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Chapitre 1 Introduction générale

I. Structure génétique et mesures de conservation

Une étape cruciale du développement et de la mise en application de mesures de conservation est la connaissance de la structure de la population grâce à l'analyse de données génétiques. En effet, les résultats des analyses génétiques sont de plus en plus souvent utilisés pour identifier et développer des mesures de gestion et de conservation (ex. Moritz 1994b; Waples 1995; Crandall *et al.* 2000; Allendorf *et al.* 2004; Palsbøll *et al.* 2007; Schwartz *et al.* 2007).

Le concept des unités évolutives importantes (UÉI ; *evolutionarily significant units* : *ESU*) a été utilisé pour la première fois par Ryder (1986) pour faciliter l'identification des populations possédant des caractéristiques importantes qui méritaient d'être préservées pour les générations présentes et futures de l'espèce. Depuis, le concept des UÉI a fait l'objet d'intenses discussions et différentes définitions ont été proposées (voir Fraser & Bernatchez 2001 pour une revue). Malgré ces divergences, ce concept est maintenant largement utilisé pour la conservation des populations sauvages et captives.

Considérant que le concept de UÉI est principalement pertinent pour les questions de conservation à long terme, Mortiz (1994b) a définit le concept des unités de gestion (*Management Units*, MUs). Ce concept se rapproche de la définition de stock et est plus orienté vers des objectifs de conservation et de gestion à court terme (voir Palsbøll *et al.* 2007 pour une revue). Ces unités de gestion constituent un niveau d'unité de conservation inférieur à celui des UÉI ; une UÉI pouvant comprendre plusieurs MU. À la différence des

UÉIs, les MUs ne représentent pas des groupes ou populations isolées du point de vue de la reproduction, mais décrivent des groupes suffisamment différenciés pour être gérés de façon indépendante (Taylor & Dizon 1999). Ainsi, les MUs semblent être un outil plus pratique, bien que moins précis, pour mettre en place des mesures de conservation pour des groupes sympatriques d'une même population (Moritz 1994a).

II. Distribution du phoque commun

Le phoque commun (*Phoca vitulina*) est un mammifère marin de l'ordre des carnivores, du sous-ordre des Pinnipèdes et de la famille des Phocidae (les « vrais » phoques). Comme son nom l'indique, le phoque commun a la distribution la plus étendue des espèces de phoques (Goodman 1998) et sa distribution est mondiale (Stanley *et al.* 1996; Figure 1). Le phoque commun est en effet retrouvé dans le nord des océans Pacifique et Atlantique (Goodman 1998). Cinq sous-espèces ont été décrites, associées à des aires géographiques distinctes, et donc isolées au niveau de la reproduction (Kappe *et al.* 1997). Au niveau de l'océan Pacifique, la sous-espèce *Phoca vitulina richardsii* fréquente la côte ouest américaine, de l'Alaska au Mexique, et la sous-espèce *P. v. stejnegeri* se rencontre le long de la côte ouest du Pacifique, du détroit de Béring jusqu'au Japon (Westlake & O'Corry-Crowe 2002). La sous-espèce *P. v. vitulina* fréquente les côtes européennes, de la Russie jusqu'au nord du Portugal, en passant par la mer Baltique, la mer du Nord, la Manche et les côtes de l'Islande (Stanley *et al.* 1996; Goodman 1998). La sous-espèce *P. v. concolor* est présente le long de la côte est du Canada et des États-Unis. Dans l'Est canadien, le phoque commun fréquente l'estuaire et le golfe du Saint-Laurent dont l'île d'Anticosti, les Îles de la Madeleine et le sud du Golfe. Il fréquente également les côtes de la Nouvelle-Écosse, de

Terre-Neuve et du Labrador. On le retrouve dans la baie d'Hudson et la mer de Baffin (Boulva & McLaren 1979; Stanley *et al.* 1996; Lesage *et al.* 2004). Dans l'est des États-Unis, le phoque commun se retrouve le long des côtes du Maine et du New Jersey. *Phoca v. mellonae* est confinée aux Lacs des Loups Marins, au Québec (Smith *et al.* 1996).

Une analyse phylogénétique à grande échelle de la séquence de la région de contrôle de l'ADN mitochondrial a montré que les quatre sous-espèces de phoque commun des océans Atlantique et Pacifique, *P. v. vitulina*, *P. v. concolor*, *P. v. richardsii* et *P. v. stejnegeri* étaient génétiquement différenciées (Stanley *et al.* 1996). La cinquième sous-espèce de phoque commun, *P. v. mellonae*, dont la distribution est restreinte aux Lacs des Loups Marins, a été mise en évidence du point de vue génétique en comparant les séquences de la région contrôle de l'ADN mitochondrial avec celles d'individus de la sous-espèce *P. v. concolor* (Smith 1999).

III. Structure de populations du phoque commun (*Phoca vitulina*)

Le phoque commun est un animal de petite taille (<100kg) qui vit en colonies discontinues le long des côtes des océans Atlantique et Pacifique. L'espèce est considérée comme étant relativement sédentaire même si certains individus, adultes principalement, peuvent effectuer des migrations saisonnières assez importantes (Boulva & McLaren 1979; Lesage *et al.* 2004). Plusieurs auteurs ont étudié la structure de populations du phoque commun à l'aide d'outils moléculaires. Certains travaux ont montré l'existence d'une structure de

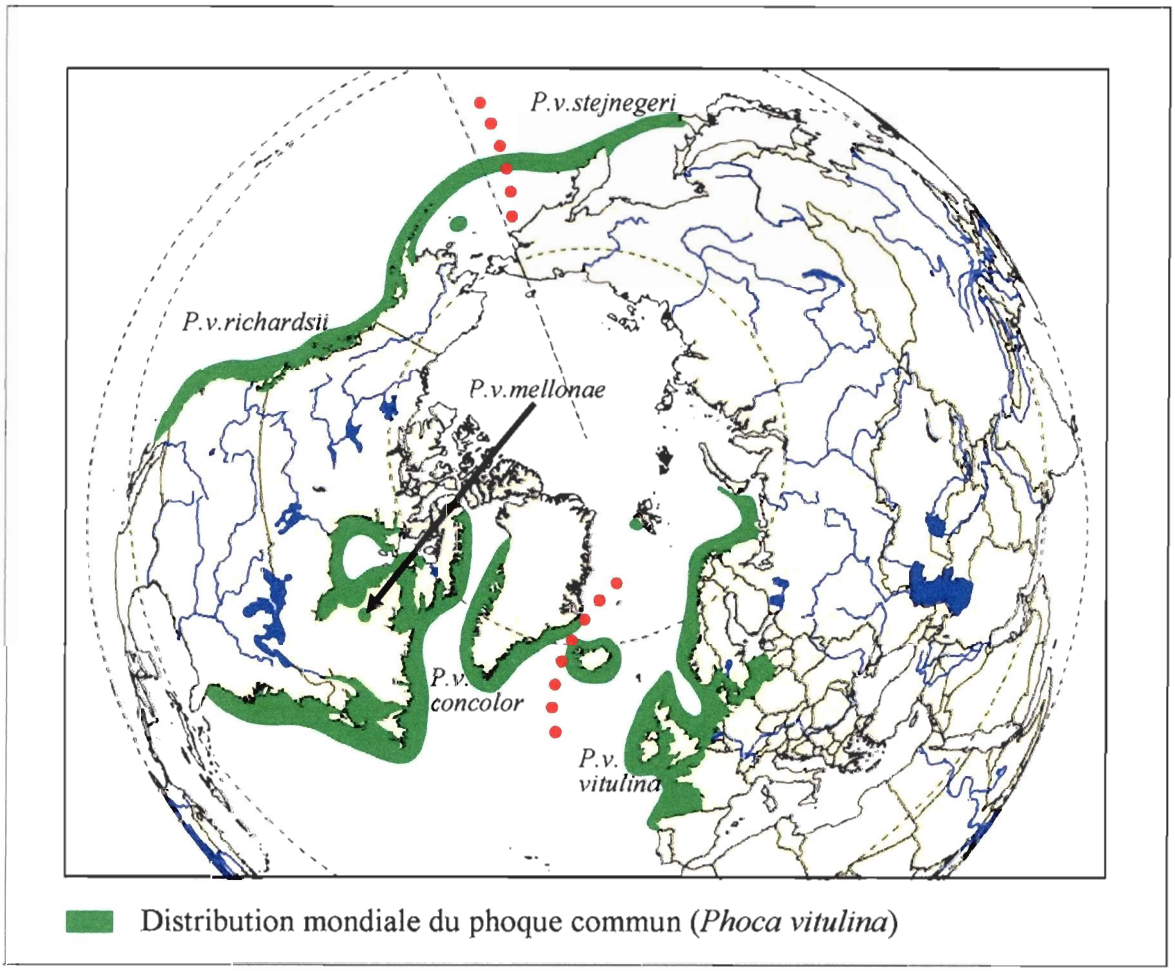

Figure 1. Carte de la distribution mondiale du phoque commun (*Phoca vitulina*) en vue polaire (modifiée de Riede 2001). La distribution des cinq sous-espèces (*P. v. richardsii*, *P. v. stejnegeri*, *P. v. vitulina*, *P. v. concolor* et *P. v. mellonae*) est géographiquement délimitée et indiquée sur la carte.

populations à l'échelle de quelques centaines de kilomètres. Ainsi, six populations (Islande, Écosse-Irlande, côte est de l'Angleterre, mer des Wadden, côte ouest de la Scandinavie et la partie est de la mer Baltique) ont été révélées à l'aide des marqueurs microsatellites chez la sous-espèce *P. v. vitulina* au niveau des côtes européennes (Goodman 1998). Dans l'océan Pacifique, la structure des populations du phoque commun a été étudiée dans différentes régions : au nord, pour les sous-espèces *P. v. richardsii* et *P. v. stejnegeri*, un schéma d'isolement par la distance au sein de l'espèce et la possibilité d'un effet fondateur ont été décelés avec l'étude de l'ADN mitochondrial (Westlake & O'Corry-Crowe 2002). Pour la sous-espèce *P. v. richardsii*, deux populations ont été mises en évidence dans le nord-est de l'océan Pacifique (sud de la Colombie Britannique et nord de la Colombie Britannique et Alaska) à l'aide de l'ADN mitochondrial et des microsatellites (Burg *et al.* 1999). Au niveau de la côte ouest des États-Unis, l'étude de l'ADN mitochondrial a montré que le flux de gènes est limité entre les populations des côtes de l'état de Washington, de l'Oregon et de la Californie (Lamont *et al.* 1996).

Chez le phoque commun, les analyses de l'ADN mitochondrial révèlent une plus grande structure des populations que les microsatellites, laissant supposer un plus fort taux de migration chez les mâles que chez les femelles (Stanley *et al.* 1996; Burg *et al.* 1999; Coltman *et al.* 2002). La philopatrie (du grec *philos* : aimer et du latin *patria* : pays du père) fait référence à l'instinct ou à la tendance d'un individu de revenir à, ou de rester dans, sa zone de naissance. À l'échelle mondiale, une certaine philopatrie régionale des femelles a été mise en évidence (Stanley *et al.* 1996). Bien que la philopatrie des phoques communs au niveau de l'Île de Sable (Nouvelle-Écosse) n'ait pas été mise en évidence,

l'analyse des empreintes génétiques a montré une forte fidélité des femelles pour les colonies de reproduction (Schaeff *et al.* 1999).

IV. Le phoque commun dans l'Atlantique nord-ouest

IV.1 Cadre du projet et Zone de Protection Marine

L'effectif de la population de l'estuaire et du golfe du Saint-Laurent ne semble pas avoir augmenté depuis l'arrêt de la chasse au phoque commun à la fin des années 1970 (Bernt *et al.* 1999). De plus, l'information pour déterminer le statut de la sous-espèce *P. v. concolor* sur la liste des espèces du Comité sur la Situation des Espèces en Péril au Canada (COSEPAC) est jugée insuffisante (« données insuffisantes ») (COSEPAC, 2004). Une mesure envisagée pour assurer la protection du phoque commun de l'Atlantique nord-ouest et des autres mammifères marins est la création d'une Zone de Protection Marine (Z.P.M.) en bordure du Parc Marin Saguenay – Saint-Laurent. Le projet de Z.P.M. a pour but d'assurer, dans l'estuaire du Saint-Laurent, la conservation et la protection à long terme des mammifères marins qui y vivent à l'année ou y transitent, de leurs habitats et de leurs ressources alimentaires. L'étude de la structure génétique des populations du phoque commun de l'Atlantique nord-ouest, *Phoca vitulina concolor*, est donc devenue nécessaire pour envisager la mise en place de mesures de conservation efficaces dans l'estuaire et le golfe du Saint-Laurent.

IV.2 Structure des populations de la sous-espèce *Phoca vitulina concolor*

La structure génétique des populations de la sous-espèce *P. v. concolor* n'a pas été étudiée à l'échelle de l'Atlantique nord-ouest. Il existe cependant des indices qui suggèrent l'existence d'une structure des populations à cette échelle.

L'analyse des polluants organiques persistants (POP) a permis de discriminer les populations de phoques communs de l'estuaire et du golfe du Saint-Laurent et de Terre-Neuve (Lebeuf *et al.* 2003). Les auteurs ont retracé la répartition des phoques sur les trois sites d'étude avec une précision de 90% suivant le gradient de concentration des POP de l'estuaire du Saint-Laurent vers Terre-Neuve.

L'analyse spatiale par suivi satellite et radio-télémétrie de phoques communs de l'estuaire du Saint-Laurent a montré des migrations saisonnières de 65 à 520 km liées à la couverture de glace hivernale de la zone d'étude (Lesage *et al.* 2004). Les patrons de déplacements ont montré que les individus de la sous-espèce *P. v. concolor* effectuent de courts déplacements le long des côtes et qu'une masse d'eau profonde telle que le chenal Laurentien représente une barrière physique (Lesage *et al.* 2004).

Des différences morphologiques, telles que la configuration du pelage et le nombre de dents postcanines, ont été observées entre les phoques communs de l'Île de Sable et ceux du continent (Boulva & McLaren 1979).

Des analyses de paternité de phoques communs de l'Île de Sable ont été réalisées grâce aux marqueurs microsatellites (Coltman *et al.* 1998; 1999). L'étude de la diversité génétique de la petite population de l'Île de Sable a montré un faible degré de variabilité génétique. Un

effet de consanguinité lié à l'isolement géographique de la population expliquerait la faible variabilité génétique (Coltman *et al.* 1998).

La structure mondiale du phoque commun a été étudiée à partir d'échantillons des quatre sous-espèces marines de *Phoca vitulina* : 19 individus de *P. v. concolor* (11 originaires de l'Île de Sable, 7 de Saint-Pierre et Miquelon et 1 de la baie d'Hudson), 38 *P. v. richardsii*, 12 *P. v. stejnegeri* et 158 *P. v. vitulina* (Stanley *et al.* 1996). Cette étude à grande échelle ne comprend aucun individu provenant de l'estuaire ou du golfe du Saint-Laurent. Lors de cette étude, seulement cinq haplotypes ont été observés. Cependant, des différences ont été observées entre les individus de l'Île de Sable, de Saint-Pierre et Miquelon et de la baie d'Hudson.

Pour discriminer la sous-espèce lacustre, *P. v. mellonae*, les séquences de la région contrôle de l'ADN mitochondrial de 6 individus ont été comparées à celles d'un échantillon de 11 individus de la sous-espèce *P. v. concolor* provenant du Groenland (2 individus), des côtes du Massachusetts (4 individus), de la baie de Fundy (1 individu), des côtes de la Nouvelle Écosse (1 individu), du New Jersey (1 individu), de Gaspé (1 individu) et de l'estuaire du Saint-Laurent (1 individu) (Smith 1999). Les séquences de la sous-espèce *P. v. mellonae* semblent plus apparentées à celles de *P. v. concolor* comparativement à celles des autres sous-espèces. Cependant, *P. v. mellonae* présente un certain degré de différenciation et l'arbre des distances génétiques, malgré une faible solidité des branches, montre une certaine structure pour la sous-espèce *P. v. concolor*.

IV.3 Utilisation des marqueurs moléculaires

Dans la présente étude, deux types de marqueurs moléculaires, les microsatellites et l'ADN mitochondrial, ont été utilisés. Ces deux types de marqueurs sont couramment utilisés en génétique des populations (ex. Sunnucks 2000; Avise 2004). Les marqueurs microsatellites sont de courtes séquences d'ADN hypervariables et répétées en tandem. Ils sont localisés sur l'ADN nucléaire et leur transmission est bi-parentale. L'ADN mitochondrial est un génome haploide qui est généralement d'héritabilité maternelle. Ce mode de transmission a pour effet de réduire la taille efficace de la population à la moitié de celle des marqueurs autosomaux. La différenciation entre les populations pourrait donc être plus rapidement détectable avec ce type de marqueurs. De plus, l'analyse conjointe de ces deux marqueurs permet de comparer l'influence tant des mâles que des femelles sur la structure des populations, et donne la possibilité de mettre en évidence une éventuelle philopatrie.

V. Objectifs

Cette étude a pour objectif de décrire la phylogéographie du phoque commun, appartenant à la sous-espèce *P. v. concolor* dans l'Atlantique nord-ouest. Au cours de cette étude, l'hypothèse nulle de la panmixie sera testée : il n'existe pas de populations génétiquement différenciées à l'échelle de l'Atlantique nord-ouest chez la sous-espèce *P. v. concolor*.

Chapitre II Population structure of the harbour seal (*Phoca vitulina concolor*) in the Northwest Atlantic based on microsatellite and mitochondrial DNA analyses

1. Abstract

The population genetic structure of the harbour seal (*Phoca vitulina concolor*) was studied at 13 sites of the Northwest Atlantic using 10 microsatellite markers and sequencing a 505-bp fragment of the mitochondrial DNA control region. Microsatellite data analyses show that Hudson Bay and Sable Island samples belong to two differentiated populations that are homogenous while all other samples belong to a third heterogeneous group. Mitochondrial DNA sequence analyses generally support the structure revealed by microsatellite analyses. Indeed, all haplotypes observed in Hudson Bay and 3 out of 4 haplotypes at Sable Island are private. Furthermore, mitochondrial DNA analyses reveal a star-like phylogeny in which the Hudson Bay haplotypes are represented on separated branches. Such a star-like phylogeny indicates a rapid expansion in recent evolutionary times. Our results suggest the existence of three population, which may represent Evolutionarily Significant Units in the Northwest Atlantic: (1) Hudson Bay; (2) Sable Island; and (3) Estuary and Gulf of St. Lawrence and Atlantic coast. Our results are in agreement with those of Stanley *et al.* (1996) suggesting that the colonization took place from the Atlantic to Pacific oceans and that the Northwest Atlantic subspecies, *Phoca vitulina concolor* occupies a central position.

