

Genetic structure of fjord- and offshore- populations of Greenland halibut *Reinhardtius hippoglossoides*, investigated using microsatellite DNA analysis.

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Part II

Paper draft

High levels of gene flow and lack of genetic structure between fjord- and offshore- populations of Greenland halibut *Reinhardtius hippoglossoides* in Greenland waters revealed by microsatellite DNA analysis.

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Preface

This study was conducted as part of my master thesis at the University of Århus in collaboration with the Danish technical University DTU (formerly DFU) in Silkeborg and financed by the Greenland Institute of Natural Resources.

During this project numerous people have been willing to help me and I therefore owe much gratitude to the many people who have assisted me during the project. I would like to thank the people involved in the project especially Helle Siegstad from The Greenland Institute of Natural Resources and my supervisors Michael Møller Hansen from DTU in silkeborg and Volker Loeschcke at University of Århus. Also a special thanks to Stuart Barker from University of New England, for comments and help on running TMVP. Thanks to the laboratory staff in Silkeborg Karen-Lise Dons Mensberg, Dorte Meldrup and Noortje de Jong for many interesting discussions and help during my laboratory work and Kaj Sünksen, Sofie Ruth Jerimiassen, Bjarne Lyberth From the Greenland Institute of Natural Resources and everybody else involved in collecting DNA samples. I would also like to thank Peter Foged Larsen, Jens Jønsson, Morten Limborg, and other fellow students for being technical and theoretical sparring partners. Finally I would like to thank my girlfriend Anja for her support and help.

In 2002 I had the great fortune of being employed as a research assistant for the Greenland Institute of Natural Resources and since then I have spent several months every summer working in the laboratory of the research vessel RV Pâmiut. During the 15 cruces (more than half a year all together) spent in the trawlers laboratory, cutting otoliths from fishes or measuring the carapaxes of thousands of shrimps, sometimes in harsh weather conditions, I have had the great pleasure of working with many kind and interesting people, whom with their great humor and good spirits have given me so many cheerful memories and happy moments. I would therefore like to thank the skipper Børge, the Greenlandic fishermen, the crew and biologists for a great time onboard Pâmiut.

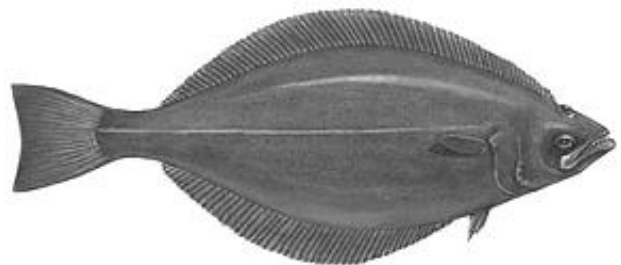
Rasmus Nygaard

Summary

The purpose of this study was to clarify the genetic relationship between the different populations of Greenland halibut in the waters around Greenland. Although Greenland halibut is an important commercial fisheries resource, many aspects of the biology of this species are still unknown, due to the deepwater distribution and harsh arctic environment, making it difficult to study. Therefore it was hoped that a population genetic study would help clarify aspects of the population structure and whether the current management units are correct. The results are however in accordance with earlier studies, both genetic, tagging, drift simulations and biological, indicating high levels of gene-flow between all areas and that Greenland halibut, at least genetically, constitutes one population throughout the entire study area from East Greenland to the Baffin bay and inside the North-West Greenlandic fjord systems. However, this further emphasize the importance of protecting the offshore Davis Strait and Iceland population, since they inhabit the only known major spawning areas and all remaining inshore- and fjord- populations may be highly dependent on recruitment from these populations.



Greenland halibut anterior view. From Smidt (1969)



Part I General Introduction

The Greenland Halibut - *Reinhardtius Hippoglossoides*

Biology

Greenland Halibut *Reinhardtius Hippoglossoides* (Walbaum, 1792) is large flatfish species belonging to the family Pleuronectidae (Righteye Flounders). It is distributed throughout the arctic areas of both the North Atlantic and the North Pacific at depths between 200 and 1600 meters (Jensen 1935, Schmidt 1969, Jørgensen 1997a), although it has been caught at depths of more than 2000 meters by commercial fishing vessels. Unlike most flatfish species, it is pigmented on both sides of the body and the left eye is positioned on the dorsal ridge of the forehead. The physical appearance suggests that it may be able to swim in a vertical position, although this could not be confirmed by video analysis of swimming behavior in front of a bottom trawl (Albert et al. 2003). The average lifespan of males is shorter than for females. Whereas males rarely reach 12 years of age and a size of 70 cm, females can reach a length of more than 130 cm and have been estimated to reach ages of more than 20 years (Bowering and Nedreaas 2001) although age validation based on otoliths is increasingly difficult for individuals above 8 years (Sünksen K. Greenland Institute of Natural Resources, personal comm.).

The topography and Ocean currents.

The seas around Greenland have a varied topography, which significantly influences the current patterns and the physical properties of the area. In the coastal areas, large deep fjord systems are separated from the offshore areas by a shelf, which sharply drops to depths of more than 1000 meters. The Baffin Bay basin is separated from the Labrador Sea by a sea ridge near the polar circle at 66°N, with a maximum depth of around 600 meters, creating the shallow Davis Strait between West Greenland and Canada.

In the arctic areas, the ocean currents are mainly driven by the formation of a thick layer of sea ice during the winter months. Upon this freezing, a heavy layer of cold and high salinity water is formed, which sinks and is replaced by large quantities of surface water including the North Atlantic current, a branch of the Gulf Current (Buch 1990). A part of the water drawn towards the polar ocean is the Irminger Current running west off Iceland where it encounters the cold East

Greenlandic current and is forced south towards Cape Farewell, the south tip of Greenland. The general Ocean circulation patterns modified from Pedersen and Smidt (2000) are illustrated below.

