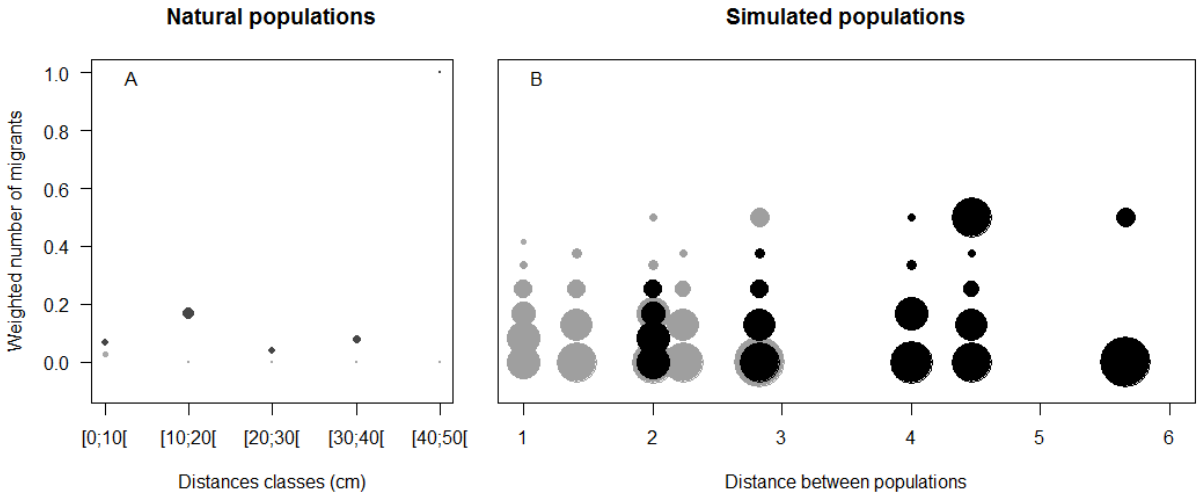


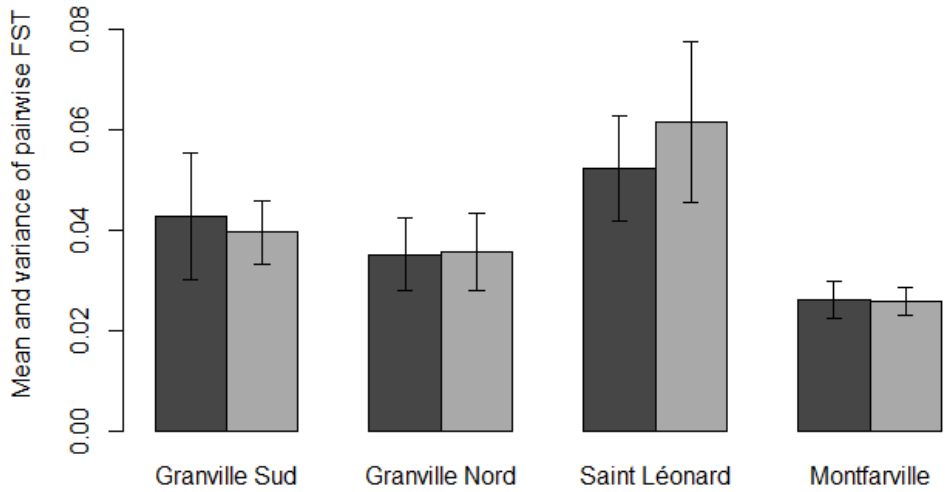
Fig. 2: Weighted number of migrants over distance classes in natural and simulated populations. Circle size is proportional to the total number of migrants in a distance class in natural (A) and simulated (B) populations (note that the absolute scales differ between the two graphs because much fewer migrants were detected in the empirical datasets). In natural populations, circles correspond to data pooled over all beaches obtained in 2012 (black) and 2013 (gray), respectively. In simulated populations, circles correspond to sampling schemes with (black) and without (gray) adjacent populations, respectively (see text for details). In simulated populations, a distance of 1 (step) corresponds to adjacent populations. Thus, circles for this value represent real migrants, and all other (black or gray) circles represent false-positive migrants (distances of more than one step). Interestingly, adjacent populations do not show more migrants than other, more distant classes. In natural populations, adjacent populations cannot be represented, but are inherently included in the smallest distance class. As observed for simulated data, there was no overrepresentation of migrants in this distant class.

Genetic drift

IBD patterns differed among sampled beaches, but this pattern remained quite stable between 2012 and 2013 (Fig. 1). Similarly, the mean and variance of pairwise F_{ST} values did not change between the two sampling sessions for all surveyed beaches (Fig. 3). We explored, through simulations, whether these patterns could all be explained by pure drift because this migrant rate seemed extremely low (see Results) and because the number of generations that elapsed between the two sampling sessions is rather low (Jan et al. unpublished data). These simulations showed that the IBD slopes we observed in 2013 were in the 95% confidence interval of what could be expected under pure drift, both for minimal and maximal effective

population sizes (Fig. 4). Likewise, the mean and variance of pairwise F_{ST} values observed in Montfarville, Granville Nord and Saint Léonard sampled in 2013 were in the 95% confidence intervals of simulated distributions of those variables after four, seven, and sometimes, ten generations, for both the lower and upper bound of effective population size (Fig. S1). For these three sampled beaches, simulations thus suggested that the observed values of IBD slopes resulted from a pure genetic drift process. Only Granville Sud showed a mean (and, to a lesser extent, variance) F_{ST} value that lay outside the lower confidence interval for all simulations tested (Fig. S1), suggesting that genetic drift was not the only evolutionary process acting on population genetic structure on this beach.

Fig. 3: Temporal change in the mean of pairwise F_{ST} values. Black and gray histograms show mean pairwise F_{ST} in 2012 and 2013, respectively. Error bars show the standard deviation of pairwise F_{ST} values in each beach and sampling year.



(Montarry et al. 2015)

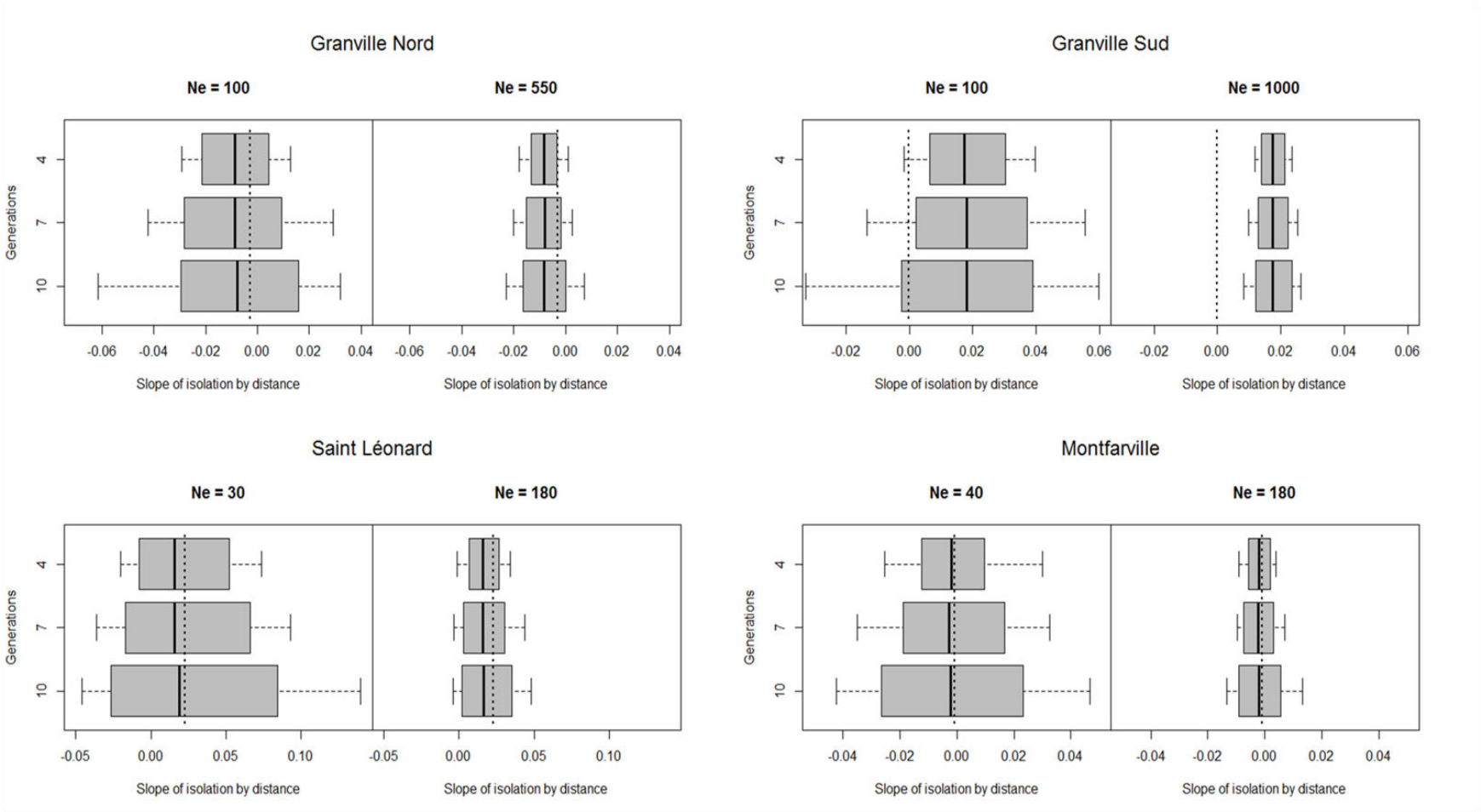


Fig. 4: Temporal change in isolation-by-distance (IBD) slopes under a pure drift process. Box and whisker plot of the distribution of the simulated IBD slope values. The central black line in the gray box is the median and the edges of the box give the upper and lower 95% confidence intervals of simulated values. Dotted lines indicate the observed IBD slope in 2013. Dotted lines located within the gray box indicate that the observed value can arise from genetic drift only.