II. Introduction

The harbour seal, *Phoca vitulina*, is the most widely-distributed Pinniped (Mansfield 1967). This species is present in temperate, sub-arctic and arctic areas on both sides of the North Atlantic and the North Pacific oceans. Five subspecies have been identified (*P. v. richardsii*, *P. v. stejnegeri*, *P. v. vitulina*, *P. v. concolor*, and *P. v. mellonae*), each of them confined to a specific geographical area (Doutt 1942; Scheffer 1958; McLaren 1966; Shaughnessy & Fay 1977; Boulva & McLaren 1979; Smith *et al.* 1994; Stanley *et al.* 1996).

In the Northwest Atlantic, the subspecies *Phoca vitulina concolor* is present on the east coast of Canada and United States. It is present in Hudson Bay and Greenland and along the coasts of Labrador, Newfoundland, Nova Scotia and Sable Island. It is also present in the Gulf and Estuary of the St. Lawrence including Anticosti Island, Magdalen Islands and the southern Gulf. In United States, the species is present along the coast of Maine to New Jersey (Mansfield 1967; Boulva & McLaren 1979; Stanley *et al.* 1996; Baird 2001; Lesage *et al.* 2004; Robillard *et al.* 2005).

The harbour seal is a relatively sedentary animal, occurring in discrete colonies dispersed along the coast (Boulva & McLaren 1979). In the Estuary and Gulf of St. Lawrence, the animals remained near the coast in shallow water areas and travelled only short distances from capture sites (Lesage *et al.* 2004). Strong site fidelity has been observed for the different subspecies in the Northeast Atlantic (Stanley *et al.* 1996; Goodman 1998; Härkönen & Harding 2001) and in the Northeast Pacific (Burg *et al.* 1999; Baird 2001) for both males and females. Such behaviour is favouring population differentiation.

Harbour seal population genetic structuring has been observed for the East and West Pacific subspecies (Lamont *et al.* 1996; Burg *et al.* 1999; Westlake & O'Corry-Crowe 2002) as well as for the East Atlantic (Stanley *et al.* 1996; Goodman 1998; Burg *et al.* 1999). Although population genetic structure of the harbour seal has not been studied at the Northwest Atlantic scale, there are indications that population differentiation exists in the area. In a study on the relationship between fitness-related traits and heterozygosity at microsatellite loci, Coltman *et al.* (1998) detected inbreeding-like effects in harbour seal pups from Sable Island, which may have been caused by the relatively small size of this population as well as by its isolation from the continent. Harbour seals from Sable Island are also characterized by different morphological characters such as pelage characteristics and the number of post-canine teeth (Boulva & McLaren 1979). Furthermore, harbour seals from the St. Lawrence Estuary (Bic), southern Gulf of St. Lawrence (Charlottetown) and southern Newfoundland (Placentia Bay and surrounding south coast) have been classified into distinct colonies on the basis of their patterns of persistent organic pollutants (Lebeuf *et al.* 2003).

Despite what the species name implies, the demographic status of some harbour seal populations is currently under examination in the Northwest Atlantic. For example, the census population size of colonies located in the St. Lawrence Estuary is low and may be decreasing despite protection from hunting since the end of 1970s (Robillard *et al.* 2005). These trends have led the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) to request information on this species and a marine protected area is planned in the St. Lawrence Estuary to protected marine mammals in the area. In this context,

obtaining information on the population genetic structure of the species represents an important step toward the development and implementation of conservation measures. Indeed, obtaining genetic data is of critical importance for the development of conservation and management strategies (Palsbøll *et al.* 2007; Schwartz *et al.* 2007). Genetic data are also important to define Evolutionarily Significant Units (ESU), a concept that was first used by Ryder (1986) to facilitate the identification of population possessing characteristics worth preserving for the present and the future generations of the species. The concept is oriented towards long-term (evolutionary) conservation and it is different from the Management Unit (MU) which is central to the short-term management and conservation of natural populations (Palsbøll *et al.* 2007). Although the concept has been the object of intense discussion and defined in several ways (see Fraser & Bernatchez for a review 2001) it is now largely applied for the conservation of wild and captive populations.

The objective of the present study is to describe harbour seal population genetic structure at the scale of the Northwest Atlantic using microsatellite and mitochondrial genetic markers. Both markers have their own advantages. Microsatellites are highly polymorphic nuclear markers allowing the detection of subtle genetic difference between populations. With these markers, the population structure is determined by both male and female gene flow. Mitochondrial DNA (mtDNA) is also a powerful marker. High genetic differentiation for mtDNA is expected from the fourfold smaller effective population size of this marker associated with maternally mode of transmission. Differences in differentiation between the two markers may indicate a sex-biased dispersal pattern (Bowen *et al.* 1992; Degnan 1993; Palumbi & Baker 1994).

III. Materials and Methods

III.1 Sample collection and DNA extraction

Samples of skin and muscle were collected between 1988 and 2004 on 259 individuals of the Northwest Atlantic harbour seal, *Phoca vitulina concolor*, from 13 locations (Table 1, Figure 2). Samples from 31 individuals belonging to the European subspecies (*Phoca vitulina vitulina*) from the Skagerrak Strait (Sweden) were also obtained. All samples were collected in accordance with local animal care guidelines.

Tissue samples were either stored in 20% dimethylsulfoxide (DMSO) at 4°C or in 100% ethanol at room temperature or at -20°C. Total genomic DNA was extracted from tissue samples using the QIAgen© DNeasy® tissue kit. DNA was eluted with 100 µL of distilled water and the solutions were stored at 4°C until analyses. DNA concentrations were determined on a Nannodrop ND-1000 spectrophotometer.

III.2 Microsatellite amplification and allele detection

Ten microsatellite loci were analysed in the present study. Two of them, Pvc 19 and Pvc 78 were originally designed for the sub-species *Phoca vitulina concolor* (Coltman *et al.* 1996) and two, SGPV10 and SGPV11, for the subspecies *P. v. vitulina* (Goodman 1997). The other 6 markers, originally developed for other seal species (Table 2) were optimised for *Phoca vitulina concolor*: HG6.1 and HG6.3 from *Halichoerus grypus*, Allen *et al.* 1995; Lw7 and Lw11 from *Leptonychotes weddellii*, Hl16 from *Hydrurga leptonyx* and Lc28

Table 1. Description of the samples used for the harbour seal (*Phoca vitulina concolor*) population genetic study based on the analysis of microsatellites and mitochondrial DNA sequences. The sample from Northeast Atlantic harbour seal (*Phoca vitulina vitulina*) is also included. ND: sex not determined.

Sampling site	Acronym	Microsatellite sample size				mtDNA sample size				Collection dates
		males	females	ND	all	males	females	ND	all	
Bic	BIC	28	20	0	48	24	15	0	39	2001-2002
Métis	MET	14	7	0	21	12	2	0	14	2002
Anticosti Island	ANI	7	9	0	16	7	10	0	17	1994; 2004
Prince Edward Island	PEI	17	12	0	29	13	10	0	23	2001-2004
West Newfoundland	WNF	12	13	0	25	11	9	0	20	2002-2003
Placentia Bay, Newfoundland	PB	9	15	0	24	9	12	0	21	2002
East Newfoundland	ENF	2	2	0	4	2	2	0	4	2002-2003
Cape Breton	CB	0	2	3	5	0	2	3	5	1992
South coast of Nova Scotia	NS	1	5	2	8	1	5	1	7	1992
Bay of Fundy	BF	8	16	0	24	6	11	0	17	1988-1989
Labrador	LAB	2	4	0	6	2	4	0	6	2003
Hudson Bay	HB	10	6	3	19	11	4	2	17	2001-2002
Sable Island	SI	15	14	1	30	15	11	1	27	1989
Total Northwest Atlantic		125	125	9	259	113	97	7	217	
Skagerrak Strait, Sweden	SW	16	15	0	31	16	15	0	31	2002

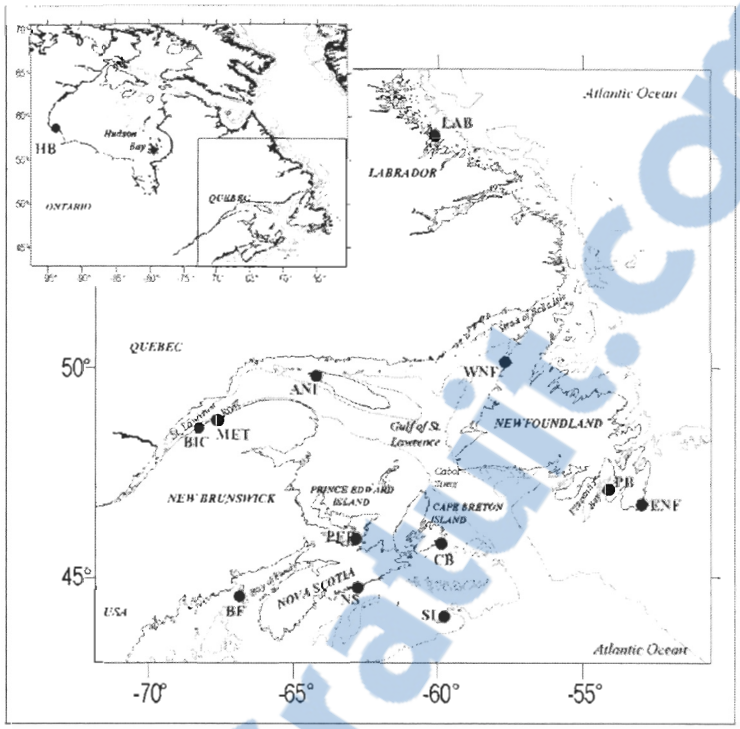

Figure 2. Map of Gulf of St. Lawrence showing the location of the sampling sites. Inset map represents the geographic location of the Gulf of St. Lawrence in the Northwest Atlantic and the sampling site in Hudson Bay. Acronyms are as in Table 1.

from *Lobodon carcinophagus*, Davis *et al.* 2002, These ten microsatellite loci were optimised and analysed with fluorescently labelled primers (Table 2).

The microsatellites were amplified in three multiplex-PCR reactions (MUX). MUX 1: Pvc19, Pvc78, Lw20 and SGPV11; MUX 2: HG6.1, HG6.3 and SGPV10 and MUX 3: Lw11, Hl16 and Lc28. Amplification conditions were the same for the three MUX groups, except for the annealing temperature which was 50°C for MUX 1 and MUX 2 and 52°C for MUX 3. Amplification conditions were the following: initial denaturation at 94°C for 3

min; 30 cycles (30 s. at 94°C, 30 s at 50 - 52°C and 30 s at 72°C) and final extension at 72°C for 3 min. All PCR reactions consisted of 10 to 100 ng of DNA template, 0.5 mM of each dNTP, 0.075 - 0.3 µM of forward and reverse primers, 0.245 Units of Expand™ High Fidelity DNA Polymerase, and 1X Expand™ High Fidelity Buffer #3 with 1.5 mM $MgCl_2$ in a total volume of 10µL. Amplifications were carried out on a Stratagene® Robocycler® 96 temperature cycler equipped with hot top.

Amplified fragments were separated by capillary electrophoreses using an ABI Prism™ 310 automated genetic analyser (PE Applied Biosystems) with a size standard GENESCAN® 400HD [ROX] (PE Applied Biosystems). Data were analysed and scored with 310 GeneScan® 3.1.2 and Genotyper® 2.5 software packages (Applied Biosystems).

III.3 Mitochondrial DNA amplification and sequencing

The primers, L15926 (5'-ACACCAGTCTTGTAAACC-3', Kocher *et al.* 1989 as modified by Rosel *et al.* 1994) and PvH00034 (5'-TACCAAATGCATGACACCACAG-3', Westlake & O'Corry-Crowe 2002 modified from Rosel *et al.* 1994) were used to amplify a 957-bp fragment of the mitochondrial DNA. The target region is located from bp 15903 to 000033 of the harbour seal mtDNA sequence (Árnason and Johnsson 1992) and included part of the cytochrome b gene, the threonine transfer RNA gene, the proline tRNA gene, and over 450-bp of the control region (Annexe 1). Amplification conditions consisted of: an initial denaturation at 90°C for 2 min 30 s, 35 cycles of [denaturation at 94°C for 45 s, annealing at 48°C for 1 min and extension at 72°C for 1 min 30 s] and a final extension at 72°C for 5 min. PCR were carried out in 25-µL cocktail consisting of genomic DNA (20 to 200 ng), 0.15 mM of each dNTP, 0.3 µM of forward and reverse primers, 1.4 Units of

Table 2. Characteristics of ten microsatellites designed from five seal species: *Halichoerus grypus* (HG6.1 and HG6.3), *Hydrurga leptonyx* (Hl16), *Lobodon carcinophagus* (Lc28), *Leptonychotes weddellii* (Lw11 and Lw20), *Phoca vitulina concolor* (Pvc19 and Pvc78) and *Phoca vitulina vitulina* (SGPV10 and SGPV11), used in the present study. The references in which microsatellites were originally described are indicated. Allele sizes and numbers are those obtained for *Phoca vitulina concolor* in the present study.

Locus	*Repeat array in cloned allele*	*Allele size (bp)*	*Allele number*	*Primer sequence (5'-3')*	*Fluorescent 5'label*	*References*
HG6.1	$(CA)_{11}TA(CA)_{10}$	140-154	7	*F*-TGCACCAGAGCCTAAGCAGACTG *R*-CCACCAGCCAGTTCACCCAG	VIC	Allen *et al.* 1995
HG6.3	$(GT)_{16}$	222-238	9	*F*-CAGGGGACCTGAGTGCTTATG *R*-GACCCAGCATCAGAACTCAAG	NED	Allen *et al.* 1995
Hl16	$(GT)_{13}$	140-150	5	*F*-CACTTATCTCGCCCTATATCCA *R*-CAGCCACAGCCAACACAA	VIC	Davis *et al.* 2002
Lc28	$(GT)_{11}$	138-150	7	*F*-TCATATAATACCCACCTCTGTAAG *R*-TGCCTCGTGATGAAAAACT	NED	Davis *et al.*, 2002
Lw11	$(GT)_{26}$	165-177	7	*F*-CTCTCCCTCTCACCTTCC *R*-GGCAAATGAGGTGATGTC	VIC	Davis *et al.* 2002
Lw20	$(GT)_{20}$	127-139	6	*F*-GACTCTTGCCCCCTTCAG *R*-GTTTCACAGACCTGCCTCTAGTG	NED	Davis *et al.* 2002
Pvc19	$(CA)_{15}$	96-104	2	*F*-GGGTGAACAGGATTTATCC *R*-GTGCTAGATAACAATCCTAC	6-FAM	Coltman *et al.* 1996
Pvc78	$(AC)_{15}$	144-148	3	*F*-GAGTATACCTCCATACTACAC *R*-AGTTGTTCTCCTGACCCAAG	VIC	Coltman *et al.* 1996
SGPV10	$(GT)_{11}$	127-137	5	*F*-TTCACTTAGCATAATTCCCTC *R*-TCATGAATTGGTATTAGACAAAG	NED	Goodman 1997
SGPV11	$(CA)_{20}$	140-166	10	*F*-GTGCTGGTGAATTAGCCCATTATAAG *R*-CAGAGTAAGCACCCAAGGAGCAG	6-FAM	Goodman 1997

ExpandTM High Fidelity DNA Polymerase, 1X ExpandTM High Fidelity Buffer #3, and 2 mM of MgCl2. The PCR was performed using a Perkin Elmer DNA Thermal Cycler 480. The 957-bp amplified fragments were purified using the QIAGEN® QIAQuick® PCR Purification Kit (250). Purified products were eluted in 70µL of sterilized distilled water. This fragment was used as a template to sequence in both directions a 505-bp fragment (part of the tRNA proline gene and over 450-bp of the control region) using the internal

primers L15829 (5'-CCTCCCTAAGACTCAAGG-3', Westlake & O'Corry-Crowe, 2002) and PvH00002 (5'-GGGCTGATTAGTCATTAG-3'). The 957-bp was first sequenced in one direction for all specimens using primer L15829. Since there was no variable site from the positions 16790 to 00030 (Árnason & Johnsson, 1992, Annexe 1) we designed the primer PvH00002 which was used to sequence the specimens in the other direction. Sequencing reaction consisted of 300ng of purified PCR product, 0.1μM primer and 4μL of BigDye® (Applied Biosystems BigDye® Terminator v3.1 Cycle Sequencing Kit) in a total reaction volume of 20μL. PCR reaction was carried out in a Perkin Elmer Thermal Cycler 480 using the following conditions: initial denaturation at 96°C for 4 min, 25 cycles of [denaturation at 96°C for 10 s, annealing at 48 or 50°C for 5 s and extension at 60°C for 4 min].

The electrophoretic separation of the sequencing reaction product was carried out using an ABI PrismTM 310 automated genetic analyser (PE Applied Biosystems). Base calling was done using the DNA Sequencing Analysis Software v.3.4.5 (Applied Biosystems). Sequences were edited using SequencherTM 4.1 (Gene Codes Corporation).

The 505-bp sequences were aligned using CLUSTAL X 1.83 (Thompson *et al.* 1997). Alignment gaps were checked visually. A total of 217 sequences were analysed on the samples from 13 locations in the Northwest Atlantic and 31 sequences on one location in the Northeast Atlantic (Table 1).

III.4 Statistical analysis

III.4.1 Microsatellite variability

Tests for independence of loci (linkage) were conducted using the software GENEPOP 3.4 (Raymond and Rousset 1995). The exact *p*-values were estimated by the Markov chain method using the following parameters: dememorization=10 000; batches=100; iterations per batch=5 000. Significance was evaluated after sequential Bonferroni correction for multiple tests (Rice 1989).

Genetic characteristics of each sample were described by calculating the number of alleles, allelic frequencies, allelic diversity, observed and non biased expected heterozygosity using the software GENETIX 4.05 (Belkhir *et al.* 1996-2004). Allelic richness (El Mousadik & Petit 1996), which takes into account variation in sample size by a rarefaction method, was first calculated for the Northwest Atlantic samples excluding the sample from Sweden using FSTAT v.2.9.3 software (Goudet 1995). When all samples from the Northwest Atlantic were included, the allelic richness calculation was based on the minimum sample size of 3 diploid individuals due to the small size of the East Newfoundland sample ($N = 4$) (Table 1). Therefore, we repeated the calculation excluding the sites East Newfoundland, Cape Breton, Nova Scotia and Labrador where the sample sizes were the smallest (Table 1). Consequently, the allelic richness calculation was based on minimum sample sizes of 14 diploid individuals.