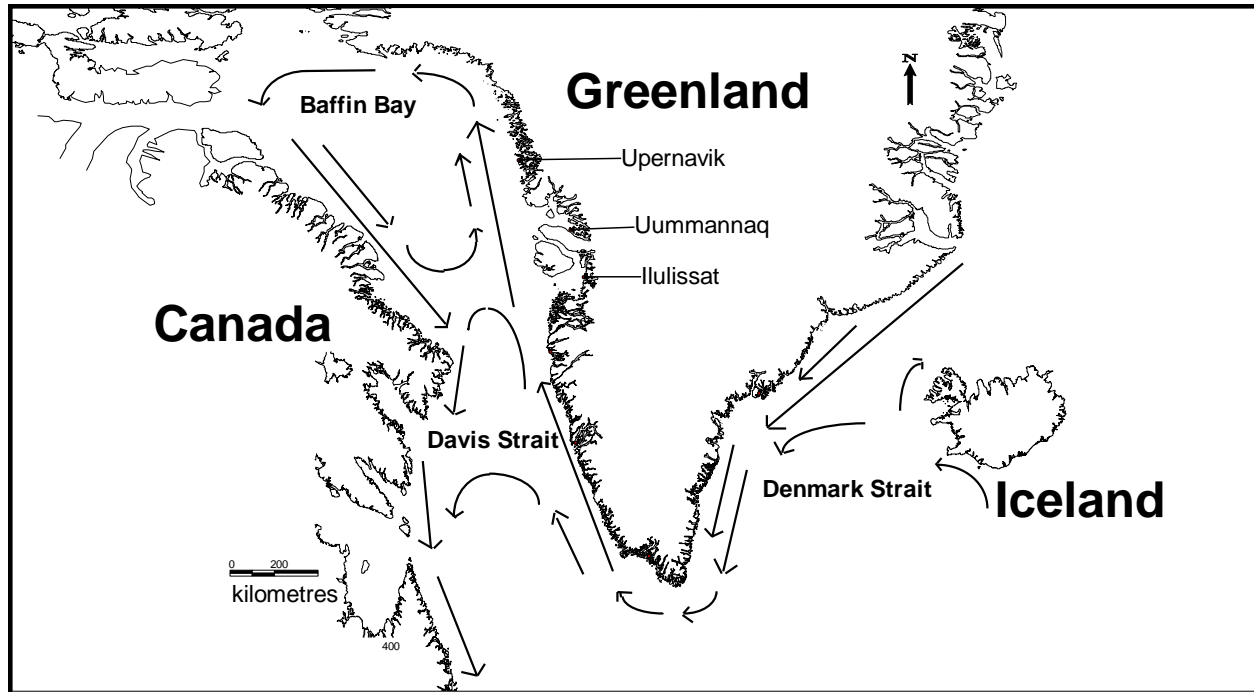


Figure 1. Ocean circulation patterns (modified from Pedersen and Smidt 2000)

Spawning and spawning areas

The main spawning area of Greenland halibut in Greenlandic waters has been identified as the Davis strait (Jensen 1935, Schmidt 1969 Jørgensen 1997a, Simonsen and Gundersen 2005), although ripe females have also been found in East Greenland near Kap Bille (Gundersen et al. 2001) the Baffin Bay (Gundersen et al. 2004) and inside the deep Uummannaq fjord in West Greenland (Schmidt 1969). However, since only a few ripe individuals have been caught in the Baffin Bay and the Uummannaq fjord there is no proof that substantial spawning takes place within these areas. Therefore, it is still unresolved whether or not Greenland Halibut spawn in the Baffin Bay and the inshore areas of West Greenland (Simonsen and Gundersen, 2005). Also, Stenberg et al. (in prep.) hypothesized that eggs and larvae spawned within the Baffin Bay would perish due to the extremely low sea temperatures within this area. Spawning takes place during the winter months (November to February) and the larvae stay pelagic until they have reached a

length of at least 65 – 70 mm before settling takes place in the autumn (August – September) (Jensen 1935; Smidt 1969).

Transport of egg and larvae

Numerous studies have indicated the importance of passive pelagic transport of fish eggs and larvae via ocean currents in shaping the genetic composition of fish populations (I will return to this subject later in this paper). The eggs and larvae of Greenland halibut stay pelagic for more than half a year and during this stage they can be transported via ocean currents for unusually long distances compared to other marine fish species.

Drift simulations of eggs and larvae of Greenland halibut performed by Stenberg et al. (in prep.) have theoretically confirmed high exchange of individuals between areas. Their simulations showed that 0-group individuals spawned in the Davis Strait can drift as far as the banks of Baffin and Labrador or recruit to Store Hellefiske Bank in the Davis Strait. And individuals spawned in East Greenland and West of Iceland can remain in these areas or be transported to SW Greenland. However, although the general transport of eggs and larvae follow the ocean current patterns (see fig 1.), they found no uniform flow direction, since egg and larvae either drifted to new areas and mixed with local populations or remained in the natal population. Based on their simulations, Stenberg et al. (in prep) suggested that this connectivity plays an important role in shaping local and metapopulation dynamics and genetic structure of Greenland halibut. Their study did however not include larval transport into the large West Greenlandic fjord systems.

Distribution and migration patterns

Especially sub-adult individuals <20cm undertake pronounced daily vertical feeding migrations towards the surface, where they feed on euphasiacea (krill) during the night (Jørgensen 1997). But also medium sized individuals are sometimes caught in Salmon driftnets at the surface probably while feeding on capelin (Smidt, 1969). These observations further emphasize the for a flatfish unusual bathypelagic behavior, at least for sub-adult individuals, which may highly increase the migration potential and exchange of individuals between areas compared to other flatfish species.