We investigated the geographical scale over which effective exchanges of migrants occurred among populations in *H. schachtii*. Temporal sampling documented the stability of observed spatial genetic structure (this study) and also served to estimate effective population sizes (see Jan et al. unpublished data), which were required for the simulations. Empirical and simulation studies show that, over time, spatial genetic structure can be stable (*e.g.* Hoffman et al. 2004; Palmé et al. 2013) or unstable (Honnay et al. 2009; Antao et al. 2011). Temporal surveys therefore deliver more information on the evolutionary processes that act on population dynamics and persistence than studies based on a single sampling session (Habel et al. 2014). In contrast to what has been suggested for agrosystems (see Plantard and Porte, 2004), levels of gene flow may be low, or even absent, among wild populations owing to spatially restricted dispersal events of cysts and/or larvae.

Isolation by distance patterns: the question of migration-drift equilibrium

The genetic differentiation among wild *H. schachtii* populations suggested contrasting patterns of population structure among beaches, sampled years and spatial scales. IBD was detected on two beaches sampled in 2012 (Granville Sud and Saint Léonard), but, interestingly, these IBD patterns were not maintained the following year. In Saint Léonard, the IBD slope was still positive, suggesting that Mantel tests were not powerful enough to confirm the spatial pattern, because fewer populations were genotyped that year. In contrast, the strong decrease in the IBD slope (0.013 in 2012 dropping to 0 in 2013) observed in Granville Sud implied that the loss of IBD signal was not due to low statistical power, but to local evolutionary processes. At higher spatial scales, the relationship between genetic differentiation and geographical distance was significant when all populations at all sampled beaches were examined. According to Hutchison and Templeton (1999), the absence of a temporally stable IBD pattern over the geographical range of our study area means that the population structure is not at equilibrium in *H. schachtii*. Therefore, observed IBD patterns may not result from current gene flow.

This hypothesis is consistent with our results on first-generation migrant detection. Assignment tests detected migrants in Granville Sud and Saint Léonard where IBD pattern were also detected, but simulated data showed that assignment tests mainly detect false-positives. Therefore, most of the (few) migrants detected in our empirical dataset were probably false-positives. This conclusion echoes that of Wang (2014) who showed that population assignment approaches tend to overestimate migration rates among poorly

differentiated populations evolving under an IBD model. Similarly, the comparison of the ranges of migrant frequencies in simulated and empirical data and the estimation of the real number of migrants showed that there were no or, at most, only one migrant observed in sampled wild *H. schachtii* populations. However, at migration-drift equilibrium, migration events should arise under IBD. Assignment tests thus lead to the conclusion that there is an insufficient number of migrants occurring in wild *H. schachtii* populations to generate and maintain the IBD patterns observed in Granville Sud and Saint Léonard. As such, IBD patterns were probably not generated by current gene flow.

Additional simulations provided further support that genetic drift alone can explain the observed variation in population genetic structures between the two sampling years for at least three of the four surveyed beaches. Only a few migrants are required to prevent population differentiation and counteract the influence of genetic drift (Slatkin 1985). As a result, if migration actually occurred, the variation in allele frequencies between the two sampling events cannot be attributed to genetic drift only on these three beaches. In Granville Sud, it seems unlikely that the loss of the IBD pattern between 2012 and 2013 results from regular gene flow, because we did not detect more migrants on this beach than on the other beaches, and because when recurrent gene flow drives IBD, this pattern should be stable over time. Overall, our results suggest that wild *H. schachtii* populations were not at migration-drift equilibrium and probably exchange few or no genes at scales ranging from 10 cm to 150 km.

The scale of gene flow in wild *H. schachtii* populations

In non-equilibrium situations, patterns of genetic structure are dominated by historical factors, such as colonization history (Ibrahim et al. 1996; Austerlitz et al. 2000). This may even be more pronounced in populations that are founded by seeds (plants) or cysts (nematodes), which are forms that can survive belowground until local conditions allow their development. Once a potential host plant grows in a place where cysts are present, the local multiplication of nematodes may generate a patchy distribution of populations and heterogeneity in infestation levels among hosts, as observed in several nematode species (Jan et al. unpublished; Gavassoni et al. 2001; Villate et al. 2008). In this case, migration of *H. schachtii* juveniles may occur among roots of adjacent host plants because *H. schachtii* juveniles are able to move at least 30 cm to reach a sugar beet plant in field conditions (Westphal 2013). Such dispersal events would be difficult to detect due to the low genetic differentiation among source populations and newly colonized host plants. Most nematode movements in coastal populations may be thus related to exceptional events such as high tides during storms. In this

case, the small number of detected migrants in this study would be thus related to the colonization history of beaches. However, further studies with an exhaustive sampling of all host plants of a beach conducted over several years are required to gain further insight into the influence of colonization processes on the observed patterns of genetic structure.

Alternatively, the small number of detected migrants may be related to the low genetic variability of microsatellites markers, which is known to limit the statistical power of assignment tests (Waples and Gaggiotti 2006). However, the concordance of results from observed population genetic differentiation with simulated data under genetic drift suggests that low migrant detection was not just an artifact. Moreover, Jan et al. (unpublished data) have shown that effective population sizes range from 50 to 400 individuals. Small effective sizes are one consequence of limited gene flow among populations. Altogether, these results strongly support the hypothesis that populations of *H. schachtii* are genetically disconnected, even at very small spatial scales. *H. schachtii* thus appears to be a species constituted of very small isolated populations in the wild.

These population genetic features are expected in parasites (Price 1980), particularly in wild plant pathosystems characterized by low host densities. In these pathosystems, genetic drift likely has a strong influence, with wild parasite populations being more vulnerable to local extinction and maladapted to their local hosts due to their lower dispersal capabilities compared with that of their hosts (Gandon et al. 1996; Thrall and Burdon 2002; Morgan et al. 2005). Interestingly, similar population genetic structure was observed in *Heterorhabditis marelatus*, an obligate parasite of a soil-dwelling insect, and was also attributed to the very low dispersal capabilities of this nematode (Blouin et al. 1999). However, small effective population sizes and low levels of gene flow are far from typical in pathosystems (*e.g.* Fournier and Giraud 2008; Glais et al. 2014), even in nematode species (Falk and Perkins 2013; Pereira et al. 2013) and among crop pests which generally have contrasted population structure.

Wild versus field populations of *Heterodera schachtii*

In agrosystems, *H. schachtii* has huge populations connected through gene flow among fields separated by distances that reach 150 km (Porte et al. 1999; Plantard and Porte 2004). These studies attributed the scale of gene flow to soil transport. Harvests of sugar beet crops lead to the loss of several tons of soil per hectare and per harvest (Ruysschaert et al. 2007). The inadvertent transport of a massive quantity of soil may passively disperse large numbers of cysts, resulting in higher levels of gene flow among cultivated fields than among wild *H.*

schachtii populations. This difference in gene flow in wild and crop plant-parasites is supported by the higher genetic differentiation occurring among the wild populations surveyed here (pairwise F_{ST} ranging from 0 to 0.3) than among crop field populations (pairwise F_{ST} ranging from -0.01 to 0.12; Plantard and Porte 2004) separated by an equivalent geographical range (0 to 150 km). Anthropogenic influence on the spread of cyst nematodes has been documented at global scale with the transport of *G. pallida* and *Globodera rostochiensis* worldwide through the potato tuber trade (Plantard et al. 2008; Boucher et al. 2013). Long-range transport has also been demonstrated at intermediate spatial scales, within and among field populations of *G. tabacum* and *X. index*, where patterns of population genetic structure matched those of agricultural practices (Villate et al. 2010; Alenda et al. 2014).

The evolutionary potential of field nematode populations, defined as the ability of a pathogen to overcome resistance (McDonald and Linde 2002), may thus be higher in agrosystems compared with the evolutionary potential of wild nematode populations. This difference in evolutionary potential may heighten the risk posed by *H. schachtii* populations for cultivated crops. For example, gene flow and high nematode densities in sugar beet fields also imply higher effective population sizes, which favor the persistence and the spread of new virulent alleles. Most of the work on wild and field pathogen populations has focused on the role played by the general characteristics of agrosystems such as host uniformity, densities and resistance genes (Stukenbrock and McDonald 2008; Rodelo-Urrego et al. 2013), but not on intrinsic characteristic of pathogens, such as their dispersal capability. However, limiting gene flow among parasite populations represents an efficient confinement strategy, which has already been strongly advocated by several authors (Burdon and Thrall 2008; Stukenbrock and McDonald 2008), and can be easily implemented because human activities are probably the main vector of gene flow among field populations of cyst nematodes and other pathogens.

Consequences for resistant varieties management

Evidence that human activities may be the main vector of the spread of *H. schachtii* populations in fields has also profound consequences for the management of resistant varieties used to control cyst nematodes. In agrosystems, dispersal capabilities of nematodes exceed those of crops, which are inherently restricted to agricultural use. Simulation studies have shown that greater pathogen dispersal results in a better adaptation of pathogen populations to their local hosts (Gandon et al. 1996; Thrall and Burdon 2002; Morgan et al. 2005). The high availability of suitable host plants also enhances the probability that *H. schachtii* populations become well adapted to sugar beet varieties (Montarry et al. 2008), and

may explain the extensive damage they cause in sugar beet crops. High nematode densities in field populations arise from the use of crops favorable to nematode development, such as tolerant sugar beet varieties (as are most currently used varieties) or alternative *H. schachtii* hosts (*e.g.* oil seed rape). The growth and yield of tolerant varieties are not affected by high nematode infestation rates, but they greatly increase the population size of *H. schachtii* (Fournet, unpublished data). Therefore, any strategy designed to limit the level of gene flow and decrease the effective population size can help limit the evolutionary potential of cyst nematodes. The use of resistant varieties that can decrease nematode population sizes may thus increase the impact of genetic drift, limiting the influence of any new virulent alleles at the local scale. Similarly, the limitation of passive human-mediated gene flow among fields will isolate avirulent from virulent populations and decrease the ability of nematode populations to overcome resistance over large spatial scales.