Tests for deviations from Hardy-Weinberg equilibrium for each locus were conducted using the Markov chain Monte Carlo method (Guo & Thompson 1992) implemented in

GENEPOP 3.4. (Raymond & Rousset 1995). The exact *p*-values were estimated by the Markov chain method with the following parameters: dememorization=10 000; batches=100; iterations per batch=5 000. Both heterozygote deficit and excess were tested. Hardy-Weinberg global tests based on 10 000 randomisations of alleles first within samples and second overall samples were performed using FSTAT v.2.9.3 software (Goudet 1995). The inbreeding index F_{IS} and associated *p*-values were calculated using GENEPOP 3.4 (Raymond & Rousset 1995).

III.4.2 Mitochondrial DNA variability

Genetic diversity of mtDNA haplotypes was described by calculating the haplotype diversity (*h*), which is equivalent to heterozygosity of a nuclear locus, and the nucleotide diversity (π), which is the average number of nucleotide differences per site between two sequences (Nei 1987). Haplotype diversity and the nucleotide diversity were estimated using Arlequin ver. 3.0 software package (Excoffier *et al.* 2005).

III.5 Population genetic structure

We used different approaches to investigate the population genetic structure of harbour seal in the Northwest Atlantic. First, for microsatellite data, genetic differentiation among all samples and between pairs of samples was estimated by calculating the F_{ST} estimator θ (theta) (Weir & Cockerham 1984). These tests were carried out using the software FSTAT (Goudet 1995). The global F_{ST} value was calculated using GENEPOP 3.4 (Raymond & Rousset 1995). For the mitochondrial DNA data, population differentiation was studied using the statistics Φ_{ST}, an estimate of genetic divergence occurring between samples which

incorporates haplotype frequencies and evolutionary distance between haplotypes. The Tamura and Nei model of evolution for mtDNA control region was used (Tamura & Nei 1993). The Φ_{ST} estimates were calculated using the ARLEQUIN version 3.0 software package (Excoffier *et al.* 2005).

Second, the Bayesian clustering method developed by Pritchard *et al.* (2000) was used with microsatellite data to infer population structure. This approach differs significantly from the analytical methods described above as individuals are assigned to clusters (populations) based on their individual genotypes exclusively. With this method, individual genotypes are assigned to clusters on a probabilistic basis in such a way as to minimize Hardy-Weinberg and linkage disequilibrium within groups. The software STRUCTURE v. 2.1 (Pritchard *et al.* 2000) was used with admixture and correlated allele frequency models (Falush *et al.* 2003). The number of presumed clusters (K) was set from 2 to 13 in order to test potential structure in a minimum of two groups to a maximum of 13 groups corresponding to the total number of samples. Five simulations were carried out for each K value and each run consisted of a burn-in period of 10^5 steps followed by 10^5 Markov chain Monte Carlo (MCMC) repetitions. The most probable number of clusters was estimated on the basis of the two ad hoc quantities, $L(K)$ (Pritchard et al. 2000) and ΔK (Evanno *et al.* 2005). The analyses with STRUCTURE were repeated with samples from the Estuary and Gulf of St. Lawrence excluding the samples from Sable Island and Hudson Bay in order to test for possible sub-structuring in this group. Individual admixture proportions, which is the part of individual genome that is derived from different clusters were also estimated with the software STRUCTURE v. 2.1.

Third, an Analysis of Molecular Variance (AMOVA) (Excoffier *et al.* 1992) was carried out on mitochondrial and microsatellite data using the ARLEQUIN version 3.0 software package (Excoffier *et al.* 2005). For mitochondrial DNA, the Tamura and Nei (1993) measure of genetic distance with a gamma correction of $\alpha = 0.50$ (Stanley *et al.* 1996) was used. The genetic variance components and the hierarchical *F*-statistic analogues were calculated and tested for significance using 10 000 permutations (Excoffier *et al.* 1992). The same AMOVA framework, which was based on the results obtained with the Bayesian clustering method of Pritchard et al. (2000), was applied to both microsatellite and mitochondrial DNA markers.

Fourth, global genetic relations between samples were displayed by drawing phylogenetic trees. For microsatellite data, a neighbour-joining tree based on Cavalli-Sforza and Edwards (1967) chord distance was build using the PHYLIP v.3.2 software (Felsenstein 1989). Support of the nodes was estimated with 1 000 bootstrap replicates. For mitochondrial DNA, the relationships among haplotypes were described using minimum spanning trees that were built using TCS v. 1.21 software (Clement *et al.* 2000). The genetic distances between haplotypes were computed assuming a gamma distribution of substitution rate across nucleotide sites (Tamura & Nei 1993), with a gamma distance parameter value of 0.5. Statistical significance was tested with 10 000 permutations.

Microsatellite and mitochondrial DNA data were analysed together using PHYLIP v.3.2 software (Felsenstein 1989) to provide a global vision of the population structure, using the approach described by Postma *et al.* (2005). For these analyses, a neighbour-joining tree was built using allelic frequencies of microsatellite data and mitochondrial DNA sequences

as an haploid locus (Postma *et al.* 2005). The original data set was resampled to generate 1 000 data sets using the SEQBOOT option of PHYLIP v.3.2 software. Cavalli-Sforza and Edwards Chord distances (Cavalli-Sforza & Edwards 1967) were calculated using the GENDIST option. Neighbour-joining trees were built for each data set using the NEIGHBOR option. The CONSENSE option was employ to generate a consensus tree. The consensus tree was drawn using the DRAWTREE option.

III.6 Mantel test of isolation by distance

Mantel tests (Mantel 1967) between pairwise Φst, calculated from microsatellite and mitochondrial DNA and pairwise geographical distances were used to test for isolation by distance with the software GENEPOP 3.4 (Raymond & Rousset 1995) with 10 000 permutations. Geographic distances between sampling sites were evaluated along the coast using Fugawi software (Northport Systems Inc. 2000-2002). Mantel tests were carried out using all samples from the Northwest Atlantic and using a selected numbers of sites that could represent migratory routes along the south shore of the Estuary and the Gulf of St. Lawrence, and Cape Breton, south coast of Nova Scotia and Bay of Fundy.

III.7 Sex-biased migration test

In order to detect sex-biased migration, we compared the genetic variation of biparentally inherited nuclear genetic markers (microsatellites) and matrilineally inherited mtDNA genetic markers. The genetic variance estimates from microsatellites (θ, theta) were compared to the Φ_{ST} estimate obtained from mitochondrial DNA analyses. The rationale behind the test is that if one sex is more prone to disperse over the other, we should observe

differences in the genetic variance estimates for the two genomes. We should however expect to see a mtDNA Φ_{ST} estimate four times that of the *microsatellite* θ, simply because the effective population size for the mtDNA is a quarter of the nuclear markers (Avise 2004). A theoretical equation, $\theta_{mito}=4\ \theta_{microsat}/(1+3\ \theta_{mito})$ (Crochet 2000), describes the case of no bias in the dispersal (Hoarau *et al.* 2004). When observed data are above the theoretical relationship, the dispersion of males is more important than the females dispersal. When observed data are located below the theoretical relationship, the dispersal of females is more important than males' dispersal. In this study, the theoretical equation used F_{ST} estimates: Φ_{ST}(mtDNA) = 4 θ (microsatellites) / [1+ 3 Φ_{ST} (mtDNA)].

III. 8 Phylogeography of the four harbour seal subspecies

In their study, Stanley *et al.* (1996) analysed 227 harbour seals from the four subspecies, mainly from the Northeast Atlantic subspecies, (*P. v. vitulina*, 158 individuals). The Northwest Atlantic harbour seal (*P. v. concolor*) sample comprised 19 individuals from Sable Island (11 individuals), Miquelon Island (7 individuals), and Hudson Bay (one individual from Churchill).

Mitochondrial DNA sequences from Stanley *et al.* (1996), which were available on GENEBANK (Accession number from U36342 to U36375), were used with those obtained in the present study to add information to the worldwide patterns of mitochondrial DNA differentiation of the harbour seal.

A total of 452-bp from the 505-bp of the sequences from the present study were aligned with the Stanley *et al.* (1996) 452-bp sequences using CLUSTAL X 1.83 (Thompson *et al.*

1997). The relationships among haplotypes were described using minimum spanning trees that were built using TCS v. 1.21 software (Clement *et al.* 2000).

IV. Results

IV.1 Statistical analysis

Linkage Disequilibrium

A total of 630 tests were carried out on microsatellite loci for each sample location. There were 25 combinations of pairs of loci (4%) that were significant for $\alpha = 0.05$ (Table 3). However, there was no significant linkage disequilibrium between any pair of loci after sequential Bonferroni correction for multiple tests (Rice, 1989).

IV.1.1 Microsatellite variability

The microsatellite markers used in this study show moderate levels of polymorphism. The descriptive statistics regarding the genetic variability of the 13 Northwest and of the Northeast Atlantic samples are presented in Table 4 and in Annexe 2. Sixty (60) alleles were detected across all loci in the Northwest Atlantic (61 alleles when including the Northeast Atlantic sample). Only 8 private alleles were observed across the Northwest Atlantic samples: 3 were observed at the site Bic, 2 at Anticosti Island and one in each sample collected along the Labrador coast, in the Bay of Fundy and Sable Island (Annexe 2). The sample from Hudson Bay shows the lowest value of allelic richness, which varies

Table 3. Summary of the results of the linkage disequilibrium tests performed between pairs of microsatellite loci. Significant *p*-values before sequential Bonferroni correction for multiple tests are shown.

Sampling site	Pair of loci	*p*-value
MET	HG6.3 – Lw11	0.00620
	Lw20 – Lc28	0.02084
NS	Lw20 – SGPV11	0.01206
	SGPV11 – Hl16	0.03545
PEI	Lw20 – SGPV11	0.01799
	Pvc19 – Lc28	0.03684
	Pvc19 –SGPV10	0.02542
WNF	Lw20 – HG6.1	0.03900
	Pvc19 –Hl16	0.02005
PB	Lw20 – Hl16	0.02484
	Lw20 – SGPV11	0.04334
	Pvc19 – Pvc78	0.03711
	SGPV11 - HG6.3	0.00109
SI	Hl16 – Lc28	0.01327
	Lw20 – Lc28	0.04122
	Pvc19 –Lw11	0.00332
HB	Lw20 – Pvc78	0.02792
	Lw20 –HG6.3	0.00693
	Pvc19 – Lc28	0.03233
SW	HG6.1 - HG6.3	0.04666
	Lw20 – Pvc19	0.01773
	Pvc19 - HG6.1	0.00336
	Pvc78 - Hl16	0.03303
	Pvc78 – SGPV11	0.03360
	SGPV11 – Hl16	0.03281

from 1.90 for Hudson Bay to 2.51 for Cape Breton when all sites are considered. As mentioned previously, the sample size varies from 4 individuals in East Newfoundland sample to 48 in Bic. When only the samples with larger sample size (≥10) were considered, R_S (≥10 in Table 4 and Annexe 2) varies from 2.60 for Hudson Bay to 3.81 for Bic. The lowest values for observed and expected heterozygosity were also observed in Sable Island sample. Indeed, observed heterozygosity (H_o) ranges from 0.355 for Sable Island to 0.522 for Cape Breton while expected heterozygosity ($H_{n.b.}$) varies from 0.366 for Sable Island to 0.539 for Bay of Fundy.

The exact tests of heterozygote excess and deficit did not reveal significant deviation from Hardy-Weinberg expectations for the 140 combinations tested (α = 0.05, Bonferroni sequential correction applied). The global tests reveal significant heterozygote deficit for Bic (F_{IS} = 0.097, *p*-value = 0.001) and for Bay of Fundy (F_{IS} = 0.094, *p*-value = 0.030) (Table 4; Annexe 2).

Table 4. Summary of the genetic characteristics of 13 *Phoca vitulina concolor* samples from the Northwest Atlantic (NWA) and one *Phoca vitulina vitulina* sample from the Northeast Atlantic (SW) surveyed at 10 microsatellite loci : number of individuals scored (N), allelic diversity (N_{all}), non biased expected heterozygosity ($H_{n.b.}$), observed heterozygosity (H_o), allelic richness based on all samples [R_S (all)] and based on sample with more than ten individuals [R_S (≥10)], F-statistics according to Weir & Cockerham (1984) (F_{IS} W&C), probability value associated to the F_{IS} (*p*-val). Significant F_{IS} values are indicated in bold characters. Detailed descriptive statistics are presented in Annexe 2. Acronyms are as in Table 1.

Genetic characteristics	Sampling sites														
	BIC	MET	ANI	PEI	WNF	PB	ENF	CB	NS	BF	LAB	HB	SI	NWA	SW
All															
N	48	21	16	29	25	24	4	5	8	24	6	19	30	259	31
N_{all}	4.7	3.4	3.8	3.2	3.8	3.7	2.4	2.3	3.0	4.0	2.8	3.1	3.0	6.0	2.7
$H_{n.b.}$	0.518	0.489	0.512	0.466	0.493	0.472	0.410	0.506	0.478	0.539	0.478	0.406	0.366	0.466	0.364
H_o	0.468	0.471	0.513	0.448	0.513	0.472	0.359	0.522	0.455	0.489	0.517	0.412	0.355	0.455	0.381
Rs (all)	2.482	2.405	2.485	2.394	2.235	2.182	2.318	2.513	2.336	2.189	2.358	1.900	2.155	2.521	1.919
Rs (≥10)	3.813	3.241	3.718	3.541	3.018	3.421	------	------	------	3.690	------	2.603	2.967	3.960	2.503
F_{IS} W&C	**0.097**	0.035	-0.001	0.037	-0.037	0.000	0.140	-0.005	0.058	**0.094**	-0.080	-0.019	0.029	0.035	-0.046
p-val	**0.001**	0.288	0.539	0.219	0.784	0.507	0.145	0.526	0.270	**0.030**	0.835	0.623	0.300	0.011	0.800

Global of F_{IS} value in the Northwest Atlantic was 0.035 (p-value = 0.0113) suggesting the existence of slight deviation from Hardy-Weinberg Equilibrium due to deficit in heterozygotes.

IV.1.2 Mitochondrial DNA variability

The 37 polymorphic sites detected in the present study define a total of 59 haplotypes in the Northwest and the Northeast Atlantic (Figure 3). These haplotypes resulted from 34 transitions, one transversion and two indels (insertion-deletion events). The variable sites show substitutions between Adenosine (A) and Guanine (G) at 14 sites. Substitutions between Cytosine (C) and Tyrosine (T) were observed at 20 sites. One site shows one substitution between Adenosine (A) and Tyrosine (T). The two indels are observed with Cytosine (site 16388 and after site 16484). The distribution of the indel in position 16388 is interesting since it is found exclusively in haplotypes of Northeast Atlantic samples and in only two haplotypes of Hudson Bay and one of Bay of Fundy samples. Sequencing of these haplotypes were determined twice to eliminate any possible error.

Of the 59 haplotypes detected, 47 (79.9%) are present only in one site and ten were found only in the Northeast Atlantic sample (Table 5). In the Northwest Atlantic, 49 haplotypes were detected, 37 (75.6%) of them are present in only one site and 21 are singletons. Cape Breton is the only site where only haplotype 1 was observed. This haplotype is the most common one with a frequency of 17.5% and is present in nine of 13 samples from the Northwest Atlantic.

The distribution of the haplotypes in the Northwest Atlantic suggests the existence of some population structure. Indeed, haplotypes 11, 12, 13, 14, 15, 16, 42 and 43 detected in the

Hudson Bay sample are not found in other locations of the Northwest Atlantic. Furthermore, in the Sable Island sample, the haplotypes 21, 31 and 40 found in 24 individuals out of 27 are also private to this site. Haplotype 57, which is relatively frequent in the West Newfoundland sample (40%), is not found elsewhere (Table 5).

Haplotype diversity is high with an overall value of 0.942 for the Northwest Atlantic (Table 6). The value of this indices varied from 0.629 for Prince Edward Island to 0.941 for Anticosti Island if the site Cape Breton where only one haplotype was detected (h=0) is excluded. Nucleotide diversity is relatively low with an overall value of 0.006 (Table 6). The nucleotide diversity value varied from 0.001 for East Newfoundland to 0.009 for Hudson Bay. As mentioned previously, only one haplotype was detected in Cape Breton, so π=0.

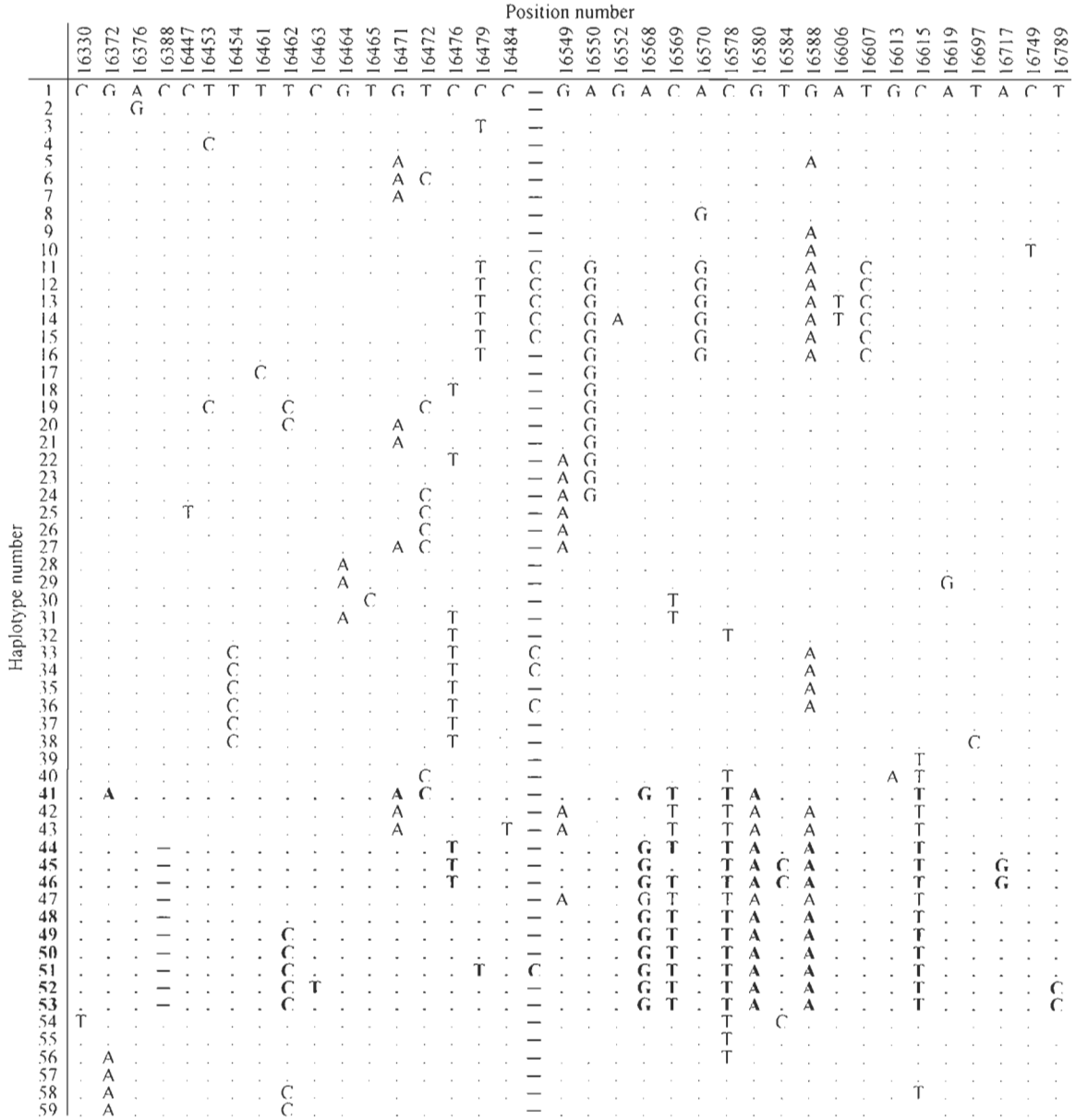

Figure 3. Description of the haplotypes observed for the harbour seal, *P. v. concolor* (49) and for *P. v. vitulina* (10 haplotypes in bold characters) from the 505-bp sequence of the mtDNA control region. Position numbers correspond to the position provided by Árnason & Johnsson (1992). Dots indicate equality with sequence 1 and dashes represent insertion-deletion events.