The spatial distribution of adult individuals also seems to change considerably throughout the year and they are known to disappear from certain areas during the year, which could indicate nonrandom migration patterns. In the Davis Strait Greenland Halibut migrates to deeper waters during the autumn, which has been interpreted as spawning aggregation (Jørgensen, 1997). Tagging experiments have demonstrated high fidelity to tagging sites, especially within the fjord areas, where around 99% of the tagged individuals were recaptured within the release area, whereas offshore individuals seem to be exchanged between areas at a higher rate (Boje 2002) and the general long distance migration patterns is in the opposite direction of the passive transport of eggs and larvae studied by Stenberg et al. (in prep.). There are therefore indications of directional migration patterns that could maintain the persistence of separated genetic populations in the offshore areas.

The existing view is that the Greenland halibut within the large West Greenlandic fjord systems function as sink populations in a metapopulation community, where recruitment is dependent on larval transport from offshore areas, and spawning within the fjords is negligible (Riget and Boje 1989; Boje and Jørgensen 1990). It is therefore presumed that the fisheries for Greenland halibut within the fjords have no negative effect on the overall recruitment and persistence of the populations and a TAC (Total Allowable Catch) is meaningless for the inshore West Greenlandic fjord areas (Boje J. and Jørgensen O.A. 1990).

Preceding population genetic studies of Greenland halibut

A variety of methods has been utilized to reveal the stock structure of Greenland Halibut. Using 3 allozymes (MDH-1, PGM and PHI-2), Fairbairn (1981) found indications, that Greenland halibut form a single homogeneous stock in the North-West Atlantic, with a partially isolated stock in the Gulf of St. Lawrence, and that Greenland halibut in the Bering Sea form an isolated stock, which differs from the North Atlantic at the subspecies level. The indications of a single North Atlantic population (West and East Greenland, Jan Mayen, The Barents Sea, The Faroe Islands and the Shetland Islands), were further supported in a study of 3 allozyme loci (GPI, IDHP and PGM) covering the North Atlantic and the Barents Sea (Igland and Nævdal 1986). However, Riget, Boje and Simonsen (1992) also using allozymes found indications that isolated populations existed in some West Greenlandic fjord systems, although the present study only included samples from one of these fjords (Disco Bay) and is therefore not directly comparable. Analysis

of mtDNA sequences identified 22 haplotypes, of which 3 are common in the North Atlantic area, and genotypic proportions revealed no significant differences between different areas (Vis et al. 1997). Parasites (Khan et al. 1982; Arthur and Albert 1993; Boje, Riget and Koie 1997), morphometrics (Bowering 1988) and meristic data (Riget and Boje 1989) have also been used in an attempt to reveal population structure of Greenland halibut.

All of these investigations suggest that Greenland halibut comprise a single panmictic stock across the entire North Atlantic Ocean, although some studies have indicated the presence of isolated populations in the West Greenlandic fjords (Riget, Boje and Simonsen 1992) and the Gulf of St. Lawrence (Fairbairn 1981). Many of these methods can however be criticized in their ability to reveal stock structure. Studies based on allozymes often rely on few loci with a few dominating alleles and corresponding low heterozygosities and therefore have weaker statistical power than highly variable microsatellite markers (Hedrick 1999; Schlötterer 2004). Also, since allozymes are important gene products, it may be unknown whether their variation reflects deterministic (selective) processes or stochastic (drift) processes (Ward 2000). However, even genetic differences created by directional selection may warrant management as separated populations in marine fishes, since such differences still indicate some degree of genetic isolation. Allozymes have been used to discriminate between eastern and western Greenlandic populations of the bathy-pelagic roughhead grenadier *Macrourus berglax* L. (Katsarou and Nævdal 2001). Morphometric data may fail to reveal stock structure since even highly genetically differentiated and completely isolated populations utilizing the same or very similar niches may exhibit morphological homogeneity due to stabilizing selection (Santos 2006). Meristic traits can also be subject to phenotypic plasticity. Although the number of vertebrae is heritable (Christiansen et al. 1988), it is generally accepted that environmental factors such as, temperature during embryonic development can strongly influence the number of vertebrae in fishes and have also been demonstrated to have profound effects on body shape (Marcil 2006). However, Rasmussen et al. (1999) concluded that the number of vertebrae is an inconclusive method for Greenland halibut in revealing population structure, since they found large deviations between identical areas sampled in different years. For these reasons neither allozymes nor morphological analysis are ideal methods for studying population structure and stock assessment. In a recent study Knutsen et al. (2007) used 9 microsatellite markers to study Greenland halibut and found indications of partially subdivided populations across the entire North Atlantic with some

evidence of isolation by distance following the ocean currents. Their study did, however, not include the Western Greenlandic fjords or temporally separated samples.

Exploitation

Greenland Halibut is very common in Greenland waters. From 1992 to 2005 the annual abundance and biomass estimates in West Greenland waters alone, have fluctuated between 260-512 mill individuals and 13000 - 40000 tons (Sünksen et al. 2005).

Since the major collapse in the cod fisheries in the 90'ies Greenland halibut have constituted the second most economic valuable fisheries resource, only surpassed by the shrimp fishery in Greenland. Whereas the shrimp fishery takes place onboard trawlers both in- and offshore, the fishery for Greenland Halibut within the fjord areas has traditionally been performed with longlines from small open boats or by dog sledges directly from the sea ice. Typically this fishery is carried out in the innermost parts of the ice-fjords at depths between 500 and 800 meters, although bigger vessels (>25 Feet) have entered the fishery during the last 10-15 years (Lyberth and Boje 2006). For this reason, Greenland halibut constitutes an important factor in upholding the traditional Inuit way of life.