CONCLUSION

This study used temporal sampling to investigate the genetic structure of wild populations of the beet cyst nematode. Wild populations of *H. schachtii* were characterized by a non-equilibrium population structure, weak levels of gene flow beyond the scale of the host plant, and a non-negligible impact of genetic drift. This pattern appeared stable over a short period of time, suggesting no isolated disturbance. Our results also suggest that the evolution of resistance in field nematode populations can be limited if pest control strategies focus on limiting passive human-mediated gene flow and field nematode densities. Data on the genetic structure of wild and field populations are still lacking for most plant-parasite nematodes. However, this study showed how population genetics is useful for (i) identifying potential improvement strategies for the control of field nematode populations and (ii) improving our general understanding of the epidemiology of diseases they cause.

ACKNOWLEDGEMENTS

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DATA ARCHIVING STATEMENT

Data for this study are available at: to be completed after manuscript is accepted for publication.

Fig. S1: Temporal change in the mean and variance in pairwise F_{ST} values under a pure drift process. Gray and dark-gray histograms represent distributions of the mean of pairwise F_{ST} values considering to the minimal and maximal values of effective population size estimated for each beach. Solid lines represent the limits of the 95% confidence interval. Dotted lines represent the observed value of the mean pairwise F_{ST} in 2013. Dotted lines located within the 95% confidence interval indicate that the observed values of mean F_{ST} can arise from genetic drift only.

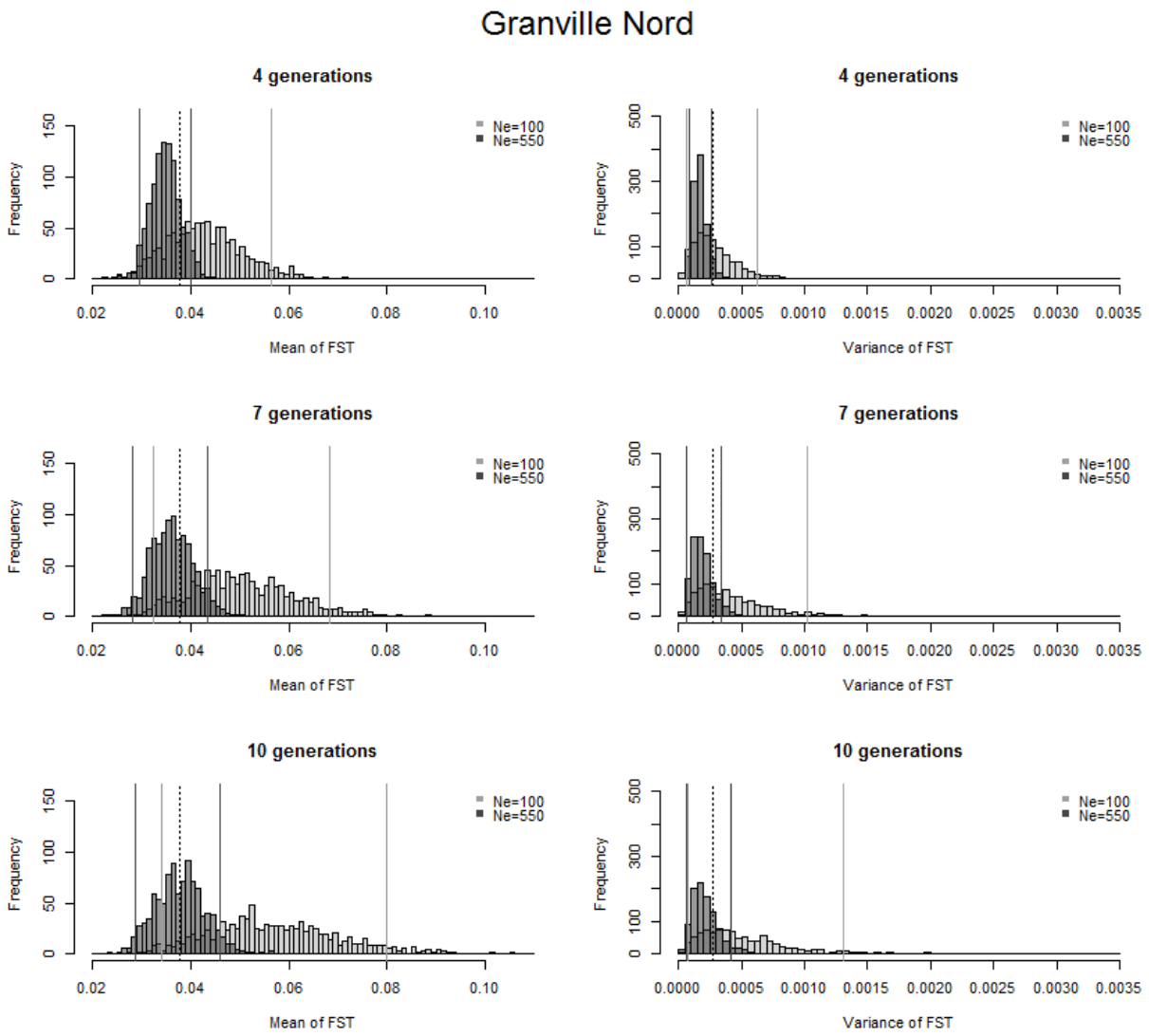
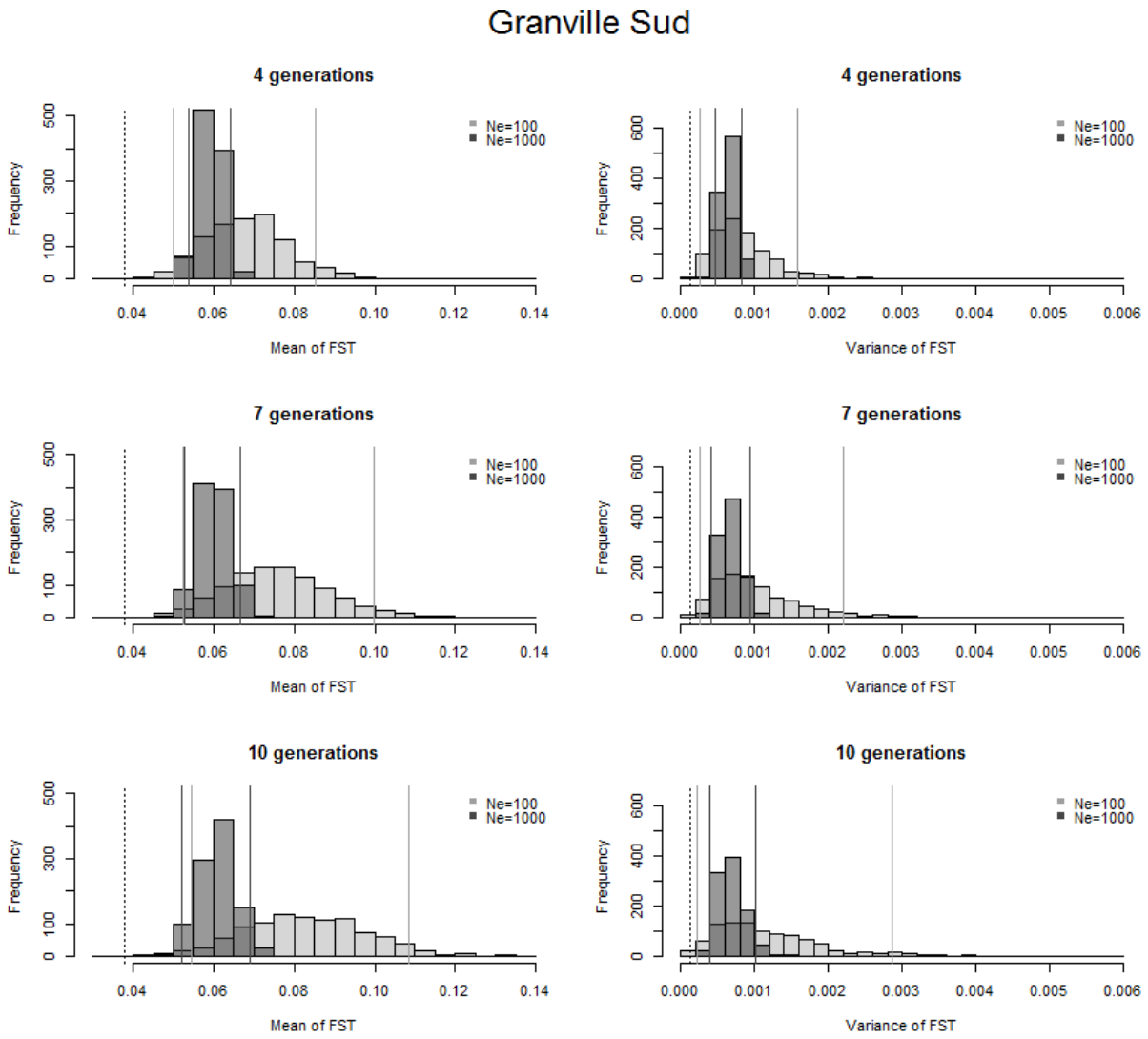


Fig. S1 (continued)