Table 5. Absolute frequency of harbour seal (*Phoca vitulina*) control region haplotypes at 13 sampling sites of the Northwest Atlantic (NWA) and one in the Northeast Atlantic (SW). Acronyms are as in Table 1.

	Haplotype number																								
Pop	1	59	3	8	27	2	37	55	18	7	28	5	26	30	32	17	22	23	54	24	58	57	10	19	35
BIC	10			5	3		1	15		1			1	1	1										
MET	5			1	7			1	1																
ANI	2	1		2	1		1	3		1						1	1	3	1						
PEI		13	4																	5	1				
WNF	2	1				4					5											8			
PB	2					1	6	2															2	5	1
ENF							1																		
CB	5																								
NS	2											2													
BF	7	1	1						1	2															
SI	3																								
LAB											1	1													
HB																									
NWA	38	16	5	8	11	5	9	21	2	4	6	3	1	1	1	1	1	3	1	5	1	8	2	5	1
SW																									
Total	38	16	5	8	11	5	9	21	2	4	6	3	1	1	1	1	1	3	1	5	1	8	2	5	1

	Haplotype number																																		Sample Size
Pop	36	38	33	34	6	39	9	25	47	56	21	31	40	20	29	4	11	12	13	14	15	16	42	43	41	44	45	46	48	49	50	51	52	53	
BIC																																			38
MET																																			15
ANI																																			17
PEI																																			23
WNF																																			20
PB	1	1																																	21
ENF			2	1																															4
CB																																			5
NS					1	2																													7
BF							2	1	1	1																									17
SI											11	7	6																						27
LAB														1	1	2																			6
HB																	3	1	1	1	1	5	3	2											17
NWA	1	1	2	1	1	2	2	1	1	1	11	7	6	1	1	2	3	1	1	1	1	5	3	2	0	0	0	0	0	0	0	0	0	0	217
SW																									1	2	1	2	12	4	1	1	1	6	31
Total	1	1	2	1	1	2	2	1	1	1	11	7	6	1	1	2	3	1	1	1	1	5	3	2	1	2	1	2	12	4	1	1	1	6	248

Table 6. Statistical summary of mitochondrial DNA sequence analyses based on 248 sequences from 217 individuals from the Northwest Atlantic (*Phoca vitulina concolor*) and 31 individuals from the Northeast Atlantic (*Phoca vitulina vitulina*). Haplotype diversity and nucleotide diversity have been calculated for each sample site and overall samples of the Northwest Atlantic.

Sample site	N	Number of haplotypes	Haplotype diversity		Nucleotide diversity	
			h	± SE	π	± SE
BIC	38	9	0.768	0.047	0.003	0.002
MET	15	5	0.705	0.088	0.004	0.003
ANI	17	11	0.941	0.036	0.005	0.003
PEI	23	4	0.629	0.084	0.005	0.003
WNF	20	5	0.763	0.060	0.003	0.002
PB	21	9	0.867	0.049	0.006	0.004
ENF	4	3	0.833	0.222	0.001	0.001
CB	1	1	0.000	0.000	0.000	0.000
NS	7	4	0.857	0.1023	0.003	0.002
BF	17	9	0.831	0.085	0.004	0.003
LAB	6	5	0.933	0.122	0.005	0.004
HB	17	8	0.875	0.053	0.009	0.005
SI	27	4	0.732	0.046	0.007	0.004
Overall NWA	217	49	0.942	0.008	0.006	0.004
SW	31	10	0.809	0.054	0.004	0.002

IV.2 Population structure

IV.2.1 Genetic differentiation from microsatellite: θ_{ST}

Two loci have high values of θ_{ST}, SGPV11 (θ_{ST} =0.176) and HG6.3 (θ_{ST} =0.160) (Table 7) and may contribute more to the observed differentiation. The highest number of alleles (10 and 9 alleles respectively) was also observed at these loci (Annexe 2). The loci with the

lowest θ_{ST} value are Pvc78 (θ_{ST} =0.022), SGPV10 (θ_{ST} =0.032) and Pvc19 (θ_{ST} =0.041). Three, 5 and 2 alleles are present at these loci.

Table 7. Global θ_{ST} estimates (Weir & Cockerham, 1984) and standard deviation of θ_{ST} permutation tests among the 13 *Phoca vitulina concolor* samples from the Northwest Atlantic.

Locus	θ_{ST} estimate	Standard Deviation
HG6.1	0.089	0.028
HG6.3	0.160	0.094
Hl16	0.084	0.041
Lc28	0.095	0.046
Lw11	0.056	0.031
Lw20	0.071	0.035
Pvc19	0.041	0.023
Pvc78	0.022	0.019
SGPV10	0.032	0.026
SGPV11	0.176	0.105
Overall	0.096	0.017

The overall θ_{ST} estimate over all loci and populations was 0.096 for the Northwest Atlantic, indicating a moderate level of genetic structure in the study area. Pairwise tests for genetic differentiation between samples resulted in θ_{ST} ranging from 0.006 to 0.285 (Table 8). Pairwise θ_{ST} values were significant for 46 of 78 tests at α = 0.05, after sequential Bonferroni correction was applied giving a corrected critical value α_c = 0.000641. Sample from Sable Island is differentiated from all other samples with θ_{ST} values ranging from 0.158 to 0.285 and *p*-values<0.0000. The Hudson Bay sample was also differentiated from the other NWA samples with the exception of Cap Breton (θ_{ST}=0.080; P=0.0098) and Labrador (θ_{ST}=0.077; P=0.0039). Pairwise comparisons with Hudson Bay ranged from

0.077 to 0.166 with *p*-value varying from 0.0000 to 0.0098. Table 8 also shows that significant results of pairwise tests were observed between various combinations of sites. It is worth mentioning that some significant results were obtained in comparisons involving pair of sites that are geographically close (e.g. Bic - Métis), suggesting genetic heterogeneity at small geographical scale. Samples from Cap Breton (CB) and south coast of Nova Scotia (NS) are exception since differentiation of these samples was only observed with the sample from Sable Island.

IV.2.2 Genetic differentiation from mtDNA: Φ_{ST}

The global Φ_{ST} value estimated for the 13 samples from the Northwest Atlantic was Φ_{ST} = 0.322, a value which is three times higher than the Φ_{ST} estimates obtained with microsatellites. Such a high value indicates that the sampling area do not represent a single homogeneous cluster. Significant differences were observed in 32 of 78 of the pairwise tests performed (Table 9). Significant differences were detected in various combinations. The highest pairwise comparison value (0.921) was observed between Cape Breton and East Newfoundland, two samples characterized by the smallest sample size (5 and 4 respectively). The high level of genetic differentiation of Hudson Bay and Sable Island sites detected with microsatellites is not so obvious with mtDNA Φ_{ST} estimates.

Table 8. Pairwise θ_{ST} values (below diagonal) between *Phoca vitulina concolor* samples and associated p-values (italic, above diagonal).

	BIC	MET	ANI	PEI	WNF	PB	ENF	CB	NS	BF	LAB	HB	SI
Bic (BIC)		*<0.0000*	*0.1748*	*<0.0000*	*<0.0000*	*<0.0000*	*0.0078*	*0.0127*	*0.0049*	*<0.0000*	*0.0371*	*<0.0000*	*<0.0000*
Métis (MET)	0.039*		*<0.0000*	*<0.0000*	*0.0010*	*<0.0000*	*<0.0000*	*0.0059*	*0.0673*	*<0.0000*	*0.0010*	*<0.0000*	*<0.0000*
Anticosti Island (ANI)	0.008	0.052 *		*<0.0000*	*<0.0000*	*<0.0000*	*<0.0000*	*0.1738*	*0.0029*	*<0.0000*	*0.0166*	*<0.0000*	*<0.0000*
Prince Edward Island (PEI)	0.047 *	0.099 *	0.052 *		*<0.0000*	*<0.0000*	*<0.0000*	*0.0156*	*0.0088*	*<0.0000*	*0.0049*	*<0.0000*	*<0.0000*
West Newfoundland (WNF)	0.027 *	0.027 *	0.032 *	0.088 *		*<0.0000*	*0.0381*	*0.0449*	*0.0088*	*<0.0000*	*0.0020*	*<0.0000*	*<0.0000*
Placentia Bay (PB)	0.061 *	0.073*	0.061*	0.088 *	0.057*		*0.0908*	*0.1055*	*0.0625*	*0.0010*	*0.0010*	*<0.0000*	*<0.0000*
East Newfoundland (ENF)	0.071	0.117 *	0.105 *	0.144 *	0.060	0.043		*0.1094*	*0.0117*	*0.0449*	*0.0205*	*<0.0000*	*<0.0000*
Cape Breton (CB)	0.055	0.067	0.018	0.060	0.040	0.029	0.078		*0.4297*	*0.5147*	*0.0332*	*0.0098*	*<0.0000*
South coast of Nova Scotia (NS)	0.046	0.025	0.044	0.046	0.048	0.027	0.090	0.010		*0.4424*	*0.0088*	*<0.0000*	*<0.0000*
Bay of Fundy (BF)	0.030 *	0.045 *	0.034 *	0.039 *	0.045 *	0.043 *	0.060	0.007	0.006		*0.0166*	*<0.0000*	*<0.0000*
Labrador (LAB)	0.035	0.087 *	0.038	0.063	0.078	0.096 *	0.108	0.058	0.082	0.049		*0.0039*	*<0.0000*
Hudson Bay (HB)	0.093*	0.166 *	0.078*	0.146 *	0.118 *	0.148*	0.135 *	0.080	0.157 *	0.113 *	0.077		*<0.0000*
Sable Island (SI)	0.161*	0.158*	0.159 *	0.179 *	0.160*	0.232*	0.285 *	0.178 *	0.173*	0.174 *	0.184*	0.280 *	

Significant at the *p*-value <0.05 level after application of the sequential Bonferroni correction, giving a corrected critical value $\alpha_c = 0.05/78 = 0.000641$.

Table 9. Pairwise Φ_{ST} values (below diagonal) between *Phoca vitulina concolor* samples and associated p-values (italic, above diagonal).

	BIC	MET	ANI	PEI	WNF	PB	ENF	CB	NS	BF	LAB	HB	SI
Bic (BIC)		*0.0012*	*0.0412*	*<0.0000*	*<0.0000*	*<0.0000*	*<0.0000*	*0.2078*	*0.0071*	*0.0212*	*0.0055*	*<0.0000*	*<0.0000*
Métis (MET)	0.206		*0.0323*	*0.0001*	*<0.0000*	*0.0004*	*0.0004*	*0.1133*	*0.0776*	*0.0145*	*0.0514*	*<0.0000*	*0.0037*
Anticosti Island (ANI)	0.050	0.099		*0.0009*	*<0.0000*	*0.0068*	*0.0004*	*0.4248*	*0.0248*	*0.2515*	*0.0558*	*<0.0000*	*0.0124*
Prince Edward Island (PEI)	0.342 *	0.305 *	0.202 *		*<0.0000*	*<0.0000*	*0.0006*	*0.0448*	*0.0035*	*0.0010*	*0.0055*	*<0.0000*	*<0.0000*
West Newfoundland (WNF)	0.244 *	0.343 *	0.179 *	0.212 *		*<0.0000*	*0.0001*	*0.1063*	*<0.0000*	*0.0015*	*0.0097*	*<0.0000*	*<0.0000*
Placentia Bay (PB)	0.214 *	0.224*	0.111	0.243 *	0.249 *		*0.0421*	*0.1243*	*0.0264*	*0.0038*	*0.0049*	*0.0032*	*0.0005*
East Newfoundland (ENF)	0.619 *	0.603 *	0.471 *	0.587 *	0.685 *	0.200		*0.0076*	*0.0033*	*0.0001*	*0.0822*	*<0.0000*	*<0.0000*
Cape Breton (CB)	0.048	0.213	0.004	0.242	0.131	0.095	0.921		*0.1263*	*0.9054*	*0.1100*	*0.0045*	*0.1232*
South coast of Nova Scotia (NS)	0.176	0.125	0.105	0.298	0.267 *	0.153	0.619	0.143		*0.3703*	*0.2982*	*0.0019*	*0.1106*
Bay of Fundy (BF)	0.055	0.132	0.014	0.191*	0.107	0.117	0.489 *	-0.070	0.006		*0.1629*	*<0.0000*	*0.0065*
Labrador (LAB)	0.202	0.178	0.075	0.243	0.187	0.536	0.098	0.082	0.030	0.041		*0.0039*	*0.1700*
Hudson Bay (HB)	0.543 *	0.468 *	0.407 *	0.504 *	0.551 *	0.481	0.438 *	0.432	0.379	0.416 *	0.377		*<0.0000*
Sable Island (SI)	0.178 *	0.147	0.093	0.284 *	0.229 *	0.410 *	0.159 *	0.102	0.074	0.111	0.063	0.407 *	

Significant at the *p*-value <0.05 level after application of sequential Bonferroni correction, giving a corrected critical value $\alpha_c = 0.05/78 = 0.000641$.

IV.2.3 Bayesian Clustering Method

The application of the Bayesian clustering method to the microsatellite data reveals the most likely number of clusters in the study area (Figure 4). The highest likelihood clustering solution was obtained for K (the number of clusters) = 4. The ad hoc quantity *L(K)* for K = 3 was -4885.8, for K = 4 was-4844.83 and for K = 5 was -4877.03. The Ln probability of K values decrease continuously for value of K higher than 5. However, the *L(K)* tends to overestimate the number of clusters (Evanno *et al.* 2005). The ad hoc quantity ΔK was used and presented the highest value for K = 3. These 3 clusters correspond to Hudson Bay, Sable Island and the Estuary - Gulf of St. Lawrence and Atlantic samples (Figure 5). The first two clusters are homogenous. The third one is more heterogeneous and the analyses with STRUCTURE were repeated excluding the samples from Hudson Bay (HB) and Sable Island (SI) in order to detect possible sub-structuring in this large area. In this case, the most probable number of cluster was K = 1 (estimated Ln probability of *K* for *K* = 1 was Ln P(*D*) = -4164). It was therefore conclude that there were 3 clusters in the study area. We therefore used K = 3 for later tests and to determine the AMOVA framework.

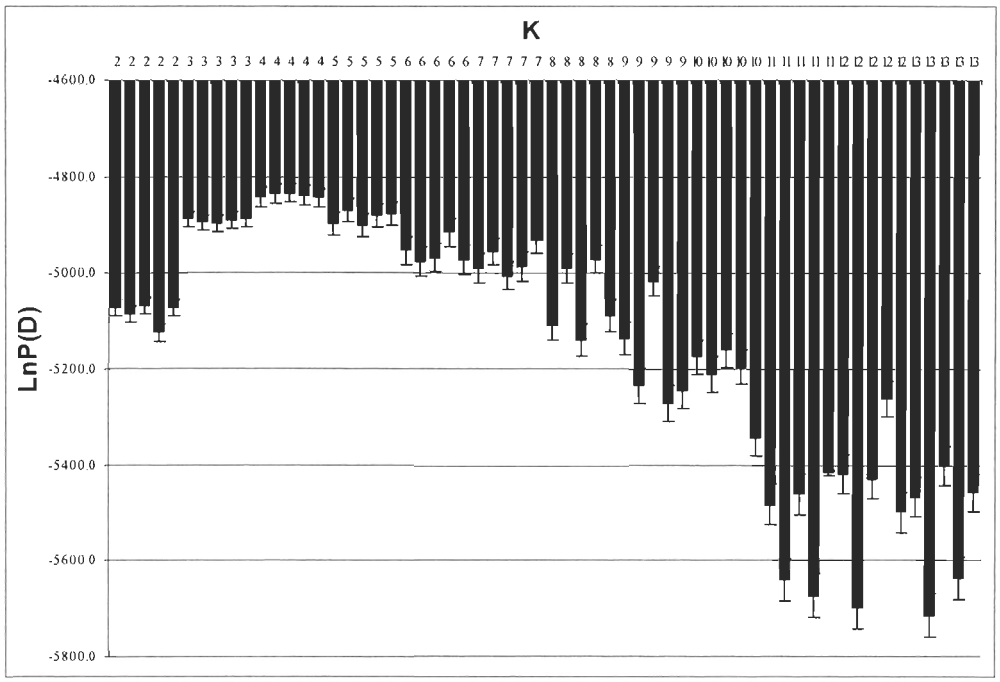

Figure 4. Graphical summary of the *p*-values logarithm for each of the five simulations carried out for K (expected number of clusters) values varying from 2 to 13 (Burn-in = 1 000 000 and Repetition = 1 000 000). LnP(D) represents the maximum likelihood for the simulations.