Conservation genetics in the marine environment

Fish is the only major human food item that is primarily harvested directly from naturally occurring wild populations. Traditionally, the biological perspective of fishery management was dominated by ecology and population dynamics and little attention was directed towards an understanding of the genetics of these populations (Ryman & Utter 1987). However, fish species rarely reproduce randomly with con-specifics throughout their geographical range, but form a series of stocks or populations that are reproductively isolated in space or time (Ryman & Utter 1987).

Normally, the major long-term goals of conservation genetics are the retention of enough genetic variation within populations, so that future adaptation to changing environmental conditions, successful expansion or reestablishment in natural habitats is possible (Hedrick and Miller 1992). In natural populations of relatively large size, environmental uncertainty and natural catastrophes appear to be the most critical factors limiting persistence of populations and demographic or

genetic factors are of relatively little concern, while in small populations all factors can be important (Hedrick and Miller 1992). Since marine species are normally characterized by large effective population sizes N_e (Ward et al. 1994, Dewoody and Avise 2000), the objective in genetic studies of marine fishes is more often to establish correct management units to ensure proper management strategies of marine resources.

In general management strategies that ignore the genetic population structure are functionally inconsistent with the principles of conservation and biodiversity maintenance (Hedrick and Miller 1992) and ignoring the genetic structure of heavily exploited marine resources can have devastating effects if diverse, locally adapted and migratory populations intermingle seasonally and are managed under the assumption of panmixia (Ruzzante 2000; Laikre 2005). An example is the mixed stock fishery on various populations of cod in the Gulf of St. Lawrence (Ruzzante 2000)

Classic Genetic Theory

Genetic theory predicts that the amount of genetic variation within species should increase with effective population size, N_e , (Frankham 1995) and in general marine species (especially fishes and invertebrates) are characterized by large effective population sizes N_e , high heterozygosities, (Ward et al. 1994, Dewoody and Avise 2000) and high potential for gene flow, both due to high migration potential of most marine species and the environmental conditions of the oceans, where direct barriers to gene flow are rare. Genetic differentiation of local populations may be caused by **mutation**, **genetic drift** due to finite population size, and **natural selection** favoring adaptations to local environmental conditions, whereas the movement of gametes, individuals, and even entire populations, collectively called **gene flow**, will oppose genetic differentiation (Slatkin 1987). Determining the relative strengths of these opposing forces is however not always straight forward, and in this context marine fishes/organisms constitutes a special case.

Genetic drift is unpredictable change in gene/allele frequencies due to finite population size (Slatkin 1987) and due to genetic drift; isolated populations will lose genetic variation at a rate of $1/2N_e$ per generation, where N_e is the effective population size. This means that small populations lose genetic variation at a high rate, whereas genetic drift is a very slow process in large populations.

Genetic divergence

The most widely used measures in population genetic studies are Wrights F-statistics, developed by Wright (1921) to calculate inbreeding coefficients of domestic animals. Wrights F-statistics measures the amount of genetic variation within and between subpopulations at migration-drift equilibrium. A useful way of describing the genetic variation among different populations is to estimate the inbreeding coefficient F_{st} of samples from different populations. One way to estimate F_{st} is through the variance in allele frequencies among populations (σ_p^2) standardized by the mean allele frequency (p) at the given locus so that:

$$F_{st} = \sigma_p^2 / [p(1-p)]$$

F_{st} takes values between 0, where the both populations are genetically identical, and 1, where the populations fixated for different alleles in all loci (Hedrick 1999; Balloux and Lugon-Moulin, 2002). An F_{st} value of 1 is however purely theoretical for multiple microsatellites loci due to the high mutation rate in these genetic markers (Hedrick 1999). In general, values above 0.05 indicates some genetic differentiation between populations (Balloux and Lugon-Moulin, 2002), but for marine organisms even values below 0.01 may still be significant. Different F-statistic estimates for Greenland Halibut can be found in the appendix, but see also the paper.

Since F_{st} is based on variance in allele frequencies, it does not account for the stepwise mutation properties of microsatellites. Therefore, Slatkin (1995) proposed the related statistic, R_{st} , which is based on variation in repeat number and therefore accounts for the memory of the SSM. However, measures of genetic differentiation based on R_{st} in general shows higher variance than F_{st} based measures (Gaggiotti et al. 1999). Also, simulations performed by Gaggiotti et al. (1999) have demonstrated that when the number of loci is less than 10 and the sample size is less than 50, as in the present study, R_{st} based measures do not necessarily perform better than F_{st} and related measures. For the present study the Estimates of R_{st} for Greenland halibut are very low and can be found in the Appendix.

Gene flow in the marine environment

Gene flow between populations of marine fish species is often thought to be substantial both due to high dispersal rates and lack of barriers to gene flow (Ward 2000), but which amount of gene flow is sufficient to prevent genetic divergence between populations? A general rule of thumb has emerged that one migrant individual per local population per generation is enough to maintain the overall genetic diversity and prevent inbreeding depression in fragmented populations (Wang 2004). The rule stems from Wrights (1931) “infinite-island model” which assumes that an infinitely large population is subdivided into an infinitely number of identical “ideal” subpopulations with an equal distance between each subpopulation. (The term “ideal” refers to the Wright-Fisher idealized population which is a population with a constant migration rate between all populations, random mating, 1:1 sex ratio, Poisson distributed variance in reproductive success, non overlapping generations and a stable population size such that $N_e = N$). Under the assumptions of Wrights (1931) island model,

$$F_{st} \approx 1/(4N_e(m+u) + 1)$$

Where N_e is the effective population size of each subpopulation and m is the migration rate between populations (u is the mutation rate and can normally be ignored). Since m is the proportion of migrants and N_e is the effective population size then mN_e is the effective (actual) number of migrants entering a population each generation (Mills and Allendorf 1996)