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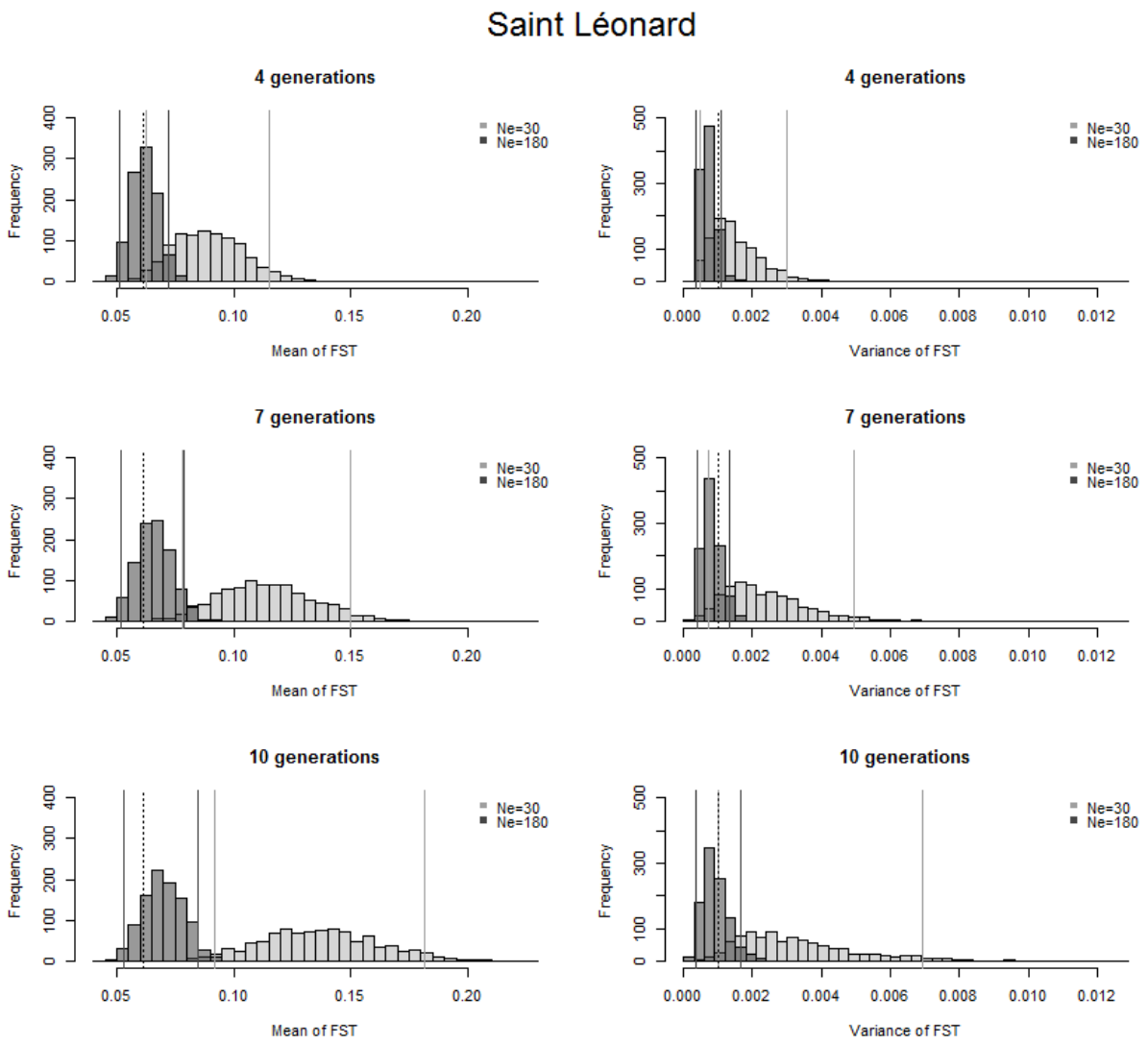

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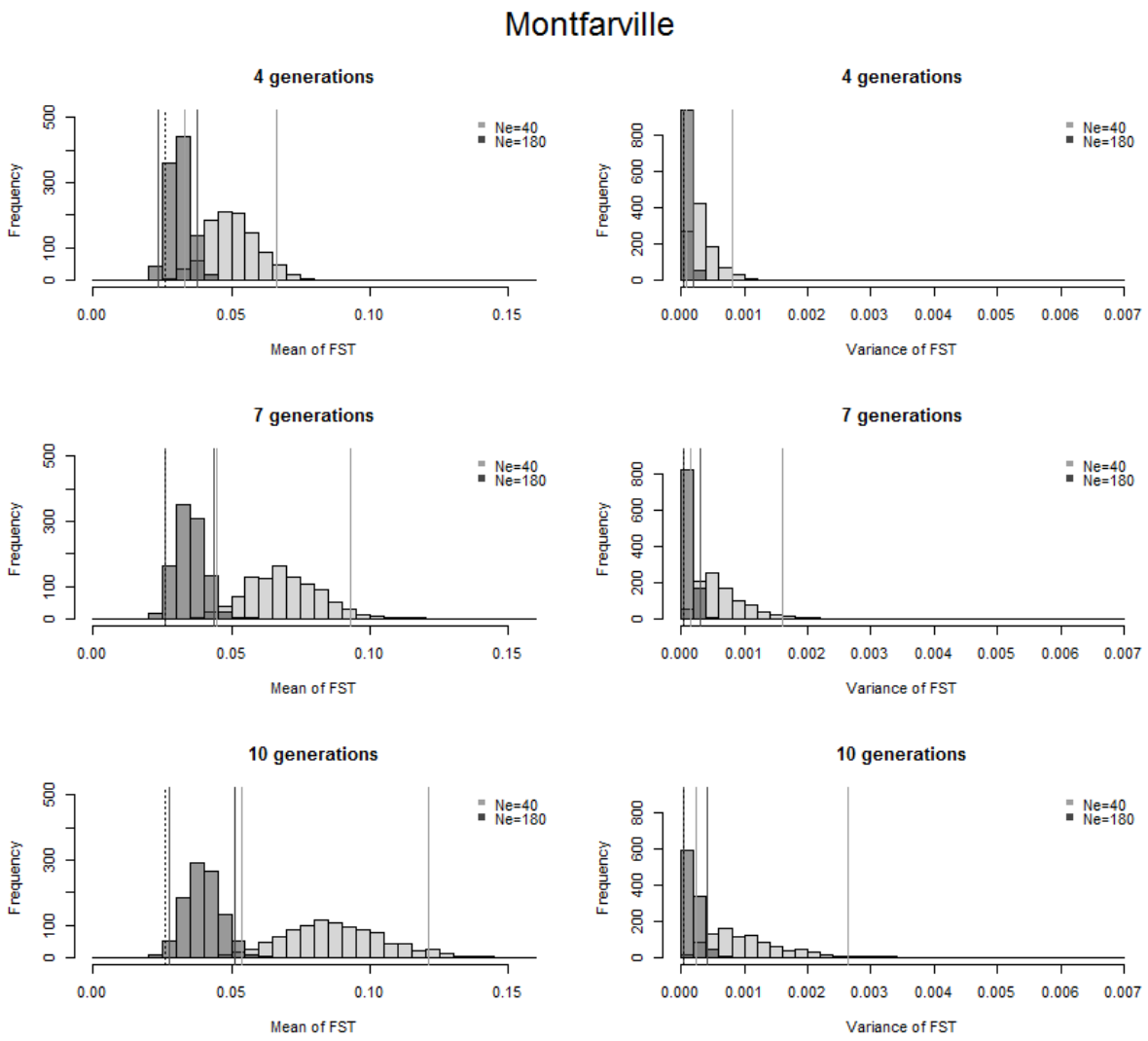

3.5. Illuminating the evolutionary biology of *H. schachtii* in the wild and in agro-ecosystems

The results obtained in this section gave new insights on the genetic structure of wild populations of *H. schachtii* at local and regional spatial scales. Spatial genetic structure appeared to involve high genetic sub-structuring at small spatial scales, moderate effective population sizes and low levels of gene flow among host-plants separated by tens of centimeters to several hundreds of kilometers from one another. These findings provide some explanations on the phylogeographical patterns observed in wild nematode populations. Our results also give information on the functioning of field populations and fuels the debate on adaptive processes involved in this pathosystem.

3.5.1. Definition of a nematode population in the wild and consequences in agro-ecosystems

At the scale of a single host-plant, populations have small effective sizes and are characterized by genetic sub-structuring. These results suggest that active dispersal of males and juveniles, which is likely to occur at this scale, may be actually very limited even among sub-populations located on the same host plant. This low mobility along with a small effective population size may induce the build-up of family structure. This fine-scale genetic structure may favor a relative reproductive isolation among sub-populations, leading to genetic erosion and inbreeding within sub-populations. Sub-populations may thus correspond to the smaller genetic unit of interbreeding individuals of the species, *i.e.* the boundaries of populations (Waples and Gaggiotti 2006). This hypothesis is also consistent with the lack of gene flow events observed at higher spatial scale, suggesting an isolation of nematode populations belonging to different host-plants.

This demic structure inside a single host-plant has been poorly documented in plant-pathogen species (but see Capelle and Neema 2005). Co-infections by several pathogen genotypes were often reported in plant pathosystems (see references in Tack et al. 2012). Infrapopulations, which include all the individuals of a parasite species in an individual host at a particular time, have also been observed in several zooparasite species (*e.g.* Bush et al. 1997; Vilas et al. 2003; Theron et al. 2004). This last observation may be the result of species life-histories specificities or intraspecific interactions within the host (Parker et al. 2003; Prugnolle et al. 2005; Keeney et al. 2007; Criscione et al. 2011). This definition implies that a population of parasite can be defined at the scale of one individual host even it is composed of

several distinct genetic entities. This concept is particularly interesting for telluric nematodes because they are difficult to sample independently according to their sub-population affiliation and because it allows considering that individuals sampled on the same single host-plant belong to the same genetic unit in future studies.