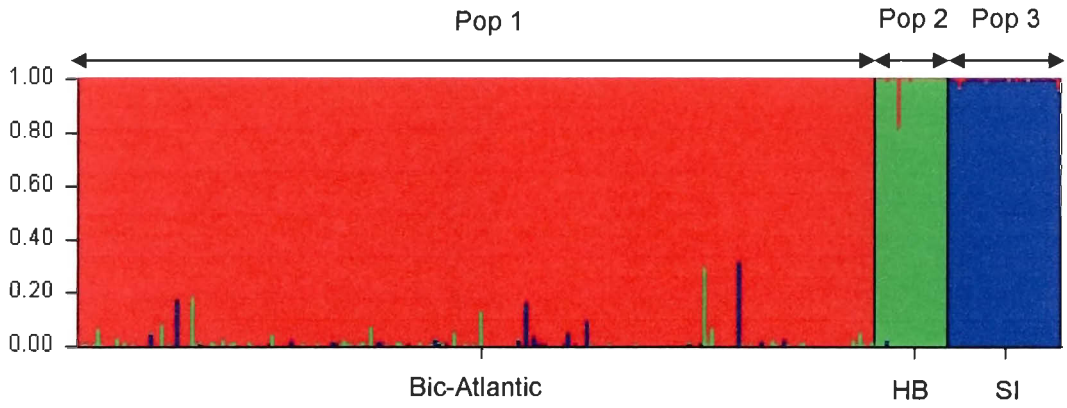

Figure 5. Graphical summary of clustering analysis (Pritchard *et al.* 2000) for 259 harbour seal (*Phoca vitulina concolor*) sampled at 13 sites of the Northwest Atlantic and scored at 10 microsatellite loci. Each individual is represented by a vertical line broken into three segments representing the estimated proportion of individual genome originating from each of the three clusters. *K* was set to three. Acronyms are as in Table 1.

IV.2.4 AMOVA on the microsatellite data

As mentioned previously the AMOVA framework was based on the most likely number of clusters obtained with the Bayesian clustering analysis. Therefore 3 groups comprising the following samples were designed: (1) Anticosti Island, Bic, Métis and Price Edward Island, West Newfoundland, Cape Breton, south coast of Nova Scotia, Bay of Fundy, Placentia Bay, East Newfoundland and Labrador; (2) Hudson Bay; (3) Sable Island.

The results of the AMOVA carried out with microsatellites data (Table 10) show that the largest proportion of the total variance (86.3%) can be attributed to variation within groups. The proportion of the total variance that can be attributed to the variation among the 3 groups defined by the Bayesian analysis is much smaller (13.7%; θ_{CT}=0.138). The fixation indice (θ) is highly significant (Table 10) indicating the existence of genetic structure in the study area.

Table 10. Summary of Analysis of Molecular Variance conducted over ten microsatellite loci for 13 samples. Three groups of samples are considered: Group 1: ANI, BIC, MET, PEI, WNF, CB, NS, BF, PB, ENF and LAB; Group 2: HB; Group 3: SI. Acronyms are as in Table 1.

Source of variation	*d.f.*	*Sum of squares*	*Variance components (V)*	*Percentage variation*	θ-statistic	*Significance test V and F (10100 permutations)*
Among groups	2	67.110	Va=0.37228	13.7	θ_{CT}=0.138	0.0000 ***
Within groups	515	1202.379	Vb=2.33472	86.3		
Total	517	1269.488	2.70700			

*** Significant values at the 0.001 level

IV.2.5 AMOVA on the mitochondrial DNA data

Results of the AMOVA carried out on mitochondrial DNA (Table 11) using the same framework as that used for microsatellites show a similar trend. Indeed, the largest proportion of the total variance (66.4%) is explained by variation within groups while

33.6% (Φ_{ST}=0.336) of the total variance is associated to variation among the three areas defined by the Bayesian clustering analysis.

In comparison with the results of the AMOVA based on microsatellites, it is worth mentioning that the AMOVA based on mitochondrial DNA data explain more genetic structure. The fixation index values associated to the broad geographic structure (the 3 groups) is larger for mitochondrial DNA data than for microsatellite data: 0.336 vs 0.138 (Tables 10 and 11). The variation within sites is less important in proportions using the mitochondrial DNA (66.4%) than using microsatellites (86.3%). We can conclude that the results of the AMOVA using the microsatellites as well as mitochondrial DNA show the existence of genetic structure and heterogeneity in the study area.

Table 11. Summary of Analysis of Molecular Variance conducted over all sampling areas using mitochondrial DNA sequence data. Three groups of populations are tested: Group 1: ANI, BIC, MET, PEI, WNF, CB, NS, BF, PB, ENF and LAB; Group 2-HB; Group 3-SI. Acronyms are as in Table 1.

Source of variation	d.f.	Sum of squares	Variance components (V)	Percentage variation	Φ-statistic	Significance test V and F (10100 permutations)
Among groups	2	60.211	Va=0.76863	33.6	Φ_{CT}=0.336	0.0000 ***
Within groups	214	324.682	Vb=1.51721	66.4		
Total	216	384.893	2.28584			

*** Significant values at the 0.001 level

IV.3 Phylogeography

IV.3.1 Geographical structure based on microsatellite data

The neighbour-joining tree with 259 individuals from the Northwest Atlantic and 31 from the Northeast Atlantic based on Cavalli-Sforza and Edwards chord distance (Cavalli-Sforza & Edwards 1967) is presented in Figure 6. Bootstrap support of the branches is generally quite low. The highest bootstrap value is observed for the branching of Bic – Métis (MET) (52.3%). Despite these low bootstrap values, some subdivisions observed in the tree are consistent with geographical repartition of the samples. Indeed, Labrador (LAB) and Hudson Bay (HD) samples are grouped, as well as Placentia Bay (PB) and East Newfoundland (ENF). There are also some unexpected results. Indeed, this tree suggests that the Northeast Atlantic sample (SW) is not very different from the samples collected in Labrador (LAB) and Hudson Bay (HB) although the individuals belong to two different subspecies. It is also interesting to notice that the sample from West Newfoundland (WNF) does not group with the other samples from the Gulf of St. Lawrence such as Anticosti Island (ANI) but with Sable Island (SI).

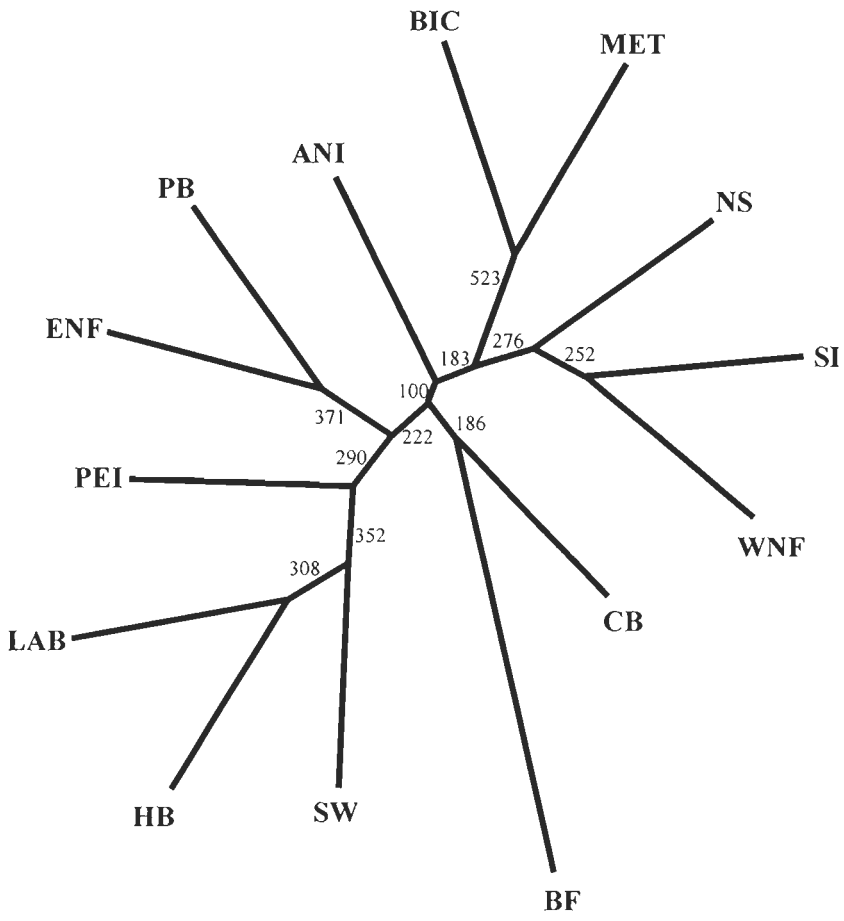

Figure 6. Neighbour-joining tree using the Cavalli-Sforza and Edwards (1967) chord distance calculated from microsatellite data collected on 259 harbour seals from the Northwest Atlantic (*Phoca vitulina concolor*) and 31 from the Northeast Atlantic (*Phoca vitulina vitulina*). Numbers at the nodes of the branches indicate support of the branch separation after 1 000 repetitions. Acronyms are as in Table 1.

IV.3.2 Phylogeography based on mitochondrial DNA sequences

A minimum spanning tree has been built among haplotypes (Figure 7). The main feature of the tree is the presence of a star-like phylogeny from which several rarer haplotypes extend. The central haplotype of the minimum spanning tree is represented by haplotype 1, which is the most common one observed in the present study. The haplotype tree shows some geographical partitioning with two branches extending from the star-like phylogeny. The first one extends to the haplotypes observed in the Northeast Atlantic sample (SW), the second one represents haplotypes 16, 11, 12, 15, 13 and 14 from Hudson Bay. Haplotypes detected in Hudson Bay sample (HB) are also represented on the branch extending to the haplotypes from Northeast Atlantic (42, 43). It is also worth mentioning that haplotype 47 detected in the Bay of Fundy is grouped with haplotypes characteristic of the Northeast Atlantic. These individuals, with mitochondrial haplotypes related to Northeast Atlantic individuals, have been sequenced twice to eliminate any possibility of error. Furthermore, microsatellite genotypes from these individuals showed alleles different from those of individuals in the Northeast Atlantic sample carrying the same mitochondrial DNA haplotypes. This observation can be attributed to migration in a recent evolutionary time or to homoplasy.

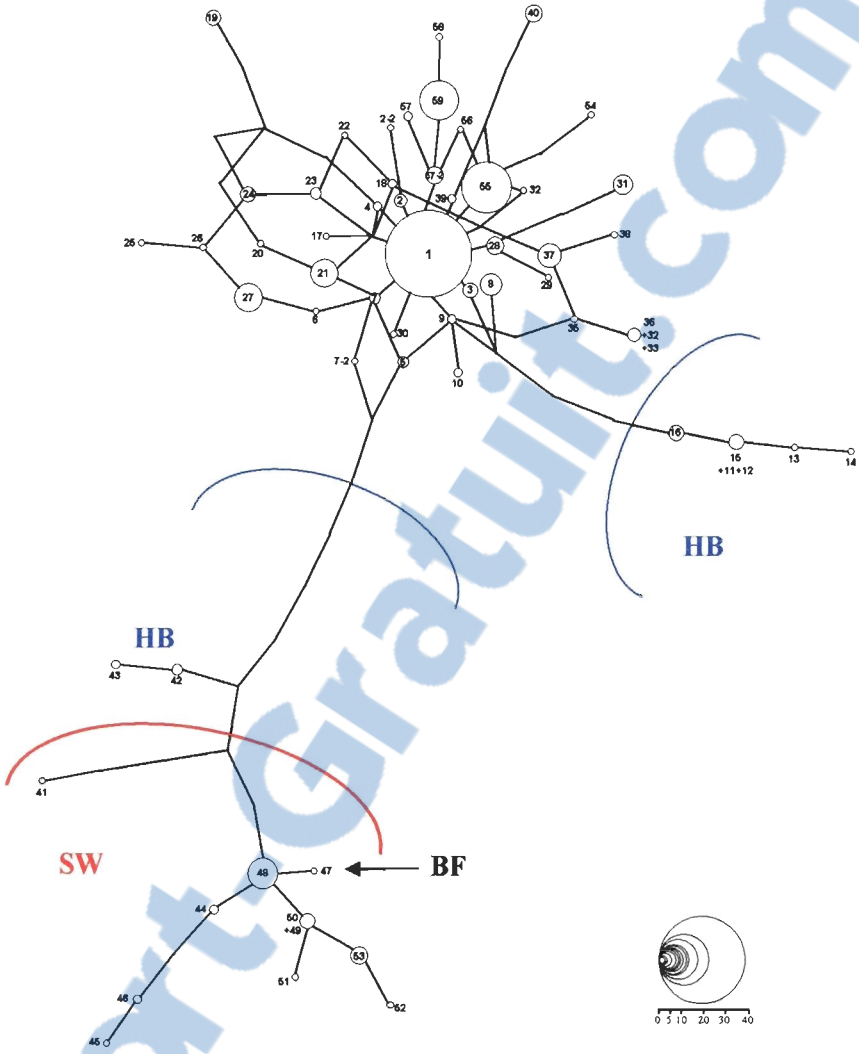

Figure 7. Minimum spanning tree based on mtDNA describing the relationship between harbour seal haplotypes observed in the Northwest Atlantic. The relationship with harbour seal haplotypes from Northeast Atlantic (SW) is also illustrated. Circle sizes are proportional to the number of individuals sharing a particular haplotype. The numbers inside or close to the circles correspond to the identification number of the haplotypes. Lengths of the branches are proportional to the number of mutations. HB: Hudson Bay; BF: Bay of Fundy.

IV.3.3 Geographical structure based on both microsatellite and mitochondrial DNA data

The neighbour-joining tree based on microsatellite and mtDNA data (Figure 8) shows some geographical organization of the samples. The first group observed on this tree comprises the neighbouring sites: Bic (BIC) and Métis (MET) (76.7%) which form a group with Anticosti Island (ANI). The support of this node, 62.3%, is relatively high. However, the other sites from the Gulf of St. Lawrence, West Newfoundland (WNF) and Prince Edward Island (PEI) do not belong to this group. Placentia Bay and East Newfoundland is the only other group for which bootstrap value is relatively high (57.6%). The support for the other branches of the tree is below 50%. However, the branching of the tree corresponds quit well to the geographic distribution of the samples as geographically-close sampling sites are grouped on the same branches of the tree: Hudson Bay (HB) and Labrador (LAB); South coast of the Nova Scotia (NS) and Sable Island (SI). However, the separation of the sample Nova Scotia (NS) from that of Cape Breton (CB) and Bay of Fundy (BF) is difficult to explain. The same observation can be made for the association between samples from Labrador (LAB), Hudson Bay (HB) with that from Prince Edward Island (PEI) and Northeast Atlantic.

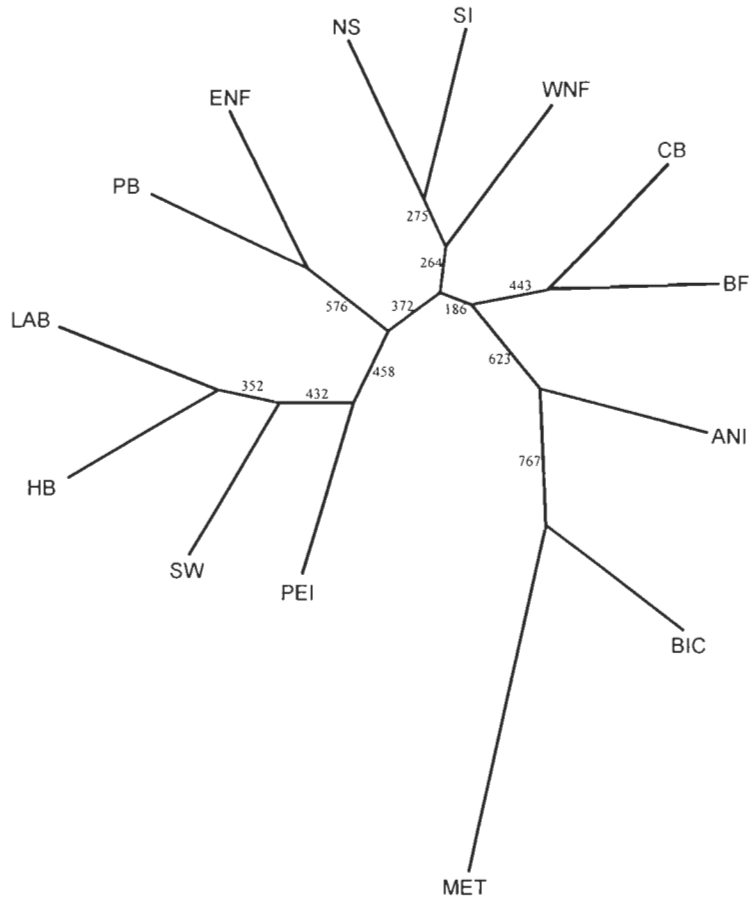

Figure 8. Neighbour-joining tree using the Cavalli-Sforza and Edwards (1967) chord distance for 293 harbour seals from 13 locations in the Northwest Atlantic (*Phoca vitulina concolor*) and one location in the Northeast Atlantic (*Phoca vitulina vitulina*), based on both microsatellite and mitochondrial DNA results. The numbers on the branches indicate support of the branch separation after 1 000 repetitions.

IV.4 Mantel tests for isolation by distance

Mantel tests were performed on the 13 samples from the Northwest Atlantic using microsatellite and mitochondrial DNA data (Figure 9). These tests were not significant for both microsatellite and mitochondrial DNA (p-value for microsatellites = 0.07678; p-value for mitochondrial DNA = 0.10687). But even if Mantel tests were not significant, the linear regression observed for microsatellite and mitochondrial DNA data indicate trends towards isolation by distance (r^2 for microsatellites = 0.0715; r^2 for mitochondrial DNA = 0.1154). Mantel tests of isolation by distance were also carried out along a hypothetical migratory route along the southern Gulf of St. Lawrence and Nova Scotia coast: BIC – MET – PEI – CB – NS – BF, using microsatellite and mitochondrial DNA data. These tests were not significant and did not show any isolation by distance (results not shown).

IV.5 Sex biased test for migration

Pairwise Φ_{ST} were compared for microsatellite and mitochondrial DNA data (Figure 10) to detect possible sex-biased migration in the study area. The correlation coefficient is low (r^2 = 0.2614) and does not appear to deviate significantly from the theoretical expression indicative of no sex-biased dispersal. Therefore, it seems that both males and females can migrate.