Barriers in the marine environment

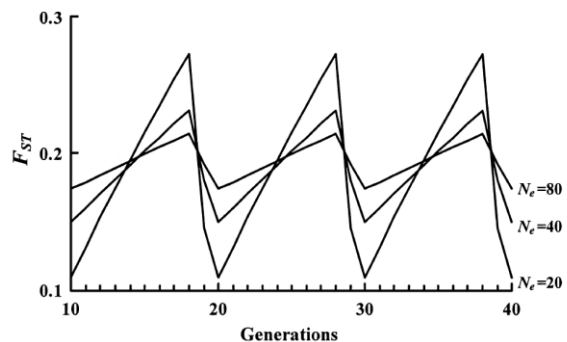
Barriers to gene flow in the marine environment have often been related to physical properties like temperature and salinity gradients (Nielsen et al. 2003; Nielsen et al. 2004; Jørgensen et al. 2005) in transition zones between oceans, seas and estuaries (Naciri et al. 1999) or shifts in ocean currents (Shaw et al. 2004; Santos et al 2006; Bernal-Ramírez and Adcock 2003), but significant genetic structuring has also been found over small geographical distances without obvious barriers to gene flow (Knutsen et al. 2003). Even behavioral differences, between fish populations or species could serve as barriers to gene flow, although this very interesting area is poorly studied in marine species. Homing behavior is mostly known from anadromous trout and salmon species, although some studies have found indications of homing behavior in marine

species. For instance Carlsson et al. 2007 proposed that the genetic structure of Bluefinn tuna is maintained by natal homing although individuals from different populations mix at feeding grounds through out the North Atlantic Ocean. For Greenland halibut the fact that migration patterns revealed by tagging studies are in the opposite direction of the passive transport of eggs and larvae could also be an indication of homing behavior (Stenberg et al. in prep.).

Since barriers to gene flow in the marine environment are often dependent on ocean current and direction, changes in these physical parameters may also lead to changes in the migration rate m between subpopulations, although such events may be rare. Common or periodic incidents of changes in the strength of ocean currents or directions are related to periodical changes in climate like El ninó in the Pacific Ocean or the North Atlantic oscilation in the North Atlantic Ocean. However, movement of water masses may also be influenced by climate change or metrological events and this could lead to sporadic and rare but intensive migration events.

Fluctuating migration rates.

If migration incidents are rare but intensive, as can be expected for marine organisms, genetic differentiation between subpopulations (F_{st}) will oscillate periodically. A small N_e within subpopulations and a large interval between migration incidents will increase the oscillations.



Changes in the degree of differentiation (F_{st}) as a function of time (in generations), assuming 10 immigrants every 10 generations into subpopulations of $N_e=20$, 40 or 80 assuming an Island model except for migration.

(After Wang 2004)

Mutation

The effect of mutation in homogenizing subpopulations is very small except when the mutation rate (u) is similar in magnitude to both the migration rate m and $1/N_e$ (Whitlock and McCauley 1999; Wang 2004). This is an unlikely case for far the majority of species, but at least the second parameter may often be fulfilled in oceanic organisms (large effective population sizes, N_e) and for microsatellite markers (high mutation rates). I will return to the subject later in this paper.

Selection

The effect of selection on genetic divergence between populations depends on the pattern of selection and on the selection intensity (Wang 2004). In general, the effect of selection is negligible compared to migration and genetic drift when the selection coefficient (s) is less than m or $1/N_e$ (Wang 2004)

Genetic differences in fitness-related traits and their plasticity may reflect adaptations to the local environment (Hutchings 2007) and indication of local adaptation in marine organisms are known. For instance Hutchings et al. (2007) used common-garden experiments, to demonstrate how different traits (larval growth and survival), and their plastic responses to food and temperature, differed across spatial scales at which microsatellite DNA failed to detect population structure. Although the evolutionary significance of population differences may be different for neutral and selective traits, even genetic differences generated by directional selection may still warrant that populations should be managed separately.

Multi-locus based analysis

A disadvantage of the traditional population genetic approaches (F_{st} and related measures) is that they rely on a priori dividing the global population into smaller entities called subpopulations, but this may not always hold for continuously distributed species (Balloux and Lugon-Moulin 2002). As an alternative to the traditional differentiation measures, computer programs that rely on Bayesian clustering and assignment methods can be used. One of the major advantages of Bayesian clustering and assignment methods is that they can be used to infer the most likely numbers of populations and pool individuals without any prior assumptions (Hansen et al. 2001; Manel et al. 2005) and differences between sampling locations or other features can be used as evidence that the correct structure has been identified. For instance, Nielsen et al. (2007) used the

Bayesian methods implemented in the computer program STRUCTURE to demonstrate departure from random mating and limited rotation of animals between zoos leading to unnecessary high levels of inbreeding within individual zoo populations of the Persian wild ass. But methods that take advantage of multi locus data have been used to define barriers and restricted gene flow at many levels both between populations (McLoughlin et al. 2003; Vähä et al. 2007) and species (Maes et al. 2006).

If separate populations are known in advance particular assignment methods (assignment tests) can be used to assign individuals to their population of origin based on the individual multi locus genotype, and this is a major advantage in for instance forensics. Assignment tests have been used to detect fishing competition fraud (Primmer et al. 2000) and illegal translocation of wild animals (Frantz et al. 2006). But assignment tests can also be used to identify recent migrants or hybrids with admixed genetic background. The relative performance of Bayesian clustering software depends on the genetic differentiation between populations and in general assignment programs like STRUCTURE and BAPS performs well at values of $F_{st} > 0.02$ (Latch et al. 2006).