Similar sub-structuring patterns were also observed in field *H. schachtii* populations, suggesting that active dispersal capabilities of nematodes are equivalent in wild and field conditions. Effective population sizes of field populations are unfortunately not available. Therefore, we cannot determine whether field sub-populations are demographically and genetically similar to what is found in the wild. However, spatially-restricted dispersal at the scale of the host-plant strongly suggest that the lower genetic differentiation observed among fields probably result from modification of levels of passive long-distance gene flow. Indeed, at spatial scale higher than a single sea beet, passive dispersal is likely to occur among populations. However, extremely low levels of gene flow were observed among plants of all surveyed beaches for the two sampling sessions. Dispersal over distances ranging from a few tens of centimeters up to hundreds of kilometers is thus limited, and probably associated to exceptional events, such as storms or high tides. This limitation of passive dispersal should prevent nematode spreading among fields. Additional factors thus facilitate passive dispersal in agro-ecosystems, which supports the contribution of human activities in disease dissemination among sugar beets within the field, and among fields within a production area.

3.5.2. Micro-evolutionary processes, host colonization, and large-scale phylogeography

Let us consider now this model of population functioning in the context of the colonization of new sea beet plants along the shoreline. In this scenario, a population of nematode corresponds to all individuals living on one sea beet, which can be grouped into several genetically distinct demes. Before the colonization event, an initial *H. schachtii* population is developing since several generations on a host plant, a sea beet or on any other suitable host-plant species for *H. schachtii*, located not too far from the shoreline. Exceptional events, such as storms, landslides or high tides then disperse cysts more or less far away from this initial source population on new host sea beets. During dispersal events, some nematodes do not survive and some of them are also not able to infest the new hosts. This results in founder events with associated bottlenecks and loss of genetic diversity. Surviving nematodes then develop on their new host and after a few generations, sub-structuring reappears at the scale of the host plant. However, small effective population sizes strengthen the influence of

genetic drift over time. Consequently population genetic differentiation increases among host-plant individuals. Genetic differentiation may also increase because of the variability in host-plant resistance. Differential resistances seem to be a widespread characteristic in several wild pathosystems and may differently affect the fitness of different nematodes (Laine et al. 2011). This hypothesis is consistent with the fact that only partial resistances were reported in the sea beet so far (Biancardi et al. 2012).

The hierarchical sampling design of the phylogeographical study implied pooled soil samples collected on several sea beets in a range of few tens of meters. Analyzing our dataset based on a pooling of genetically distinct units may lead to the loss of the genetic signature of any colonization process and result in unstructured genetic patterns. The erasing of subtle spatial genetic structure may be also amplified by temporal evolution of the initial population source of nematodes. It should be noticed that neither mutation, nor recombination are included in this hypothetic model of population dynamics because (i) there is no information on these two forces in *H. schachtii* and (ii) because the influence of genetic drift limit the invasion of new alleles in small population (see below). However, this model of population functioning allows building a colonization scenario of beaches consistent with large-scale phylogeographical patterns of *H. schachtii* along the Atlantic coastline. In this case, coastal population of *H. schachtii* may have recolonized Europe by the coast along with the sea beet, suggesting the two species may have interacted more than expected from phylogeographical results alone.

4. Discussion and perspectives

4.1. What can be learned from our results?

4.2. Evolutionary potential of *H. schachtii* populations and co-evolution with *B. vulgaris* ssp. *maritima* populations

4.3. Relationships between wild and field populations in *H. schachtii*: a missing point in the estimation of the evolutionary potential of this nematode

4.4. Alternative host species of *H. schachtii* as a blank page for future research

4.1. What can be learned from our results?

4.1.1. Objectives and main results

Cyst nematodes are one of the most damaging pathogens of sugar beet crops. Knowledge provided by wild populations of pathogens may be informative to assess long-term risk associated with crop pests. To generate information facilitating the prediction of the durability of genetic resistance used to control cyst beet nematode populations, this PhD project investigated their evolutionary potential (McDonald and Linde 2002). In this work, the evolutionary potential is defined as the capability of a pathogen to evolve to overcome genetic resistance of its host. Such adaptation capabilities mainly depend on the history of the pathogen and the current evolutionary dynamic of its populations over space and time. Consequently, we investigated the evolutionary history of wild populations of *Heterodera schachtii* and *H. betae* over the species distribution area along with more local surveys devoted to disentangle the respective effects of gene flow and genetic drift. This work highlighted numerous features of the biology and of the evolutionary dynamics of beet cyst nematodes.

(1) *Heterodera schachtii* and *H. betae* showed a large distribution along the Atlantic shoreline. The colonization history of coastal habitats was presumably influenced by climatic fluctuations occurring since the Last Glacial Maximum. We also cannot rule out the effect of marine currents in driving cyst dispersal, which was an unexpected result given the terrestrial nature of these nematodes species. Dispersal of these species may thus be more complex than expected in cysts nematode species.

(2) The lack of congruence between the phylogeographical patterns observed for the sea beet and *Heterodera schachtii* suggested a non-shared evolutionary history. This situation may be related to a larger spectrum of host-plant species allowing for direct inland recolonization of northern Europe. Alternatively, fast local evolutionary dynamics of nematode populations may blur any genetic signatures of past colonization events.

(3) *Heterodera betae* did not originate from The Netherlands, as previously suspected. Populations were strongly genetically differentiated, suggesting both a geographical isolation of populations and the influence of their parthenogenetic mode of reproduction. This reproduction mode may explain the differences observed in spatial genetic structure when compared to *H. schachtii*. However, ecological preferences and host range may also result in

similar patterns of genetic structure. Overall, microsatellite data suggested that a classical coastal recolonization scenario was more likely for this nematode than for *H. schachtii*.

(4) Wild populations of *H. schachtii* were characterized by moderate effective population sizes, geographical isolation, and genetic structuring at the level of the host plant. Active and passive dispersal capabilities may be spatially-restricted over short distances in natural ecosystems. Long-distance episodes of gene flow may probably be mainly related to exceptional events such as high tides or storms. These general features implied a substantial influence of random genetic drift and also underlined stochastic demographic events like recurrent extinction/colonization events on the evolution of population genetic structure over time.

4.1.2. Evolutionary potential of beet cyst nematodes and durability of resistances

Altogether, our results are not yet satisfactory to definitively conclude on the evolutionary history of *H. betae* and of its adaptive capacity to evolve under selective pressures like host resistances. However, the physical isolation of populations along with parthenogenetic reproduction may lead to a lower evolutionary potential as compared to *H.* schachtii and according to the criteria defined in the study of McDonald and Linde (2002) (Figure 14). Nevertheless, parthenogenesis and limited gene flow may also favor local adaptation in wild populations because nematode cannot escape to selective pressures imposed by the host. More studies are thus required to understand how *H. betae* populations evolve on wild hosts. This is also true for field populations because there is no data available to make any comparison between evolutionary characteristics of field and wild populations.

According to the study of McDonald and Linde (2002), small levels of gene flow and small effective population sizes are thought to result in low adaptive value for wild populations of *Heterodera schachtii*. Specifically, the strong influence of genetic drift limit the strength of selection pressures as well as the impact of mutation (see § 4.2). Consequently, resistance breakdown in wild populations is probably difficult suggesting that occurrence of virulent individuals adapted to *B. v.* ssp. *maritima* may be a rare event. Moreover, low levels of gene flow among wild nematode populations suggested they do not represent a great risk for new *H. schachtii* virulences occurring in fields, though the question of gene flow among wild and field nematode populations remains to be investigated (see § 4.3).

In fields, the situation is a little bit different. The high density of *H. schachtii* juveniles observed in sugar beet fields is expected to generate large effective population sizes, which in

turn are also favored by high levels of gene flow. Large effective population sizes reduce the influence of genetic drift and may increase the strength of natural selection. Additionally, in large populations, the same favorable mutation may appear several times, decreasing its probability to be lost. In fields, where resistant crops represent a strong selective pressure for nematode populations and where effective population sizes of pathogens are supposed to be high, new virulent alleles thus have a high probability to spread over large spatial scales. The study of local genetic structure of nematode species also highlighted the importance of consanguineous mating in several cyst nematode species. McDonald and Linde (2002) supposed that less new genotypes are created in inbreed pathogens than in outcrossing species. In their model, inbreeding is thus expected to reduce the adaptive capabilities of pathogens. However, inbreeding can also lead to increased homozygosity, including loci governing avirulence/virulence, which favors the expression of virulence when underlying genes are recessives, and thus the adaptation of host genetic resistance. Field nematode populations may thus have a higher evolutionary potential because they may have higher levels of genetic diversity and a higher capacity to face resistances as compared to wild populations. In this context, a careless use of genetic resistances would probably favor adaptation of *H. schachtii* populations and lead to resistance breakdown. As a result, all management strategies of resistant varieties devoted to reduce gene flow events among fields and to reduce nematodes densities should improve their durability.

Durable crops' protections against telluric pathogens may thus gain at developing management methods considering higher spatio-temporal scales than one field during a given cropping year. Such an approach has already been proposed for aerial fungi and would be suitable to maintain the whole pathogen metapopulation maladapted to its hosts and to prevent epidemics (Bousset and Chèvre 2013; Bousset 2014). Nonetheless, despite the general view assuming that field populations of nematode are large, it may be interesting to clearly estimate their effective population sizes. In practice, such a study would be difficult to conduct in fields because point estimation methods are not adapted to the biology of nematodes and because temporal methods are challenging to implement in annual crops, such as sugar beet. Nevertheless, in multivoltine species such as *H. schachtii*, two sampling at the beginning and the end of the growing season may be possible to describe the evolution of allelic frequency variations. Sampling may be performed at the plant and/or the field scale to apprehend effective population sizes at different spatial scales.