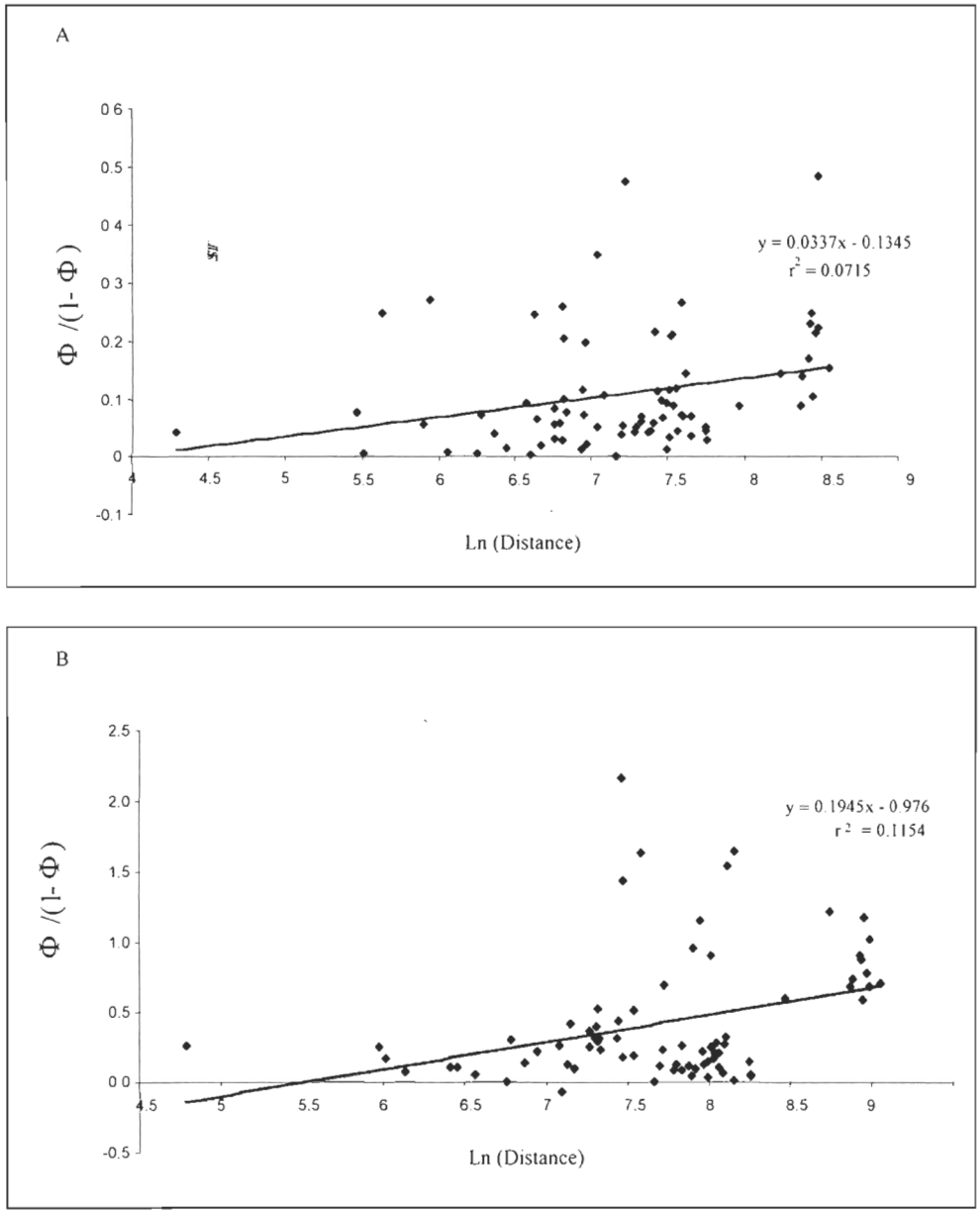

Figure 9. Relationship between pairwise F_{ST} estimates from microsatellites (A) and mitochondrials DNA (B) and geographical distances (Km) for the 13 *Phoca vitulina concolor* samples collected in the Northwest Atlantic. Equations of the linear regression and squared correlation coefficient are indicated.

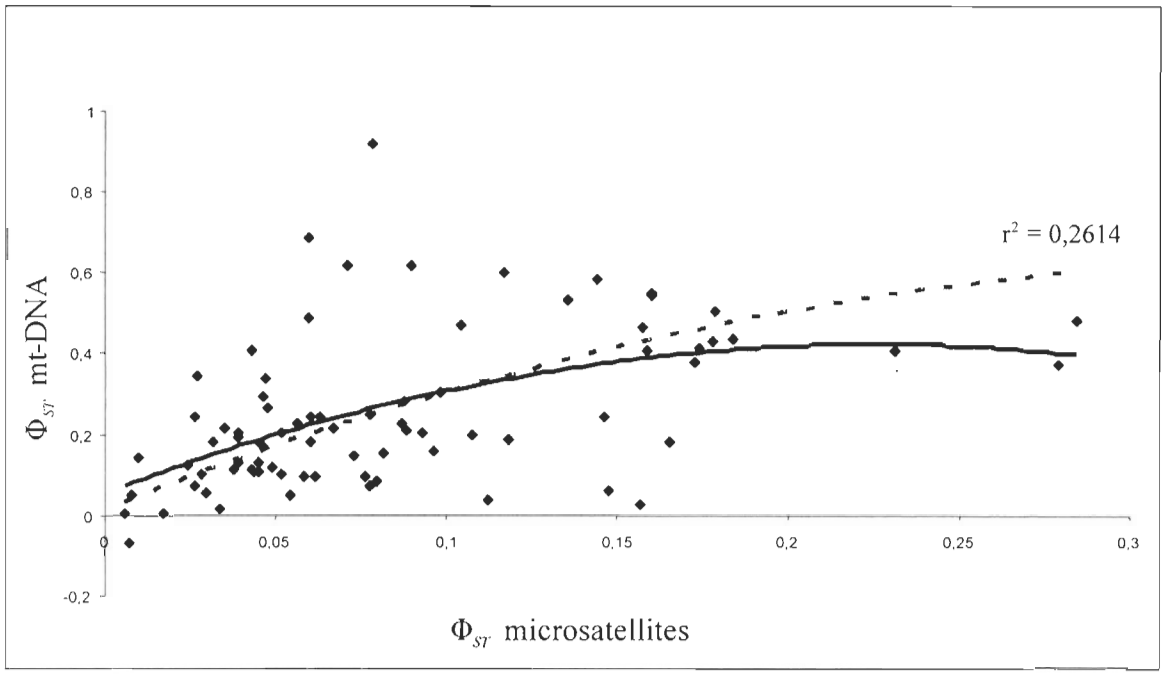

Figure 10. Test for sex-biased migration in *Phoca vitulina concolor*. Pairwise Φ_{ST} (mitochondrial) versus Φ_{ST} (microsatellite). The dotted line represents the theoretical expression: Φ_{ST} (mitochondrial)=4 Φ_{ST} (microsatellite)/[1+3 Φ_{ST} (microsatellite)] (Crochet 2000), indicative of no sex-biased dispersal. Black diamonds (♦) represent data from this study.

IV.6 Phylogeography of the four harbour seals subspecies

The worldwide pattern of the harbour seals haplotypes phylogeny from Stanley *et al.* (1996) is described in building a Minimum Spanning Network (Figure 11). Harbour seal haplotypes from the Northwest Atlantic are found in central position from which haplotypes of the other subspecies extend. Haplotypes from the Northeast Pacific and the Northwest Pacific appear on different branches as do haplotypes from the Northwest and Northeast Atlantic. These results are in agreement with those of Stanley *et al.* (1996). As mentioned previously, some haplotypes detected in individuals sampled in the Northwest Atlantic grouped with haplotypes from the Northeast Atlantic. This is the case, in the present study, for haplotypes 42, 43 (Hudson Bay) and 47 (Bay of Fundy). The same observation applies to haplotype G7 from the Stanley *et al.* (1996) study.

V. Discussion

The present study represents the first attempt to describe the population genetic structure of the harbour seal *Phoca vitulina concolor* at the scale of the Northwest Atlantic. Microsatellite loci and mitochondrial DNA sequences were used to analysed samples that were collected between 1988-1989 (Bay of Fundy, Sable Island) and 2004 (Anticosti Island and Prince Edward Island). Some samples collected in the 1990s, Anticosti Island (N= 7), South coast of Nova Scotia (N = 8) and Cape Breton (N = 5) represent 7.7 % of the

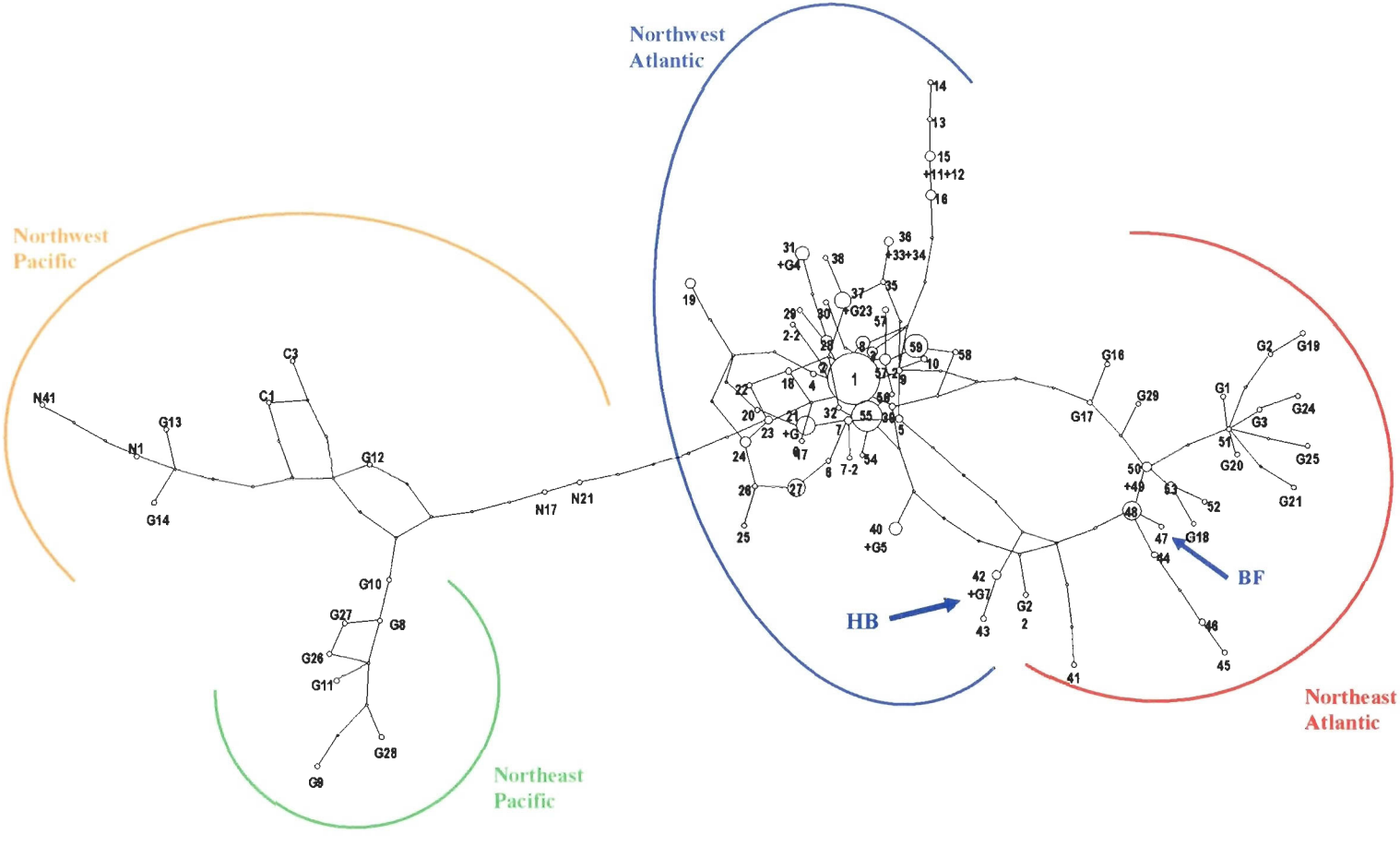

Figure 11. Minimum spanning network of the north Atlantic and north Pacific harbour seal (*Phoca vitulina*) haplotypes based on our analysed data and Stanley *et al.* (1996) mitochondrial D-Loop sequences. Name of the haplotypes are the same as in Fig. 3. Black circles indicate ancestral extinct haplotypes.

samples analysed. All other samples were collected between 2001 and 2004. Despite the discrepancies in the sampling periods, we believe that the results of the present study provide a good overall picture of the harbour seal phylogeography at the scale of the Northwest Atlantic and that our results are not influenced by difference in the sampling years.

V.1 Genetic diversity

The analyses of microsatellites and of the mitochondrial DNA nucleotide sequence reveal relatively high level of genetic variability in this species. In the Northwest Atlantic, the average observed heterozygosity of microsatellite loci was 0.455, a value close to that of 0.47 observed for *Phoca vitulina richardsi* in the Northeast Pacific (Burg *et al.* 1999) and of 0.501 for *Phoca vitulina vitulina* in the Northeast Atlantic (Goodman 1998). These values are higher than that estimated for the Weddel seal (*Leptonychotes weddelli*, >0.347) and lower than that observed for other pinniped such as the leopard seal (*Hydrurga leptonyx*, >0.619), the crabeater seal (*Lobodon carcinophagus*, >0.800) (Davis *et al.* 2002) and the hooded seal (*Cystophora cristata*, 0.75) (Coltman *et al.* 2007).

V.2 Population structure

The analyses of the two types of markers used in this study reveal the existence of population structure at the scale of the Northwest Atlantic. They also suggest the existence of heterogeneity in the study area.

The results of different statistical analyses carried out with microsatellites are convergent and pointed at the existence of at least three populations at the scale of the Northwest

Atlantic: Hudson Bay, Sable Island, Estuary-Gulf of St. Lawrence and Atlantic. First, pairwise F_{ST} comparisons revealed significant differences between the sample from Sable Island and all the other samples included in the present study (Table 8). Further, the same test also revealed that the sample from Hudson Bay differs from the other samples except those collected along Labrador coast and Cape Breton. The other F_{ST} pairwise tests revealed the existence of heterogeneity in the study area but no other systematic differences were detected. Second, the application of the Bayesian clustering method (Pritchard *et al.* 2000) revealed the existence of three clusters across the Northwest Atlantic (Figure 5). However, the estimated Ln probabilities for K = 3, 4 and 5 are very close to each other. In such case, it is difficult to determine the real number of clusters and it might be more appropriate to select the smaller number of populations (Pritchard *et al.* 2000). We can therefore conclude that three clusters are detected by the Bayesian analysis of microsatellites. These clusters are (1) Hudson Bay, (2) Sable Island, (3) Estuary and Gulf of St. Lawrence and Atlantic. The Hudson Bay and Sable Island clusters are homogenous while the third one is more heterogeneous.

The broad scale geographic structure described above with microsatellite data is also supported by the geographic distribution of haplotypes (Table 5). Indeed, the 8 haplotypes observed in Hudson Bay sample are private. Only 3 individuals from Sable Island share the haplotype 1, the most common one in the study area, with the individuals from other sites. The haplotype 21, which is present in almost 41% of the individuals in Sable Island, is private.

Our analyses of microsatellites and mitochondrial DNA also suggest the existence of heterogeneity or sub-structuring at a smaller geographical scale within the Estuary-Gulf of St. Lawrence and Atlantic cluster. Indeed, the results of the AMOVA based on 3 groups (Tables 10, 11) show that all fixation indices are highly significant and a large proportion of the variation is explained by variation within groups. The phylogenetic tree topology based on both markers also suggests some grouping among samples although the support of branching is generally low. In these trees, samples from Bic, Metis and Anticosti Island are grouped, as it is also the case for Labrador and Hudson Bay, Nova Scotia and Sable Island, Cape Breton and Bay of Fundy. However, Bayesian analyses with STRUCTURE did not detect additional clusters within this group when the analyses were repeated without Hudson Bay and Sable Island samples.

In the present study, we did observe a trend of isolation by distance which was not however significant. In this respect, our results differ from those of other studies, which showed isolation by distance in the Pacific Ocean (Stanley *et al.* 1996; Westlake & O'Corry-Crowe 2002) as well as in the Northeast Atlantic (Stanley *et al.* 1996; Goodman 1998). The absence of relation between genetic differentiation and geographical distance might be partly explained by the small size of some samples, particularly in the northern Gulf of St. Lawrence and Labrador. The absence of samples from the north coast of the St. Lawrence Estuary and Gulf may have prevented us from detecting such relation.

The genetic differentiation of the Sable Island harbour seal population is most likely related to 165 km of open ocean separating this island from the continent. Indeed, Lesage *et al.* (2004) have shown that harbour seals generally tend to remain near the coast (less than 11

km from shore) in shallow water areas (less than 50m deep). Our results are in agreement with those of previous studies on morphology (Boulva & McLaren 1979) and genetic characteristics (Coltman *et al.* 1998) pointing at possible isolation of this population. Furthermore, the reduction in population size in the 70s (Bowen *et al.* 2003) may have accentuate the genetic differentiation of this population (Coltman *et al.* 1998). In the present study, the lowest heterozygosity and allelic richness values were observed in this sample (Table 4; Annexe 2). It has been suggested that harbour seal immigration may have taken place in the 80s (Bowen *et al.* 2003). However, we did not detect much evidence of admixture in the population (Figure 5). However, our sample size is rather small to allow rejecting this hypothesis.

Our study reveals more population structure at the scale of the Northwest Atlantic than for other pinniped species. Although differentiation was observed between Northwest and Northeast Atlantic for the harp seal (*Pagophilus groenlandicus*) and the grey seal (*Halichoerus grypus*), there was no differentiation within the Northwest Atlantic (Boskovic *et al.* 1996; Perry *et al.* 2000). For the grey seal, the population from Sable Island was not differentiated from that of the southern Gulf of St. Lawrence (Boskovic *et al.* 1996). In a recent study, Coltman et al. (2007) tested for genetic differentiation between four hooded seal (*Cystophora cristata*) breeding herds using microsatellites and mitochondrial markers. They found that both markers indicate panmixia for this species. Such panmixia was attributed to the fact that hooded seal breeds on pack of ice, a behaviour that does not favour philopatry.

The star-like phylogeny observed with the minimum spanning tree, as well as the low nucleotide diversity (0.006) relative to the high haplotypic diversity (0.942) observed in our data, are consistent with recent colonisation followed by rapid population expansion in recent evolutionary time (Westlake & O'Corry-Crowe, 2002; Coltman *et al.* 2007).

V.3 Evolutionarily Significant Units

Evolutionarily Significant Units are the populations or groups of populations (metapopulations) that are "evolutionarily" different from one another. A population that is evolutionary different is one that possesses unique adaptive genetic variation. Such unique genetic variation arises through local selection, genetic drift and environmental isolation (Avise 2004). An ESU is a group of organisms that has undergone significant genetic divergence from other groups of the same species (Moritz 1994b; Waples 1995). Identification of ESUs is based on natural history information, range and distribution data, and results from analyses of morphometrics, cytogenetics, allozymes, nuclear and mitochondrial DNA. Concordances of those data, and the indication of significant genetic distance between sympatric groups of organisms, are critical for establishing an ESU. In the present study, the individuals analysed belong to three different clusters. According to the definition of ESUs from Moritz (1994b), the mitochondrial DNA analyses identified two ESUs: one in the Hudson Bay, and one in the rest of the study area. However, the individuals from the second groups (all samples except) Hudson Bay there is a clear separation between Sable Island and the St. Lawrence-Atlantic samples. These two populations are highly differentiated based on microsatellite data but are located on the

same branch of the mtDNA phylogenetic tree (Figure 7). Although it is not clear how many ESUs can be defined for harbour seal at the scale of the Northwest Atlantic, it appears that populations from Hudson Bay, Sable Island and St. Lawrence – Atlantic should be managed separately.