Molecular genetic Markers

Allozymes – (Allelic variants of enzymes).

Protein electrophoresis was one of the most widely used techniques for analyzing genetic variation in plants and animal species, because it was relatively inexpensive and allowed screening of many individuals. Today the technique is somewhat outdated as a population genetic marker and has been replaced by microsatellites, SNPs or direct sequencing. As previously mentioned, protein variants are often monomorphic, especially in endangered species and often low statistical power is gained from these markers, when used for population genetic studies (Hedrick 1999; Schlötterer 2004).

Mitochondrial DNA

Mitochondrial DNA (mtDNA) is maternally inherited and therefore allows determination of maternal lineages but can also be used in studying female or male biased dispersal rates in conjunction with nuclear DNA markers. For instance Waits et al 2000 subscribed discrepancies in the genetic structure revealed by mitochondrial DNA and 19 microsatellites to male mediated dispersal in Swedish populations of Scandinavian brown bear.

Microsatellites as genetic markers

Marine fishes often show weak but significant population structure at neutral loci and microsatellites have proved to be informative for resolving population genetic structure in numerous studies. Microsatellites are simple repeated sequences distributed approximately evenly throughout the Euchromatic part of genomes (see Box on microsatellites). Compared to allozymes, microsatellites are much more common and show much higher levels of polymorphism. They are co-dominant genetic markers, meaning that a progeny inherits one allele from the male parent and one from the female parent and both alleles are visible upon amplification. Due to the high mutation rates of microsatellites, they show high levels of polymorphism and these properties have made them popular genetic markers for mapping, paternity testing and population genetics (Schlötterer 2004; Chistiakov et al 2006). In general microsatellites are considered neutral genetic and therefore, no deviation from the expectations

under the neutral model is expected by selection acting on the microsatellite itself (Chistiakov et al. 2006).

Microsatellites are mostly composed of short stretches of DNA of around 100-300 bases and for this reason they are relatively robust to degradation. Since they can be amplified by standard PCR conditions, preserved pieces of tissue like scales and otoliths can be used for molecular analysis. For instance, DNA from old samples of fish scales have been used to characterize historical populations of pike *Esox lucius* (Larsen et al. 2005) and brown trout *Salmo trutta* (Hansen et al. 2002).

Microsatellites

Microsatellites are simple tandemly repeated sequences of one to five base pairs. They are distributed throughout the genomes of both eucaryotes and chloroplast and can be classified as pure, compound and interrupted repeats.

Pure: ----TGTGTGTGTGTG----

Compound: ----TGTGTGTCTCTCTC----

Interrupted: ----TGTGCCTGCCTGTGTGTG----

The most common types are di- (TGTG), tri- (GGTGGT), and tetra- (GATAGATA) nucleotides.

Modified from Jarne and Lagoda 1996

Disadvantages of microsatellites

The high levels of polymorphism that characterize microsatellites should lead to increased statistical power (Hedrick 1999), but may also lead to constrain estimates of genetic differentiation because F_{st} cannot exceed the homozygosity of the genetic markers used to estimate it (Hedrick 1999). However this rarely seems to be problematic since estimates of F_{st} or related measures tend to fall well below the theoretical maximum F_{st} values (O'Reilly et al. 2004).

Also, the expectation of selectively neutrality may not always be valid, since microsatellite loci may be linked to genes under selection. For selection to have a significant effect, the intensity of selection needs to be on the order of m or $1/N_e$ (Wang 2004). This is unlikely for the majority of loci in small populations (Wang 2004). However, even a minimal selective disadvantage of some loci in very large populations may significantly influence allele frequency distributions of microsatellite loci. If this selective disadvantage increases with increasing repeat number this

could seriously influence the mutation process and therefore the estimate of genetic divergence. Other disadvantages like size homoplasy and constraints on the mutation patterns will be discussed below.

Mutation rates in microsatellites

The mutation rate is an important factor since mutation is the source of genetic variation and therefore mutation rate is a major determinant of the level of genetic variation maintained within populations (Jarne and Lagoda 1996). Unique eukaryotic DNA sequences mutate at a rate of approximately 10^{-9} /nucleotide/ generation, whereas the mutation rate of microsatellites are several orders of magnitude higher and most often in the range of 10^{-5} - 10^{-2} /locus/generation (Jarne and Lagoda 1996; Ellegren 2000). In general, the vast majority of microsatellite mutation events involve either gaining or losing a single repeat, thus following the classical stepwise mutation model (Ellegren 2000). The mutation process is however highly heterogeneous and show distinct differences between species, repeat type, loci and even within alleles (Ellegren 2000).

Mutation models

IAM – the infinite alleles model (Kimura and Crow 1964).

Under the IAM each mutation event creates a new allele and consequently identical alleles can only be identical by descent and. The IAM does therefore not allow size homoplasy (Balloux and Lugon-Moulin 2002)

SSM – The stepwise mutation model (Kimura and Ohita 1978)

Under the SSM model each mutation creates new allele by either adding or deleting a single repeat unit of a microsatellite with a rate of $u/2$ in either direction. As a consequence, alleles which are very different are more distantly related and therefore the SSM has a memory.

KAM – the K-allele model

Under the KAM there are k possible allelic states and any allele have a probability $(k-1)$ of mutating to a different allele. The KAM was originally developed for protein genetic markers like allozymes because these markers were separated on basis of their electric mobility on starch gels and substitutions (mutations) either added or deleted a charge from the amino-acid chains of the proteins.