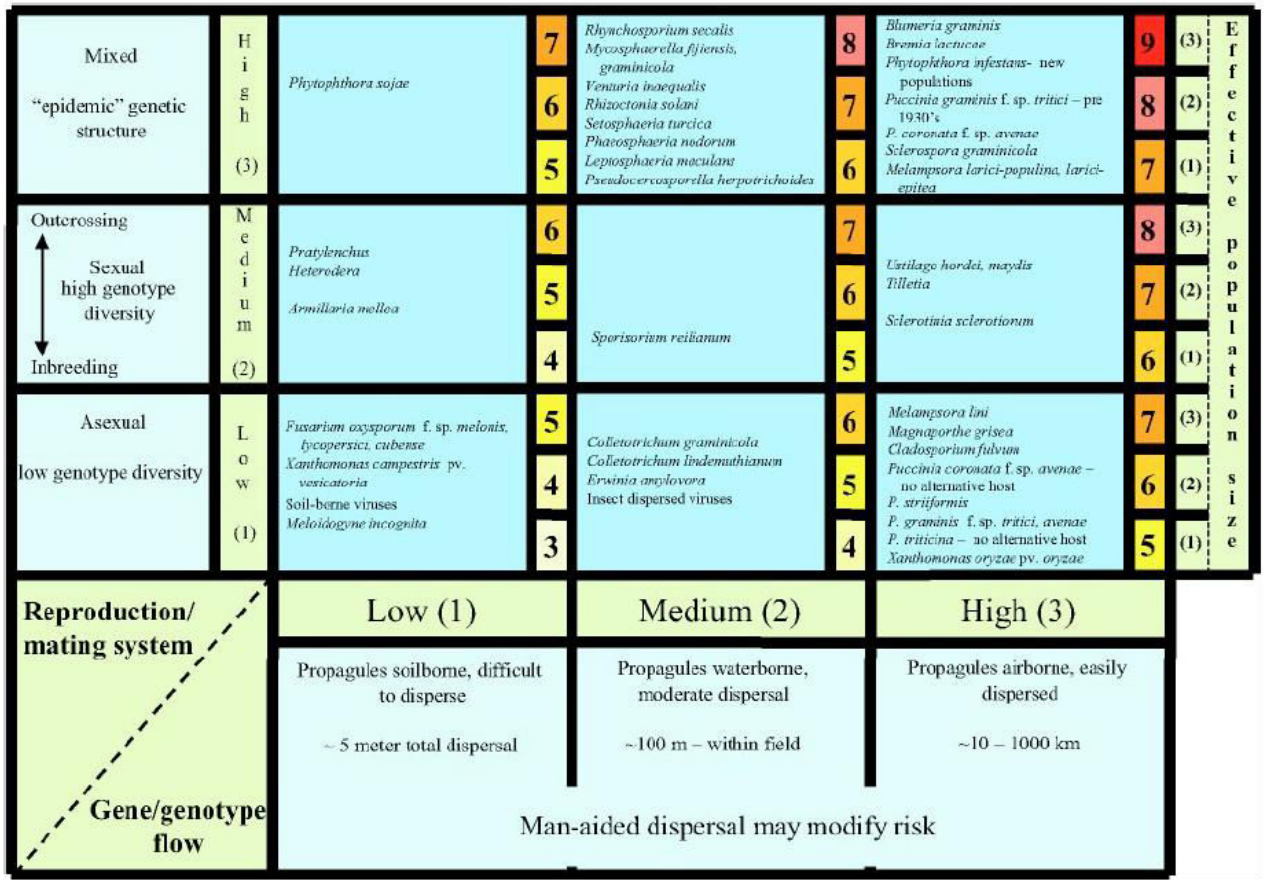

Figure 14 : Estimation of the evolutionary potential of a pathogen and the risk it represents for crops according to reproduction/mating system, gene/genotype flow and effective population size (from McDonald and Linde 2002). Effective population size (Ne) is on a 1–3 scale where 1 is small Ne, 2 is average Ne, and 3 is large Ne. Assignment of total risk value assumes that all effects are additive. This risk model assumes that mutation rates are constant and that selection is efficient for all pathogens. In the case of natural populations of *H. schachtii* sexual reproduction characterized by inbreeding associated to low gene flow and small effective population size give an evolutionary potential of 4, which is one of the lowest value on this scale. In fields, gene flow mediated by man activities among fields at large geographical scale and higher population sizes raises the evolutionary potential to a value ranging from 6 to 8. In the case of *H. betae*, asexual reproduction associated to isolation of population would result in a low evolutionary potential ranging from 3 to 5.

4.2. Evolutionary potential of *H. schachtii* populations and co-evolution with *B. v.* ssp. *maritima* populations

Our results suggested that the specialization of *H. schachtii* to sea beets may not be straightforward. Indeed, in all surveyed populations, any new favorable allele is likely to be quickly lost through the stochastic effects of genetic drift (Kimura 1957, 1962; Otto and Whitlock 1997). In small populations, such as wild *H. schachtii* populations, natural selection cannot counteract the influence of genetic drift which may prevent the emergence of virulent alleles specific to sea beets. Similarly, the limitation of gene flow among populations may prevent the spread of virulent alleles from distant populations, reducing also the adaptive capabilities of *H. schachtii* populations. In addition, the frequent extinction/recolonization

events characterizing sea beet populations also represent a highly changing environment for nematodes, where the distribution of genetic resistances among sea beet populations may be highly heterogeneous over space and time.

This spatio-temporal instability may thus limit the development of specific genotype-genotype interactions between sea beets and *H. schachtii*, and may prevent the development of specific control method in the host, like major gene resistances (Harrison et al. 2013). Indeed, recurrent localized extinction/recolonization events observed in sea beet populations (see De Cauwer et al. 2012) may correspond to a renewal of local host genotypes for nematodes. In this situation, nematodes adapted to previous sea beet generations may have lower infective capabilities on their new hosts, as observed in Susi and Laine (2013). The non-specialization of nematode populations may thus represent an advantage for long term-persistence. This hypothesis may explain why no major resistance genes against *H. schachtii* were reported so far in *B. v.* ssp. *maritima* accessions, which, in contrast, exhibited partial resistances. It is generally assumed that the polygenic nature of these resistances affect a larger range of pathogen genotypes, enhancing the probability of the host plant to reduce pathogen pressures. However, quantitative resistance can also vary among host populations, and only some of this variation is adaptive (Salvaudon et al. 2008). To understand the role of partial resistance in the evolution of this pathosystem, it would be particularly interesting to estimate how much this quantitative resistance differentiation result from the action of local selective pressures or from other neutral processes such as genetic drift or gene flow.

The hypothesis of non-specialization of nematodes has also consequences on their capacity to evolve through space and time. This non-specialization prevents the appearance of pre-adapted nematode individuals able to deal with new resistance gene. In this case, gaining insights into phylogeographical patterns in the wild may not give meaningful information into the adaptive capacity of nematodes to evolve in man-made habitats like the agro-ecosystems. However, population genetic structure of sea beet populations is also influenced by the occurrence of long-lived seed banks which mainly stay in the neighborhood of the mother plants and can stay dormant in the soil (Sester et al. 2006; Arnaud et al. 2010). Long-lived seed banks can be viewed as sleeping genes that buffer changes in genetic composition of the host plant population over time. Therefore, this life-history trait may favor longer interactions between related host genotypes and cyst nematodes. In this case, adapted genotypes may occur and initiate parasitic interactions.

It is thus difficult to conclude on the relative influence of non-adaptive processes, such as genetic drift and demographic fluctuation, and of natural selection in the evolution of host-

pathogen association between *Beta vulgaris* ssp. *Maritima* and *Heterodera schachtii*. Because our study only considered neutral variation in sea beet and nematode populations, the understanding of adaptive dynamics of this pathosystem would gain from further investigations considering genetic and/or phenotypic characterization of host and nematode populations for virulence and resistance. This approach was difficult to implement so far, because virulence factors of *H. schachtii* and associated genetic resistances were not available. Genotyping for resistance was thus impossible and the phenotyping of wild host plant would be difficult to interpret because the qualitative or quantitative nature of the resistance would be unknown. Nevertheless, the recent publication of a genetic marker in sea beet related to resistance to *H. schachtii* may facilitate the screening of wild host plant for nematode resistance and the association of genotypes with phenotypic characterization (Stevanato et al. 2014). The concomitant development of virulence markers in *H. schachtii* using for example, new sequencing technologies, would definitively open a new area of investigation in this pathosystem.

4.3. Relationships between wild and field populations in *H. schachtii*: a missing point in the estimation of the evolutionary potential of this nematode

Wild pathogen populations represent a reservoir of inoculum and genetic diversity when they are connected through gene flow with field populations (McDonald and Linde 2002; Burdon and Thrall 2008). Our findings described above suggest this situation is unlikely to arise in the case of *Beta vulgaris* ssp. *maritima* and *Heterodera schachtii*. Wild nematode populations are connected by low levels of gene flow and non-adaptive processes limit the appearance of individuals adapted to genetic resistances of the host plant. Nonetheless, it is difficult to determine whether the evolutionary dynamics of nematode populations living in habitat adjacent to fields is more influenced by field or wild nematode population dynamics (high *vs* low gene flow). It is also difficult to determine whether there are gene exchanges between the two habitats. It would be thus interesting to clearly examine this question. This issue is currently investigated by considering two different approaches.

First, we sampled one wild nematode population on sea beet hosts in a beach along the shoreline, and four field nematode populations located within sugar beet fields in inland areas: one just next to the sampled beach and three others localized far away from the shoreline and every 5 km from each other. We proceeded similarly for two additional sampled beaches. Cysts were extracted from soil samples and genotyped with the same set of microsatellite loci

used in the work previously presented. Analyses are still running. With this dataset, we would like to estimate whether dispersal events can be detected among field and wild nematode populations. We also would like to evaluate the intensity of gene flow among fields. Additionally, this study may give clues on how field nematode populations may influence the population genetic structure of surrounding wild nematode populations. Numerous studies investigated the occurrence of gene flow between wild and cultivated plant species, such as carrots and apples (Cornille et al. 2013; Rong et al. 2013), and among wild and field populations of aerial pathogens (Clarkson et al. 2013) and plant virus (Webster and Adkins 2012; Almeyda et al. 2014). However, no studies documented this kind of interactions for soil organisms as they are not expected to disperse over large geographical distances.