V.4 Phylogeography of the four harbour seals subspecies

Hudson Bay haplotypes are grouped in two separated branches. There is one branch extending from the central haplotypes, and the second branch is extending from the branch with haplotypes from Sweden. On this branch with Sweden haplotypes, there is a singleton from Bay of Fundy. Such observations were found but not discussed in previous studies. The G7-called haplotype from Hudson Bay of Stanley *et al.* (1996), and the NJ-94-01-called haplotype from Smith (1999) belong to this European harbour seal's branch. There are two possibilities to explain this similarity: migration or homoplasy (homoplasy refers to similarity of traits or genes for reasons other than coancestry, Sanderson & Hufford 1996). The large geographical distance between individuals that carried these haplotypes could support the hypothesis of homoplasy in the Northwest Atlantic harbour seal. However, there is no obvious barrier to gene flow between the Northeast and the Northwest Atlantic subspecies and the populations in Iceland or in Greenland have not been sampled. This could support the hypothesis of migration. A more detailed sampling could help to elucidate this question.

The Minimum Spanning trees using Stanley *et al.* (1996) haplotypes, show that harbour seal from the Northwest Atlantic occupies a central position, with one branch extending to

the Northeast Atlantic and one branch extending to the North Pacific. These results are in agreement with those of Stanley *et al.* (1996) suggesting that in both the Atlantic and the Pacific, colonization of the ocean basins took place from west to east. These analyses include 227 individuals: 158 from the Northeast Atlantic, 19 from the Northwest Atlantic, 38 from the Northeast Pacific and 12 from the Northwest Pacific. This study extends the Stanley *et al.* (1996) study.

VI. Conclusion

Three distinct populations were identified at the scale of the Northwest Atlantic some of which may represents Evolutionarily Significant Units (ESUs): Sable Island, Hudson Bay, coasts of Atlantic and Gulf of St. Lawrence.

The present study emphasizes some behavioural habits of the harbour seal. A pattern of migration along the coasts has been shown. There are trends to philopatry, but no sex-biased dispersal has been revealed.

Combined analyses using data from both Stanley *et al.* (1996) and from the present study corroborates the patterns of colonization from west to east in both the Atlantic and the Pacific Ocean basins previously shown by Stanley *et al.* (1996).

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Chapitre III Conclusion générale

Pour la présente étude, six marqueurs microsatellites ont été optimisés à partir de loci développés pour d'autres espèces de phoques : Lw11, Lw20, Hl16 et Lc28 développés pour des phoques de l'Antarctique puis testés pour le phoque commun (Davis *et al.* 2002); HG6.1 et HG6.3 développés pour le phoque gris (Allen *et al.* 1995). Deux loci microsatellites ont été développés pour le phoque commun de la sous-espèce de l'Atlantique nord-est (Goodman 1997) et deux loci ont été développés pour le phoque commun de la sous-espèce de l'Atlantique nord-ouest (Coltman *et al.* 1996). Ainsi, dix loci microsatellites polymorphes ont permis d'appréhender la structure génétique des populations de phoque commun de l'Atlantique nord-ouest. L'analyse d'un grand fragment, 505 paires de bases comprenant une portion du gène de l'ARNt-Proline et une portion de la région contrôle, a été réalisée en optimisant les amorces définies pour le phoque commun du Pacifique (Kocher *et al.* 1989; Westlake & O'Corry-Crowe 2002) et en définissant une amorce spécifique pour la présente étude. Cette amorce a été définie dans une zone préalablement amplifiée et séquencée avec les amorces utilisées par Westlake & O'Corry-Crowe (2002), dans une région de la séquence où aucun site variable n'était détecté, et définissant un fragment de plus de 500 paires de bases.

Cette étude révèle l'existence, chez le phoque commun, d'une structure génétique à une grande échelle géographique. En effet, trois populations distinctes ont été observées à l'échelle de l'Atlantique nord-ouest : 1) la baie d'Hudson ; 2) l'Île de Sables ; 3) l'estuaire, le golfe du Saint-Laurent et la côte de l'Atlantique. Dans cette étude, les populations de la

Baie d'Hudson et de l'Île de Sables ne sont représentées que par un seul échantillon. La distribution géographique de la troisième population est très étendue. En effet, cette population regroupe les échantillons de l'estuaire et du Golfe du Saint-Laurent et ceux de la côte atlantique de la baie de Fundy au Labrador. Il n'est pas possible d'exclure totalement l'existence d'une sous-structure dans cette grande population même si les analyses bayesiennes effectuées avec le logiciel STRUCTURE n'ont pas permis de détecter de groupes distincts à l'intérieur de cette population (K = 1). L'existence de cette sous-structure serait compatible avec les résultats des analyses de la variance moléculaire (AMOVAs) effectuées avec les données de microsatellites et d'ADN mitochondrial. Ces analyses ont montré qu'un pourcentage important de la variance était associé aux variations intra-groupes. (Tableaux 10 et 11). De plus, l'arbre phylogénétique effectué à partir des données de microsatellites et de l'ADN mitochondrial (Figue 8) met en évidence un regroupement de certains sites qui est compatible avec la biologie de l'espèce même si le support statistique de certains embranchements est parfois faible. Ainsi, les échantillons provenant de Bic, Métis et de l'île d'Anticosti se retrouvent sur des branches rapprochées de l'arbre. La même observation s'applique aux échantillons de Terre-Neuve et ceux de la baie de Fundy et du Cap Breton. D'une manière générale, la topologie de l'arbre phylogénétique suggère que les individus se déplacent vers les sites les plus rapprochés.

L'analyse des dix marqueurs microsatellites et des 505 paires de bases de la région contrôle de l'ADN mitochondrial montre que l'Île de Sable, d'une part, et la baie d'Hudson, d'autre part, sont fortement différenciées. L'éloignement géographique et la configuration semi-fermée de la baie d'Hudson expliquent cet isolement génétique. L'Île de Sable n'est

éloignée de la côte de la Nouvelle Écosse que de 165 km. Cependant, le phoque commun semble se déplacer le long de la côte et une masse d'eau profonde paraît constituer une barrière physique à ses déplacements (Lesage *et al.* 2004). De plus, la population de l'Île de Sable semble fragile, son effectif était en déclin (Bowen *et al.* 2003). Ainsi, l'isolement géographique et la fragilité de la population expliqueraient le degré de différenciation génétique observé pour les échantillons de l'Île de Sable.

L'analyse de l'ADN mitochondrial met en évidence plus de structure que l'analyse des marqueurs microsatellites. L'ADN mitochondrial est de transmission maternelle et les marqueurs microsatellites sont de transmission biparentale, l'information portée par l'ADN mitochondrial concerne donc plus particulièrement la structure supportée par les femelles. La comparaison des Φ_{ST} des deux types de marqueurs (Hoarau *et al.* 2004) ne montre pas que les mâles se dispersent davantage que les femelles. Dans l'Atlantique nord-est, les femelles de phoque commun montrent de la philopatrie (Stanley *et al.* 1996; Coltman *et al.* 1998). Dans le Pacifique, les travaux de Westlake et O'Corry-Crowe (2002) ont également montré de la philopatrie chez le phoque commun. La présence d'haplotypes particuliers à certains sites, parfois en grande proportion (l'haplotype 57 est rencontré chez 40% des individus de l'ouest de Terre-Neuve, WNF), laisse supposer une tendance à la philopatrie des femelles. Cette tendance pourrait être confirmée en augmentant les effectifs des petits échantillons (NS, CB, ENF, et LAB).

Lors de l'analyse des arbres phylogénétiques et des arbres recouvrant de longueur minimum (*minimum spanning tree*), deux haplotypes observés au niveau de la baie d'Hudson (HB) et un haplotype observé dans la baie de Fundy (BF) semblaient apparentés

aux haplotypes observés en Europe, pour la sous-espèce *Phoca vitulina vitulina*. La présence d'haplotypes de la sous-espèce *Phoca vitulina concolor* apparentés à des haplotypes de la sous-espèce *P. v. vitulina* a déjà été relevée, mais n'a pas été expliquée, pour des individus provenant de la baie d'Hudson (Stanley *et al.* 1996, haplotype G7) et du New Jersey (Smith 1999, haplotype NJ-94-01). Ces observations convergentes pourraient indiquer l'existence d'homoplasie entre les échantillons de la baie d'Hudson et de la baie de Fundy et ceux observés dans les échantillons de Suède. L'homoplasie fait référence à une similarité de traits ou de gènes qui n'est pas liée à un ancêtre commun (Sanderson & Hufford 1996). En effet, les échantillons de l'Atlantique nord-est analysés pour la présente étude proviennent du détroit de Skagerrak, à l'entrée de la mer Baltique, et les haplotypes de l'Atlantique nord-ouest, qui leur semblent apparentés, proviennent de Churchill, à l'intérieur de la baie d'Hudson et de Gand Manan dans la baie de Fundy. La possibilité de migration entre ces différents sites d'échantillonnage semble peu plausible, mais ne peut cependant être totalement rejetée.

Lorsque des analyses de séquences de l'ADN mitochondrial de la présente étude sont associées aux résultats du patron mondial décrit par Stanley *et al.* (1996), la phylogénie de l'espèce se précise. La forme en étoile de la phylogénie des haplotypes de la région de contrôle de l'ADN mitochondrial indique une expansion rapide et récente de la la population de cette sous-espèce. Les résultats obtenus sont en harmonie avec les observations de l'étude de Stanley *et al.* (1996) en accord avec une colonisation de l'ouest vers l'est dans chaque océan. Au cours de la présente étude, 49 haplotypes ont été mis en évidence pour le fragment incluant une portion de la région contrôle de l'ADN

mitochondrial, à partir de 248 individus échantillonnés dans l'Atlantique nord-ouest. Dans l'Atlantique nord-est, 158 individus analysés ont défini 12 haplotypes pour le même fragment d'ADN mitochondrial (Stanley *et al.* 1996). Cependant, dans le Pacifique, 225 haplotypes ont été identifiés, grâce à l'analyse de 778 individus, pour ce même fragment (Westlake & O'Corry-Crowe 2002). Cette différence dans le nombre d'haplotypes observés s'explique par le nombre d'individus échantillonnés, mais également par l'étendue de la zone géographique couverte. En effet, l'étude de Westlake & O'Corry-Crowe (2002) s'étend sur les deux côtes du Pacifique, de la côte est du Canada jusqu'au Japon, alors que l'échantillonnage de l'Atlantique nord-est de l'étude de Stanley *et al.* (1996) est restreinte aux côtes du Royaume Uni, à la mer du Nord et à la mer Baltique, ainsi qu'un site en Islande. La présente étude se limite à la côte est du Canada et à un site dans la Baie d'Hudson.

Le phoque commun de l'Atlantique nord-ouest est protégé depuis la fin des années 1980 (ROMM 2004). Les trois populations qui ont été observées au cours de cette étude peuvent servir de base à la mise en place d'un plan global de protection de cette espèce dans l'Atlantique nord-ouest. Certaines faiblesses, particulièrement au niveau de l'échantillonnage, limitent la portée de cette étude. Il serait judicieux d'accroître à environ 20 individus les effectifs d'échantillons provenant de la côte sud de la Nouvelle Écosse (NS), du Cap Breton (CB), de l'est de Terre-Neuve (ENF) et de la côte du Labrador (LAB), afin d'augmenter la puissance des analyses. De même, il serait intéressant d'analyser des échantillons provenant de la côte nord du golfe du Saint-Laurent, de la côte atlantique des États-Unis, du nord-est de Terre-Neuve et du nord du Labrador, ainsi que des échantillons

de phoques communs de la sous-espèce des Lacs des Loups Marins, *Phoca vitulina mellonae*, sur la Péninsule d'Ungava. Ainsi, l'aire de distribution de l'espèce serait couverte et l'analyse de ces échantillons pourrait permettre de mieux appréhender la structure génétique de cette espèce et éventuellement des facteurs (ex comportement) qui la détermine.

Le but de la présente étude était de décrire la structure des populations de phoque commun de l'estuaire et du golfe du Saint-Laurent et les populations de la côte Atlantique. Elle montré l'existence d'un flux de gènes entre ces deux zones rejetant de ce fait l'hypothèse de l'isolement génétique des populations de l'estuaire et du golfe du Saint-Laurent. Cette étude visait également à décrire la structure de la population de phoque commun de l'Atlantique nord-ouest dans la perspective de la création d'une Zone de Protection Marine (ZPM) en bordure du Parc Marin Saguenay—Saint-Laurent pour la conservation du béluga et du phoque commun. Le phoque commun de l'estuaire et du golfe du Saint-Laurent est protégé depuis la fin des années 1970 mais la population de l'estuaire n'a pas augmenté depuis (ROMM 2004). Les sites Bic et Métis (inclus dans le projet de ZPM) présentent une forte variabilité génétique. Les chiots de phoque commun avec une forte variabilité génétique présentent une mortalité néonatale plus faible (Coltman *et al.* 1998). Il semble donc que la raison de la stagnation de l'effectif de phoques communs de l'estuaire du Saint-Laurent ne soit pas liée à la variabilité génétique. Des mesures de conservation, notamment la création d'une ZPM, sont cependant nécessaires pour préserver cette forte variabilité génétique observée à Bic et Métis.

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Annexe 1. Mitochondrial DNA sequence (Árnason & Johnsson 1992) analysed in the present study. The PCR- and the sequencing-primers are underlined. Positions of the mitochondrial genes are indicated. The names of primers do not correspond to the position numbers because they are related to the positions in the human genome.

15781 ctaggggccctacttctcattctagtcctaacactactagtgctattctcacccgacctg

15841 ttaggagacccagacaactatatccctccgaatcccctaagcaccccaccacatatcaaa L15926

15901 cccgaatggtacttcctatttgcctacgcaatcttacgatccatccccaacaaactagga

15961 ggagtactagccctagtactctccattcttgtcctcgctatcatacccctactccacaca

16021 tcaaaacaacgaggaataatattccgacccatcagccaatgcctattctgattcctagta

16081 gcagacctactcacactaacatgaatcggaggacaaccagtcgaacacccttatatcacc

16141 gttggtcaactagcctcaatcctatactttacaatcctcctagtactcatacccattgcc

Cytochrome *b* ┐┌ tRNA Thr

16201 agcatcatcgaaaataacattctaaaatgaagagtctttgtagtatactatattaccttg

┐┌ tRNA Pro

16261 gtcttgtaaaccaaaaatggaggacacaactctccctaagactcaaggaagaggtaaaca L15829

┐ control region

16321 accccaccaccagcacccaaagctgacattctaattaaactattccctgacacccaccca

16381 atccccccttcactcctcaattcatataatagtaccaccttactgtgctatcacagtatt

16441 cacgcacctggcttatgtacttcgtgcattgcatgccccccccccatcctcggacccccta

16501 tgtatatcgtgcattaatggtttgccccatgcatataagcatgtacatgaagtggttgat

16561 tttacatatatggcatataattgtaacaccaagttctaaagcataattacctgttatgaa

16621 cgcatttcacctagtccacgagccttaatcaccatgcctcgggaaatcagcaacccttgt

16681 gaaacgtgtacctagatctcgctccgggcccataacatgtgggggtttctatactggaac

16741 tatacctggcatctggttcttacttcagggccatgaaatctctagaattcaatcctacta

16801 acccttcaaatgggacatctcgatgg

PvH00002 actaatgactaatcagcccatgatcacacataactgtggtgtcatgcatttggtatcttt PvH00034

61 taaatttttaggggggaaagcggtatcactcagctatgaccgtaaaggtctcgacgcagt

Annexe 2. Summary statistics for 13 *Phoca vitulina concolor* samples collected in the Northwest Atlantic (NWA) and one *Phoca vitulina vitulina* sample from the Northeast Atlantic, surveyed at ten microsatellite loci: number of individuals scored (N), number of alleles (N_{all}), non biased expected heterozygosity ($H_{n.b.}$), observed heterozygosity (H_o), allelic richness based on all samples [R_S (all)] and based on sample with more than ten individuals [R_S (≥10)], F-statistics according to Weir and Cockerham (1984) (F_{IS} W&C), probability value associated to the F_{IS} (*p*-val). Private alleles are shown in bold characters. Acronyms are as in table 1.