Although the mutation process is heterogeneous, several features about it seem to be consistent. First, several studies have found a correlation between variability and mean/or maximum repeat number, indicating that long loci mutate more often than short loci (Ellegren 2000; Dettmann and Taylor 2004). Studies examining individual mutational events have also found alleles with more repeats to be more likely to mutate than alleles with fewer repeats (Weber and Wong 1993; Amos and Rubinsztein 1996). This positive correlation could be caused by the fact that replication slippage can take place at more locations in repetitive sequences with higher numbers of repetitive segments (Ellegren 2000). And finally, di-nucleotide repeat loci appear to evolve at a rate 1.5-2 times greater than the tetra-nucleotide loci and tri-nucleotide which have intermediate mutation rates (Chakraborty 1997). However evidence is accumulating to suggest that other processes are involved and that assumptions of the classical SSM are violated. For instance, some di-nucleotide loci show bias in favor of expansion (Ellegren 2000) and this process can be balanced by repeat losses or even large deletions in long alleles (Primmer 1996) or selective pressure elimination larger alleles (Jarne and Lagoda 1996). Also, studying human tetranucleotide repeat loci, Xu et al. (2000) found the rate of expansions to be constant and independent of repeat number, whereas the rate of repeat contractions increases exponentially over repeat length. The result is that at a certain critical repeat number, the rate of expansion and contraction mutations are equal. A mutation process like this would therefore create a “bell shaped” distribution of alleles at equilibrium, with a mode at some intermediate level, since short alleles are likely to expand and long alleles are more likely to contract (Xu et al. 2000).

In relation to the current study of Greenland Halibut, it seems possible that mutation patterns could have influenced the allele frequency distributions observed in some loci. For instance, in the tetranucleotide microsatellite Stpf1001, a clear bell shaped distribution of alleles (Appendix II) is obvious in all populations, and one might worry if such a pattern could have been maintained independently within subpopulations by constraints on the mutation process, even if they had been separated for long periods. Also the frequency distribution of several of the di-nucleotide loci used in this study show a clear tail of long alleles at low frequency. It is perhaps unlikely that the frequency of these alleles would drift to higher frequencies within the subpopulations, when N_e is large, if the alleles with a high mutation rate would either gain a

repeat or be subject to a larger deletion. Thus, if the effective population size N_e within subpopulations is very large and stable over time, the mutation process is nonrandom and the mutation rate is very high, then the results of this study may be seriously biased. If for instance we imagine a metapopulation structure where a subpopulation originally was formed by a large number of individuals, from a panmictic population followed by long periods of complete isolation, restrictions in the mutation process could obscure the effect of genetic drift in the microsatellite, by maintaining the genetic variation within the large subpopulations.

The problem of size homoplasi

Surprisingly enough, the highly variable and presumably neutral microsatellites have sometimes partially failed to demonstrate genetic differentiation, where both meristic characters and other genetic markers have proven useful.

An example is the genetic distinction between the American eel *A. rostrata* and the European eel *A. Anguilla*. In these catadromous species both morphological characters (number of vertebrae), allozymes (Mdh-2^a) and mitochondrial DNA are diagnostic molecular genetic markers for distinguishing between these two species, whereas microsatellite allelic frequency histograms overlap extensively and private alleles are rare (Mank and Avise 2003). Therefore, Mank and Avise (2003) hypothesized that this is mainly due to extensive size homoplasy associated with mutation-driven saturation effects. Size homoplasy occurs when different copies of a locus are “identical in state” but not “identical by descent” and can therefore introduce error in genetic studies. In a comparative study of electromorph size homoplasy (ESH) in a tropical tree and an anadromous fish, Adams et al. 2004 found the amount of ESH to differ both between both taxa and loci. In the anadromous fish no ESH were observed, but in the tree 7 of the 12 loci showed ESH. It is however worth noticing that both of the species had less variable loci (1-4 alleles in the fish and 3-12 alleles in the tree) than most of the loci used in the present study. In general, size homoplasy seems to be problematic in situations where the mutation rate is high together with strong allele size constraints in large populations and the effect is to limit estimates of population differentiation and the inference of recent population history (Estoup et al. 2002). It is also worth noting that there should be no effect of homoplasy on F_{is} and no simple effect on F_{st} (Rousset 1996). In relation to the present study at least the effective population size seems to be large and there are some indications of a high mutation rate and allele size constraints at least in the

tetramer Stpf 1001, and this could affect gene flow estimation between subpopulations (Gaggiotti et al. 1999). That size homoplasy can pose a problem for estimates of genetic differentiation measures in marine fishes has been demonstrated. For instance, O'Reilly et al. (2004) found single locus estimates of F_{st} in the marine fish walleye Pollock (well below the theoretical maximum F_{st} values for the given locus) to declined with locus polymorphism and allelic richness, and subscribed this to the particular mutational properties of the microsatellites and size homoplasy.

In summary, selection against large alleles or constraints on the mutation processes could render microsatellites unsuitable population genetic markers for studies of genetic differentiation in highly abundant and stable populations.

N_e – The effective population size.

To understand the strength of genetic drift in natural populations it is important to know N_e , the effective population size.

Estimating N_e

N_e can be estimated in several ways, but one of the most commonly used is the temporal method. In this study, I have attempted to estimate the effective population size N_e using the software TMVP1p (a variant of TMVP, Beaumont 2003) because only two temporal periods were covered in the study. The program has been used for instance to estimate the effective population size of the Finnish wolf population (Aspi et al. 2006). TMVP1p uses gene frequency data to obtain a single estimate of the harmonic mean of N_e over the time period of the two samples. The program assumes that the data is obtained from a closed population sampled at two time points separated for a sufficiently short time so that mutation can be ignored. Due to the low genetic differentiation between populations, I have pooled all individuals from the different populations into the two periods 1995 and 2004-2006, to maximize the number of samples in each time period. This pooling could violate the assumptions of sampling in one isolated population with random mating and no immigration. However, the low genetic differentiation between populations indicates that pooling samples from the same period and different areas is equivalent of random sampling in one population with random mating. Also, the pair-wise F_{st} value estimated via FSTAT for the two pooled periods is low (-0.0002) indicating that this pooling should not be a problem. (A graphic visualization of different runs can be viewed in the appendix IV).