Second, the aim of this PhD project was also to investigate how genetic diversity is partitioned among field nematode populations at higher geographical scale. We decided to sample one to three fields located in several European countries and to genotype *H. schachtii* populations using the same set of microsatellite loci (Figure 15). These two steps are still in progress and only a part of the dataset is already available. This dataset may help to trace back the origin and spread of field nematode populations. We initially hypothesized that *H. schachtii* populations were recently transferred to fields from natural ecosystems because the sugar beet cultivation only exists since the 19th century. A comparison between field and wild nematode populations may help to determine whether this hypothesis is relevant and may allow the identification of potential wild sources of *H. schachtii* infesting fields. Alternatively, *H. schachtii* may be a species already present in the soil at the establishment of cultural practices, and field populations may not be related to each other. Therefore, the study of population genetic features of field populations may help to distinguish among these two hypotheses. We also plan to phenotype these field nematode populations to investigate the potential correlations between neutral genetic structure and virulence profiles. This investigation would be a first step to understand the adaptive dynamic of this pathosystem and a good source of information for the use of genetic resistance at large geographical scale.

4.4. Alternative host species of *Heterodera schachtii* as a blank page for future research

Heterodera schachtii is a pathogen with tens of alternative host species occurring both in wild and cultivated habitats (Goodey et al. 1965). For practical purposes, we did not take into account this large spectrum of hosts in this work, selecting only one host plant species with

agronomical and ecological interests. However, alternative host species may also impact the evolutionary history of *H. schachtii* and investigating the population genetic structure of *H. schachtii* among alternative hosts may be thus interesting at different levels.

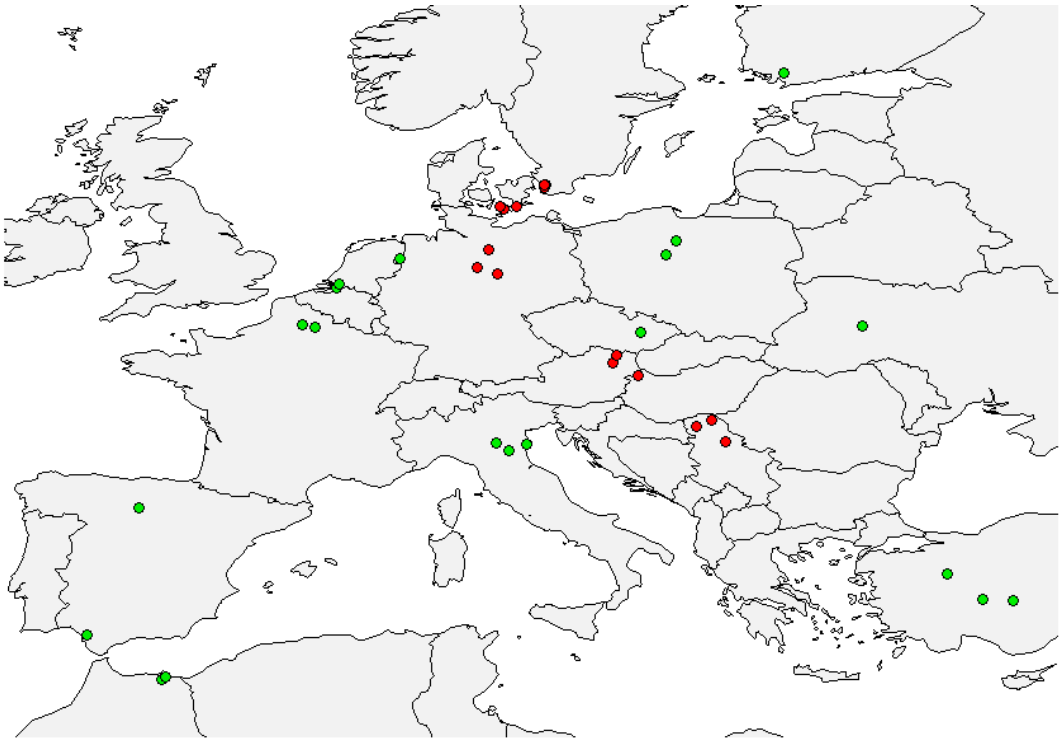

Figure 15: Distribution of field populations of *H. schachtii* in Europe. Green: samples with genotyped populations (microsatellites); Red: samples in process.

First, the consideration of alternative host species may help to better understand the phylogeography of this nematode. Similar approaches as the one used in this PhD applied on another host plant species may help (i) to determine whether *H. schachtii* recolonized Europe on inland host species, (ii) to explain the southward recolonization pattern observed in *H. schachtii*, (iii) to determine whether *B. v.* ssp. *maritima* is a favorite host of this nematode or just a dead end, *i.e.* the last suitable species occurring on beaches before unhospitable habitats, *i.e.* the sea. These hypotheses were not considered in our phylogeographical study but should be investigated to understand how this telluric pathogen evolved under climatic fluctuations and how strong was its interaction with the sea beet. Indeed, the inefficiency of *B.v.* ssp. *maritima* resistances on *H. betae* and the existence of major resistance genes controlling *H. schachtii* in another *Beta* species suggest that *Heterodera* spp. and *Beta* spp. species may have interacted and/or co-evolved more than expected based on our results alone. A large-scale comparative study comparing *H. schachtii* populations developing on the host-

plant community associated with several *Beta* species would be very helpful to have a larger understanding of the history and the evolution of this nematode.

Second, the study and the comparison of *H. schachtii* populations developing on the sea beet and alternative host-plant species may also be interesting to refine our understanding of local dynamics of wild populations of this nematode. Indeed, theoretical expectations assumes that generalist pathogens are able to maintain larger populations than specialists because the population dynamics of the pathogen does not depend anymore on the population dynamics of a unique host species (Dobson 2004; Barrett et al. 2008). This theoretical assumption recently received empirical support from a study on a zoo-parasitic nematode (Archie and Ezenwa 2011). This suggests that the evolutionary dynamics of *H. schachtii* may also be more complex than expected. Higher effective population size would result in a lower influence of genetic drift and demographic processes on the genetic structure of populations, which may lead to very different evolutionary dynamics as the one described in our results. Moreover, the occurrence of gene flow among populations developing on different host species may have contrasting consequences for the adaptive dynamics of *H. schachtii* populations. On the one hand, the specialization to a particular host species maybe difficult due to the spread of alleles adapted to different host species. On the other hand, there is empirical evidence that gene flow among populations does not always prevent pathogen adaptation to the host (Spottiswoode et al. 2011; Laine et al. 2014). As a result, the adaptive capabilities of nematode populations may vary among populations due to local conditions.

Third, the consideration of alternative host plant species may be interesting to take into account potential host-shifts or host-jumps in the history of *H. schachtii*. These events cannot be detected using phylogeographical studies because they occur on longer evolutionary time scales (Hoberg and Brooks 2008; de Vienne et al. 2013). However, they may impact the understanding of the recent evolutionary history of populations. For example, several emergent pathogens resulted from the contemporary colonization of new host species (Stukenbrock and McDonald 2008). In this situation, focusing on populations only developing on the new host would only give a part of the history of the pathogen, which may obscure the localization of its native area or the understanding of its adaptive dynamic. As a result, our study may probably give only a partial picture of nematode evolutionary history, but is a good starting point for the development of additional investigations in wild populations of telluric nematodes.

Appendix 1: sequencing protocol

Nematodes

To complement the nematode microsatellite dataset, we sequenced 7 nuclear loci, and 2 mitochondrial loci according Roche's amplicon pyrosequencing procedures described in the protocols handbook associated with a GS FLX 454 pyrosequencer (Roche technologies). Amplicon sequencing is based on two sequential PCR. The first one is dedicated to gene amplification, and the second one associates a specific MID (*e.i.* barcode) to each sample for identification in subsequent bioinformatic analyses. All procedures, from the first PCR to the final pools were performed twice, from the same DNA samples and sequenced independently on two different quarters of run plate to estimate consistency of results.

a. Primers design

H. schachtii and *H. betae* are non-models species without reference genome. Very few genes were already described in literature for both species and we selected thus genes with sequences already available in GenBank® (http://www.ncbi.nlm.nih.gov) database. Primers were then designed using the web software Primer3Plus (Untergasser *et al.* 2007), and were listed in the Table S1. All markers were designed to be between 430 and 530 bp after the two PCRs.

Table S1: Forward and Reverse primers.