LOCUS	BIC	MET	ANI	PEI	WNF	PB	ENF	CB	NS	BF	LAB	HB	SI	NWA	SW
HG6.1															
(N)	48	21	16	26	25	24	4	5	7	22	6	19	27	250	31
140	0.000	0.000	0.000	0.000	0.080	0.042	0.000	0.000	0.071	0.000	0.000	0.000	0.000	0.014	0.081
142	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.016
144	0.875	0.952	0.906	0.673	0.920	0.729	0.750	0.600	0.857	0.705	0.750	0.842	1.000	0.838	0.000
146	0.031	0.000	0.063	0.000	0.000	0.083	0.000	0.400	0.071	0.227	0.000	0.026	0.000	0.050	0.032
148	0.010	0.000	0.000	0.289	0.000	0.146	0.250	0.000	0.000	0.046	0.250	0.105	0.000	0.068	0.871
152	0.083	0.048	0.000	0.039	0.000	0.000	0.000	0.000	0.000	0.023	0.000	0.026	0.000	0.028	0.000
154	**0.000**	**0.000**	**0.031**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.002**	**0.000**
N_{all}	4	2	3	3	2	4	2	2	3	4	2	4	1	6	4
$H_{n.b}$	0.229	0.093	0.179	0.471	0.150	0.448	0.429	0.533	0.275	0.460	0.409	0.286	0.000	0.290	0.237
H_o	0.229	0.095	0.188	0.462	0.160	0.542	0.500	0.400	0.286	0.318	0.500	0.316	0.000	0.268	0.258
R_s(all)	1.656	1.268	1.532	2.105	1.411	2.295	1.964	1.995	1.857	2.202	1.909	1.829	1.000	1.863	1.692
R_s(≥10)	2.885	1.894	2.863	1.968	2.792	3.806	------	------	------	3.510	------	1.000	3.471	3.549	3.112
F_{IS} W&C	-0.002	-0.026	-0.047	0.021	-0.067	-0.215	-0.200	0.273	-0.043	0.313	-0.250	-0.108	------	0.077	-0.088
p-val	0.550	1.000	1.000	0.513	1.000	1.000	1.000	0.601	1.000	0.065	1.000	1.000	NA	0.022	1.000

Annexe 2. Continued

LOCUS	BIC	MET	ANI	PEI	WNF	PB	ENF	CB	NS	BF	LAB	HB	SI	NWA	SW
HG6.3															
(N)	48	21	16	28	25	24	4	5	8	23	6	19	30	257	31
222	0.010	0.000	0.063	0.000	0.120	0.125	0.125	0.000	0.063	0.065	0.000	0.026	0.000	0.041	0.000
224	0.063	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.065	0.083	0.211	0.000	0.035	0.048
226	0.167	0.095	0.125	0.304	0.020	0.167	0.000	0.300	0.125	0.261	0.333	0.316	0.067	0.169	0.000
228	0.010	0.000	0.094	0.054	0.060	0.000	0.000	0.000	0.000	0.022	0.000	0.000	0.000	0.021	0.145
230	0.073	0.214	0.063	0.143	0.060	0.354	0.375	0.300	0.563	0.326	0.000	0.000	0.000	0.148	0.065
232	0.177	0.357	0.313	0.179	0.360	0.125	0.125	0.400	0.125	0.109	0.333	0.132	0.933	0.298	0.484
234	0.500	0.333	0.344	0.321	0.380	0.208	0.250	0.000	0.125	0.152	0.167	0.316	0.000	0.282	0.258
236	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.083**	**0.000**	**0.000**	**0.002**	**0.000**
238	0.000	0.000	0.000	0.000	0.000	0.021	0.125	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.000
N_{all}	7	4	6	5	6	6	5	3	5	7	5	5	2	9	5
$H_{n.b}$	0.689	0.724	0.776	0.763	0.718	0.788	0.857	0.733	0.675	0.799	0.803	0.758	0.127	0.779	0.683
H_o	0.646	0.714	0.688	0.714	0.840	0.917	0.500	1.000	0.750	0.739	0.833	0.526	0.133	0.658	0.742
R_s(all)	3.189	3.134	3.607	3.441	3.221	3.672	4.214	2.929	3.249	3.778	3.712	3.375	1.351	3.611	3.068
R_s(≥10)	5.380	3.991	5.975	5.398	4.879	5.577	------	------	------	6.494	------	1.926	4.736	5.887	4.756
F_{is} W&C	0.063	0.013	0.118	0.065	-0.173	-0.167	0.455	-0.429	-0.120	0.077	-0.042	0.312	-0.055	0.157	-0.088
p-val	0.281	0.539	0.270	0.336	0.961	0.981	0.088	1.000	0.907	0.301	0.744	0.019	1.000	0.002	0.846
HI16															
(N)	47	21	16	28	17	21	3	4	8	24	6	16	30	241	31
140	0.011	0.167	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.016
144	0.734	0.310	0.875	0.821	0.706	0.714	0.667	0.875	0.625	0.521	0.750	1.000	0.683	0.701	0.984
146	0.075	0.191	0.031	0.054	0.177	0.143	0.167	0.000	0.250	0.313	0.250	0.000	0.017	0.114	0.000
148	0.075	0.143	0.094	0.125	0.088	0.143	0.167	0.125	0.125	0.167	0.000	0.000	0.300	0.129	0.000
150	0.106	0.191	0.000	0.000	0.029	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.039	0.000
N_{all}	5	5	3	3	4	3	3	2	3	3	2	1	3	5	2
$H_{n.b.}$	0.443	0.803	0.232	0.312	0.476	0.460	0.600	0.250	0.567	0.616	0.409	0.000	0.450	0.478	0.032
H_o	0.319	0.667	0.188	0.286	0.471	0.429	0.333	0.250	0.500	0.542	0.500	0.000	0.467	0.386	0.032
Rs (all)	2.324	3.716	1.663	1.862	2.349	2.257	3.000	1.750	2.509	2.589	1.909	1.000	1.995	2.394	1.097
Rs (≥10)	4.122	4.999	2.874	3.820	2.877	2.999	------	------	------	3.000	------	2.467	1.000	4.078	1.452
F_{is} W&C	0.282	0.173	0.196	0.087	0.012	0.070	0.500	0.000	0.125	0.123	-0.250	------	-0.037	0.193	0.000
p-val	0.006	0.105	0.193	0.380	0.597	0.416	0.212	1.000	0.477	0.275	1.000	NA	0.620	0.000	1.000
Lc28															
(N)	48	21	16	22	24	24	4	4	6	22	6	14	28	239	31
138	0.073	0.071	0.000	0.000	0.167	0.042	0.000	0.000	0.000	0.023	0.000	0.000	0.000	0.044	0.000
140	0.000	0.000	0.000	0.000	0.000	0.000	0.125	0.000	0.000	0.000	0.000	0.357	0.000	0.023	0.000
142	0.448	0.595	0.313	0.341	0.583	0.750	0.750	0.500	0.667	0.432	0.250	0.000	0.375	0.456	0.210
144	0.323	0.167	0.406	0.659	0.146	0.167	0.125	0.375	0.250	0.341	0.667	0.321	0.482	0.337	0.645
146	0.115	0.167	0.250	0.000	0.042	0.042	0.000	0.125	0.000	0.182	0.083	0.321	0.036	0.107	0.145
148	0.042	0.000	0.031	0.000	0.063	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.107	0.029	0.000
150	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.083	0.023	0.000	0.000	0.000	0.004	0.000
N_{all}	5	4	4	2	5	4	3	3	3	5	3	3	4	7	3
$H_{n.b.}$	0.682	0.599	0.696	0.460	0.618	0.415	0.464	0.679	0.530	0.679	0.530	0.691	0.625	0.665	0.527
H_o	0.646	0.524	0.813	0.500	0.750	0.375	0.500	0.750	0.333	0.682	0.500	0.786	0.714	0.623	0.484
Rs (all)	3.018	2.757	2.927	1.932	2.891	2.161	2.500	2.750	2.409	2.904	2.409	2.807	2.651	2.942	2.398
Rs (≥10)	4.656	3.968	3.875	4.764	2.000	3.663	------	------	------	4.273	------	3.743	3.000	4.887	2.997
F_{is} W&C	0.054	0.129	-0.175	-0.090	-0.219	0.098	-0.091	-0.125	0.394	-0.005	0.062	-0.144	-0.145	0.063	0.084
p-val	0.333	0.247	0.911	0.819	0.990	0.354	1.000	0.768	0.265	0.608	0.508	0.856	0.907	0.011	0.335

Annexe 2. Continued

LOCUS	BIC	MET	ANI	PEI	WNF	PB	ENF	CB	NS	BF	LAB	HB	SI	NWA	SW
Lw11															
(N)	48	21	16	28	24	22	4	3	8	22	6	14	28	244	31
165	0.000	0.000	0.063	0.000	0.000	0.046	0.000	0.000	0.000	0.023	0.000	0.000	0.000	0.010	0.000
167	0.115	0.191	0.125	0.071	0.042	0.023	0.000	0.000	0.125	0.046	0.167	0.000	0.018	0.076	0.000
169	0.354	0.405	0.188	0.429	0.229	0.136	0.000	0.000	0.313	0.341	0.417	0.036	0.643	0.328	0.645
171	0.250	0.167	0.281	0.161	0.292	0.477	0.500	0.500	0.250	0.341	0.000	0.107	0.268	0.262	0.129
173	0.250	0.214	0.313	0.161	0.354	0.205	0.500	0.500	0.250	0.227	0.417	0.536	0.054	0.250	0.226
175	0.031	0.024	0.031	0.161	0.083	0.114	0.000	0.000	0.063	0.023	0.000	0.107	0.018	0.059	0.000
177	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.214	0.000	0.014	0.000
N_{all}	5	5	6	6	5	6	2	2	5	6	3	5	5	7	3
$H_{n.b.}$	0.743	0.743	0.792	0.747	0.744	0.713	0.571	0.600	0.808	0.729	0.682	0.667	0.521	0.753	0.525
H_o	0.667	0.762	0.813	0.714	0.667	0.727	0.500	0.333	0.625	0.591	0.667	0.643	0.250	0.631	0.548
Rs (all)	3.303	3.332	3.669	3.460	3.302	3.296	2.000	2.000	3.712	3.204	2.758	3.072	2.368	3.405	2.379
Rs (≥10)	4.632	4.667	5.863	4.807	5.442	5.504	------	------	------	5.146	------	3.882	5.000	5.383	2.995
F_{IS} W&C	0.104	-0.026	-0.026	0.044	0.106	-0.021	0.143	0.500	0.239	0.194	0.024	0.037	0.525	0.162	-0.046
p-val	0.142	0.668	0.685	0.412	0.267	0.648	0.775	0.599	0.199	0.101	0.646	0.530	0.000	0.000	0.713
Lw20															
(N)	48	21	16	29	25	24	4	5	8	23	6	19	29	257	31
127	0.250	0.452	0.313	0.224	0.320	0.063	0.125	0.400	0.500	0.239	0.167	0.132	0.621	0.296	0.000
129	0.031	0.000	0.031	0.000	0.040	0.188	0.000	0.000	0.000	0.044	0.167	0.026	0.017	0.041	0.000
131	0.458	0.238	0.281	0.655	0.340	0.417	0.750	0.300	0.313	0.478	0.333	0.290	0.310	0.403	0.194
133	0.240	0.310	0.313	0.121	0.300	0.333	0.125	0.300	0.188	0.217	0.250	0.553	0.052	0.249	0.807
135	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.022	0.083	0.000	0.000	0.008	0.000
139	**0.000**	**0.000**	**0.063**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.004**	**0.000**
N_{all}	5	3	5	3	4	4	3	3	3	5	5	4	4	6	2
$H_{n.b.}$	0.676	0.659	0.744	0.515	0.705	0.691	0.464	0.733	0.658	0.679	0.833	0.609	0.525	0.688	0.317
H_o	0.729	0.667	0.750	0.483	0.600	0.708	0.500	1.000	0.875	0.609	0.833	0.632	0.655	0.665	0.323
R_s (all)	2.925	2.717	3.256	2.353	2.971	2.966	2.500	2.929	2.725	2.981	3.924	2.645	2.291	2.949	1.741
R_s (≥10)	4.150	3.000	4.863	3.811	2.993	3.934	------	------	------	4.461	------	3.351	3.736	3.983	2.000
F_{IS} W&C	-0.080	-0.013	-0.008	0.063	0.151	-0.026	-0.091	-0.429	-0.361	0.106	0.000	-0.038	-0.255	0.033	-0.017
p-val	0.838	0.613	0.626	0.398	0.186	0.675	1.000	1.000	0.978	0.302	0.672	0.684	0.971	0.334	0.731
Pvc19															
(N)	47	21	16	29	25	24	4	5	8	24	6	19	30	258	30
96	0.277	0.381	0.406	0.500	0.300	0.604	0.000	0.500	0.500	0.271	0.250	0.132	0.367	0.357	0.550
104	0.723	0.619	0.594	0.500	0.700	0.396	1.000	0.500	0.500	0.729	0.750	0.868	0.633	0.643	0.450
N_{all}	2	2	2	2	2	2	1	2	2	2	2	2	2	2	2
$H_{n.b.}$	0.405	0.483	0.498	0.509	0.429	0.489	0.000	0.556	0.533	0.403	0.409	0.235	0.472	0.460	0.503
H_o	0.340	0.476	0.438	0.517	0.600	0.292	0.000	0.600	0.250	0.375	0.500	0.263	0.467	0.411	0.633
R_s (all)	1.865	1.955	1.968	1.977	1.898	1.959	1.000	2.000	1.993	1.868	1.909	1.599	1.943	1.928	1.972
R_s (≥10)	2.000	2.000	2.000	2.000	2.000	2.000	------	------	------	2.000	------	2.000	1.999	2.000	2.000
F_{IS} W&C	0.160	0.015	0.125	-0.017	-0.412	0.408	------	-0.091	0.548	0.072	-0.250	-0.125	0.012	0.107	-0.264
p-val	0.220	0.670	0.505	0.677	1.000	0.056	NA	0.866	0.191	0.538	1.000	1.000	0.623	0.058	0.969

Annexe 2. Continued

LOCUS	BIC	MET	ANI	PEI	WNF	PB	ENF	CB	NS	BF	LAB	HB	SI	NWA	SW
Pvc78															
(N)	48	21	16	29	25	24	4	5	8	24	6	19	30	259	30
144	0.802	0.976	0.719	0.862	0.820	0.917	0.875	0.800	1.000	0.792	1.000	0.790	0.967	0.859	0.967
146	0.146	0.024	0.156	0.103	0.160	0.083	0.125	0.200	0.000	0.188	0.000	0.211	0.033	0.116	0.033
148	0.052	0.000	0.125	0.035	0.020	0.000	0.000	0.000	0.000	0.021	0.000	0.000	0.000	0.025	0.000
N_{all}	3	2	3	3	3	2	2	2	1	2	1	2	2	3	2
$H_{n.b.}$	0.336	0.048	0.458	0.249	0.308	0.156	0.250	0.356	0.000	0.345	0.000	0.341	0.066	0.248	0.066
H_o	0.250	0.048	0.500	0.207	0.360	0.167	0.250	0.400	0.000	0.292	0.000	0.421	0.000	0.224	0.000
Rs (all)	1.903	1.143	2.258	1.695	1.790	1.425	1.750	1.867	1.000	1.859	1.000	1.785	1.192	1.666	1.192
Rs (≥10)	2.824	1.667	3.000	2.559	2.722	1.975	------	------	------	2.583	------	1.720	2.000	2.529	1.720
F_{IS} W&C	0.258	0.000	-0.096	0.172	-0.174	-0.070	0.000	-0.143	------	0.157	------	-0.241	1.000	0.099	1.000
p-val	0.031	1.000	0.798	0.267	1.000	1.000	1.000	1.000	NA	0.234	NA	1.000	0.015	0.221	0.015
SGPV11															
(N)	48	21	16	29	25	24	4	5	8	23	6	19	28	256	31
140	0.073	0.024	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.083	0.000	0.000	0.018	0.000
146	**0.010**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.002**	**0.000**
152	0.000	0.000	0.000	0.000	0.000	0.021	0.250	0.000	0.000	0.000	0.000	0.000	0.071	0.014	0.000
154	0.417	0.500	0.469	0.707	0.480	0.396	0.000	0.400	0.563	0.565	0.167	0.079	0.214	0.422	0.726
156	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.022**	**0.000**	**0.000**	**0.000**	**0.002**	**0.000**
158	0.021	0.119	0.031	0.017	0.020	0.000	0.000	0.100	0.000	0.044	0.000	0.000	0.000	0.025	0.000
160	0.417	0.333	0.406	0.276	0.400	0.563	0.750	0.500	0.375	0.370	0.750	0.658	0.000	0.387	0.274
162	0.063	0.024	0.094	0.000	0.000	0.021	0.000	0.000	0.000	0.000	0.000	0.263	0.018	0.043	0.000
164	0.000	0.000	0.000	0.000	0.100	0.000	0.000	0.000	0.063	0.000	0.000	0.000	0.679	0.086	0.000
166	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.018**	**0.002**	**0.000**
N_{all}	6	5	4	3	4	4	2	3	3	4	3	3	5	10	2
$H_{n.b.}$	0.650	0.639	0.625	0.431	0.611	0.537	0.429	0.644	0.575	0.554	0.439	0.505	0.497	0.663	0.405
H_o	0.625	0.667	0.625	0.379	0.640	0.542	0.500	0.400	0.750	0.609	0.500	0.579	0.571	0.578	0.484
Rs (all)	2.816	2.760	2.620	1.974	2.556	2.207	1.964	2.595	2.348	2.322	2.273	2.275	2.369	2.922	1.867
Rs (≥10)	4.593	4.331	3.874	3.548	2.483	3.167	------	------	------	3.461	------	3.944	2.986	5.061	2.000
F_{IS} W&C	0.039	-0.045	0.000	0.123	-0.048	-0.008	-0.200	0.407	-0.333	-0.102	-0.154	-0.151	-0.154	0.129	-0.200
p-val	0.413	0.707	0.600	0.335	0.693	0.613	1.000	0.238	0.913	0.783	1.000	0.870	0.915	0.001	0.955
SGPV10															
(N)	47	21	16	29	25	24	4	5	8	24	6	19	30	258	30
127	**0.011**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.002**	**0.000**
131	0.011	0.000	0.000	0.000	0.040	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.000
133	0.819	0.952	0.938	0.879	0.900	0.979	1.000	1.000	0.938	0.917	0.833	0.974	0.750	0.890	0.783
135	0.149	0.048	0.063	0.121	0.060	0.021	0.000	0.000	0.063	0.083	0.167	0.026	0.250	0.101	0.217
137	**0.011**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.002**	**0.000**
N_{all}	5	2	2	2	3	2	1	1	2	2	2	2	2	5	2
Hn.b.	0.310	0.093	0.121	0.216	0.189	0.042	0.000	0.000	0.125	0.156	0.303	0.053	0.381	0.199	0.345
Ho	0.213	0.095	0.125	0.241	0.040	0.042	0.000	0.000	0.125	0.167	0.333	0.053	0.300	0.155	0.300
Rs (all)	1.822	1.268	1.345	1.555	1.552	1.125	1.000	1.000	1.375	1.425	1.773	1.158	1.837	1.531	1.785
Rs (≥10)	2.889	1.894	1.988	2.733	1.993	1.583	------	------	------	1.975	------	2.000	1.737	2.246	2.000
F_{IS} W&C	0.315	-0.026	-0.034	-0.120	0.791	0.000	------	------	0.000	-0.070	-0.111	0.000	0.216	0.221	0.133
p-val	0.020	1.000	1.000	1.000	0.001	1.000	NA	NA	1.000	1.000	1.000	1.000	0.243	0.000	0.413

Annexe 2. Continued

LOCUS	BIC	MET	ANI	PEI	WNF	PB	ENF	CB	NS	BF	LAB	HB	SI	NWA	SW
All															
N	48	21	16	29	25	24	4	5	8	24	6	19	30	259	31
N_{all}	4.7	3.4	3.8	3.2	3.8	3.7	2.4	2.3	3	4	2.8	3.1	3	6	2.7
$H_{n.b}$	0.518	0.489	0.512	0.466	0.493	0.472	0.410	0.506	0.478	0.539	0.478	0.406	0.366	0.466	0.364
H_o	0.468	0.471	0.513	0.448	0.513	0.472	0.359	0.522	0.455	0.489	0.517	0.412	0.355	0.455	0.381
Rs (all)	2.482	2.405	2.485	2.394	2.235	2.182	2.318	2.513	2.336	2.189	2.358	1.900	2.155	2.521	1.919
Rs (≥10)	3.813	3.241	3.718	3.541	3.018	3.421	------	------	------	3.690	------	2.603	2.967	3.960	2.503
F_{is} W&C	0.097	0.035	-0.001	0.037	-0.037	0.000	0.140	-0.005	0.058	0.094	-0.080	-0.019	0.029	0.035	-0.046
p-val	0.001	0.288	0.539	0.219	0.784	0.507	0.145	0.526	0.270	0.030	0.835	0.623	0.300	0.011	0.800