Gene	Forward primer	Reverse primer
CoI	GCAGGTATTAGTTCTATCGGAGGCA	GTATTCCAACAACAAATATATGATGAGC
CoII	GGTAGTTGGTCATCAATGATATTG	GCCACGGGTATAAAGGAATGA
CLE2	TGGCAAAAGTAAAATATTGAAATAA	GTGGAACCTGGTAAAAATGAGTT
CM2	GAGCAACTCTGACTGCGCT	GTCTCCATAATTGTCCCCAATG
VAP2	RTCGCCCTCTTTTTCATGCT	GAAAATGTTCCGCCATTCA
PENG1	GTCTCGTGGACCGATGTG	GAGCTTGGCACCAAGATCACT
PENG2	CTGAGTGACGCGAACACC	GGCAACTTTGGTGAAGAATTCAA
ENG2	ATGCACAGTCGGAGATAACG	GTCGTAGTGATCTGGTGTCAAG
AD	TTGGAGAATACGGAGAACAATC	GTTTTTGTACTCGGCACAGC

b. Gene amplification

Gene amplification was performed on pools of DNA coming of all 27 and 21 populations of *H. schachtii* and *H. betae*. Nematode larvae used in DNA extraction are composed with a small number of cells, limiting the DNA concentration of samples. As a result, the use of one single larva is insufficient to sequence some genes. We decided thus to pool DNA coming

from 1 to 10 larvae, according to the number of individuals available in populations, to perform PCR. Because of this specific number of cells and because DNA extractions were performed in the same conditions with the same protocols, initial DNA concentrations were expected to be homogenous among all selected nematodes individuals. PCRs were performed in 26µL of reaction volumes with the Go Taq® Flexi (Promega) kit, H_2O and 0.6 µM of forward and reverse primers. Thermal cycling protocol was similar for all amplicons with 94°C denaturation for 5 min, 30 cycles of 94°C for 1 min, 57°C for 1 min, and 72°C for 1 min, then followed by 5 min of extension at 72°C.

c. MIDs incorporation and sample pooling

Previous PCR products contained a universal tail allowing their subsequent fusion with 454 adaptors and MIDs in a second PCR. Pyrosequencing was unidirectional with only one MID to tag one population of one nematode species. We chose to tag populations and not individuals to reduce the number of MIDs and so sequencing costs of the study. As a result, we selected 48 MIDs from Roche protocols and previous studies (Table S1, Gompert et al. 2010, Boers et al. 2012). PCRs were performed in 25µL of reaction volumes, with the Go Taq® Flexi (Promega) kit, H_2O and 0.15µM of forward and reverse primers. The cycling procedure was the same than for the first PCR.

Emulsion-based clonal amplification performed during a 454 pyrosequencing run preferentially amplifies small DNA fragments than longer ones. As a result, amplicons should exhibit similar size and samples should be purified of all residuals fragments coming library preparation, as primers dimers. As a result, PCR products were separated with an electrophoresis on a 2% agarose gel. DNA fragments with expected sizes were manually excised from the gel with a scalpel and purified with the Quiagen MinElute® Gel Extraction Kit. Concentrations of pools were estimated using the analysis of pictures of E-Gels® 96 Agarose gels 1% calibrated with the E-Gel® Low Range Quantitative DNA Ladder with the software ImageJ version 1.47. Indeed, the Quantitative DNA ladder allows computing a linear correlation between intensity of samples and their DNA concentrations. Amplification was homogenous among genes. Estimations of concentrations of samples allowed thus to pools them in equimolar concentrations.

d. Data pre-processing

Raw data were extracted from SFF files and filtered according their length and quality scores using the Mothur toolkit version 1.24.0 (Schloss et al. 2009). Reads smaller than 150pb and with an average base quality score below 20 were removed. The Mothur Toolkit was also

used to list reads associated with each MID. Reads identifiers of fasta and quality files were then modified according this list to include codes of populations using homemade Python scripts. Primers, adapters, universal tails and MID were then removed using Galaxy tools and Cutadapt (Giardine et al. 2005; Blankenberg et al. 2010a; Blankenberg et al. 2010b; Goecks et al. 2010; Martin 2011). Output files were then used to build consensus sequences for each amplicons using the *de novo* assembling option of the software Mira version 4.0.2 (Chevreux *et al.* 1999). Reads were then mapped on those consensus sequences with the mapping option of Mira. Mapping results were output under the ace format to be used with the software Haplotyper 454 version 2.10 to detect SNPs (Boutte unpublished data). This software considers all sites of a sequence as a potential variant and can generate haplotypes with different allele for several sites of the sequences. The analysis can be performed on the global dataset and per populations to detect local variations. All analyses, except assemblies with Mira, were performed using the Galaxy instance of the Genouest plateform (Le Bras *et al.* 2013) and were independently conducted for the two quarters of run.

Sea beet

Three chloroplastic one mitochondrial regions were selected for sequencing to complement the microsatellite dataset including a part of the *matK* gene, the *trnD-trnT* and the *trnL-trnF* intergenic spacers and the *cox1-cox2* intergenic region. The matK region was amplified in three overlapping fragments because of its size (~2000 pb). Primers pairs were K1-F (GTTGCCCGGGATTCGAA)/MatK1-R (ATTAGGGCATCCCATTAGTA) (annealing temperature (T_a)=54°C, modified from Fénart et al. 2006), MatK2-F (CTAGCACAAGAAAGTCGAAG)/Matk6B-R (GGATTTCTAACCATCTTGTT) (T_a=50°C, modified from Fénart *et al.* (2006)), and MatK2-F/k6B2-R (GGTTCAAACTCTTCGCTAC) (T_a=50°C). *trnD-trnT* and the *trnL-trnF* intergenic spacers were amplified with primers and annealing temperatures described in Fénart *et al.* (2006). PCR amplification was performed in conditions detailed in Fénart *et al.* (2006) with the DreamTaq polymerase (5U/µl) kit (Thermo scientific). Cycling procedures were as follow: 1 min at 94°C, 30 cycles with 45 seconds at 94°C, 45 seconds at the T_a temperature and 1 to 1 min 30 seconds (depending on the fragment length) at 72°C and 10 min at 72°C for a final elongation step. The *cox1-cox2* intergenic region was amplified using primers detailed in Nishizawa et al. (2007). PCR amplification was performed in 10µL of reaction volumes containing 3 mM of $MgCl_2$, 200 µM of each dNTP, 0.2µL of BSA and DMSO, 0.2µM of each primer and 0.9 $U.\mu L^{-1}$ of DreamTaq (DreamTaq polymerase (5U/µL) kit (Thermo scientific)). Thermal cycling was composed of 5

min at 94°C, 30 cycles with 30 seconds at 94°C, 60 seconds at 63°C and 45 seconds at 72°C, and 10 min at 72°C for a final extension step. All PCR products were then purified with the NucleoFast® 96 PCR (Macherey-Nagel) and sequenced with a BigDye Terminator v3.1 Cycle sequencing Kit on a 3130xl Genetic Analyzer (Applied Biosystems). Raw data were read, checked and aligned with the software SeqScape version 2.1 (Applied Biosystems).

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RÉSUMÉ

De la génétique des populations à la gestion durable des résistances : Intérêt de l'étude des populations sauvages des pathogènes des cultures. Cas de deux nématodes à kystes et de leur hôte sauvage commun.

La gestion durable des variétés génétiquement résistantes aux pathogènes des cultures nécessite de tenir compte de leurs capacités évolutives. Celles-ci découlent de leur histoire évolutive et de la dynamique actuelle de leurs populations qui peut être modifiée par les activités humaines inhérentes au milieu agricole. La description et l'évaluation des capacités évolutives d'un pathogène ne sont donc possibles que sur des populations issues d'environnements non soumis à des perturbations d'origine anthropique. Les objectifs de ce travail sont donc (1) de reconstruire les histoires évolutives de deux nématodes à kystes, Heterodera schachtii et Heterodera betae et de leur hôte sauvage commun, Beta vulgaris ssp. maritima, à partir de populations sauvages distribuées sur le littoral du sud de l'Espagne à la Suède ; (2) de décrire, à une échelle plus fine, le fonctionnement et la structure génétique de populations sauvages d'H. schachtii. Nos résultats montrent que la colonisation de la côte Atlantique par les deux nématodes a probablement été influencée par les fluctuations climatiques survenues depuis le dernier Maximum Glaciaire et les courants marins. Les patrons phylogéographiques observés entre H. schachtii et la plante suggèrent des histoires évolutives disjointes contrairement à ceux observés chez H. betae. A fine échelle spatiale, les populations d'H. schachtii sont isolées entre elles, structurées à l'échelle de la plante hôte et présentent de petites tailles efficaces. Ces résultats sont discutés dans le contexte général de la protection des cultures.

Mots-clefs : phylogéographie, flux de gène, dérive génétique, populations sauvages de pathogènes, Heterodera schachtii, Heterodera betae, Beta vulgaris ssp. maritima, gestion des résistances génétiques

ABSTRACT

From population genetic to sustainable management of resistances: benefit of the study of wild populations of crops pathogens. Case of two cysts nematodes and their common wild host

The sustainable management of genetic resistances to crop pathogens needs to consider their evolutionary potential. The evolutionary potential results both from the evolutionary history and the current population dynamics of pathogens, which can be affected by human activities occurring in agro-ecosystems. Therefore, they should be described and evaluated on wild pathogen populations free of human-mediated disturbances. The aim of this work is twofold (1) assessing the evolutionary history of two cysts nematodes, Heterodera schachtii and Heterodera betae, and their common wild host, Beta vulgaris ssp. maritima, in wild populations sampled on the coast from the South of Spain to Sweden; (2) investigating at a fine spatial scale the population genetic structure of H. schachtii. Results show that the colonization of the Atlantic coastline by the two nematodes was probably influenced by climatic fluctuations occurring since the Last Glacial Maximum, along with marine currents. Phylogeographical patterns of H. schachtii and the host-plant suggest a non-shared evolutionary history, which contrasts with the coastal recolonization of Europe observed in H. betae. The fine-scale study evidenced that wild populations of H. schachtii are genetically sub-structured at the level of the host plant, strongly isolated and characterized by small effective population sizes. All these results are discussed in the framework of the sustainable management of nematodes populations in cultivated fields.

Keywords: *phylogeography, gene flow, genetic drift, populations of wild pathogens, Heterodera schachtii, Heterodera betae, Beta vulgaris ssp. maritima, control of genetic resistances*

AGROCAMPUS OUEST • Institut supérieur des sciences agronomiques, agroalimentaires, horticoles et du paysage
65 rue de Saint-Brieuc – CS84215 – F-35042 Rennes Cedex
Tél. : 02 23 48 50 00
www.agrocampus-ouest.fr