Unfortunately, under all tested parameters, the estimate of N_e shows bad convergence towards a fixed value. The reason for this is unknown, but may have to do with the large number of rare alleles that is present in the dataset even after pooling the samples into two periods (see the infile for TMVP1p in appendix IV). It would require running perhaps ten times as many samples to get to get a descent estimate of all these low frequency alleles. Also, limiting the dataset to populations or removing alleles that showed signs of null-alleles had no effect. However, limiting N_{e-Max} to 10.000 or less, give a highly skewed distribution towards the higher N_e value. Varying the parameters ‘maxit’ and ‘max pop size’ or increasing the number of updates and iterations did not improve the convergence. Even for a maximum population size of 1.000.000, the mode of N_e

is still near the maximum population size and the distribution of N_e is always very broad and flat. Therefore, it seems safe to conclude that N_e for Greenland halibut within the study area is very large (at least in the order of several thousands) since the two samples are genetically very similar and there has been little genetic drift between them, but TMVP1p cannot obtain a good estimate of N_e .

Factors that can limit the size of N_e

Wright (1938) recognized that the effective population size is usually less than the apparent number of breeding individuals in a population, and subscribed this to fluctuations in population size within subdivided populations. Indeed, in a review of published data of effective population sizes, Frankham (1995) estimated the ratio of the effective population size to the ecological census population size, N_e/N , to be approximately 0.11 averaged over a large number of species. However, even though the ecological numbers N of some marine organisms, insects and some plant species can be several millions, estimates of the effective population size N_e is often several orders of magnitude lower (Hedgcock 1994; Turner 2002; Hedgcock 2007). Also, the effect of genetic drift due to finite population size may still be significant in species with high gene flow potential via dispersing pelagic larvae, since slight but statistically significant genetic differentiations between local populations are sometimes found. These paradoxes led Hedgcock (1994) to suggest that in species with high fecundity and very high mortality rates in early life stages (typical of marine organisms), the entire population may be replaced by offspring from a small minority of spawning individuals, thus lowering the effective number of breeders N_b in the population. The hypothesis makes two predictions. First of all, random genetic drift resulting from the low N_e/N ratios, imposed by large variance in the number of progeny per parent V_k , ought to be measurable in natural populations of marine animals (Hedgcock 1994). And secondly, specific cohorts of new recruits, to the extent that they represent the output of a reproductively successful minority, should have less genetic diversity than the total adult population (Hedgcock 1994).

The Hedgecock effect.

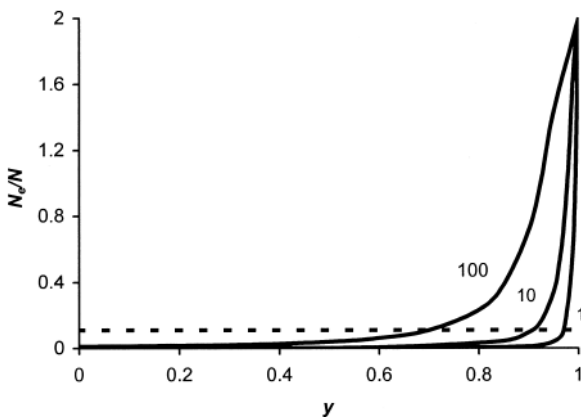
In the “ideal” diploid population with constant population size, progeny have equal probability of descending from each parent, resulting in a Poisson distribution of progeny, where both the mean number \bar{k} and the variance in the number of progeny per parent V_k are equal to 2. However when $V_k > 2$ then the ratio of the effective population size to the adult census size N_e/N is less than unity since:

$$\frac{N_e}{N} \approx \frac{4}{V_k + 2} \quad \text{(Wright 1938)}$$

If we imagine proportion of parents y that produce 2 progeny each ($V_k=0$), another proportion of very successful breeders N_b each contributing the same number of progeny $(1-y)2N/N_b$ and finally a proportion of unsuccessful breeders $(N-N_b-yN)$ that have 0 progeny, then:

$$\frac{N_e}{N} \approx \frac{N_b}{N(1 - y)^2} \quad \text{(See Hedrick 2005 for calculations.)}$$

The figure illustrates the N_e/N ratio for $N_b = 1, 10$ and 100 in an adult population of 10,000 individuals using the above formula. When y is low the N_e/N ratio is also low. The high variation in reproductive success where a few individuals produce most of the progeny is quite robust to the introduction of a high proportion of individuals with limited reproductive success.



The N_e/N ratio when a proportion of parents y have 2 progeny, there are one, 10 or 100 parents that contribute equally in a population of 10,000 adults. The broken line indicates the N_e/N ratio of 0.11 estimated by Frankham (1995). After Hedrick (2005).
(Modified from Hedrick 2005)

The key aspects of this process is that some species are subject to “sweepstakes” survival, where the vast majority of individuals produce no offspring that survive to adulthood (Turner 2002, Waples 2002). The mechanisms can stem from environmental, behavioral and human made factors like fishing (Birkeland and Dayton 2005) or habitat alteration (Turner 2002). Sweepstake survival could be related to the highly variable marine environment where clouds of eggs and larvae are at risk of drifting to unsuitable areas or larvae could experience periods of low prey densities (Cushings match mismatch hypothesis). Indications of this process have been found in several studies (Hedgecock 1994; Planes and Lenfantalt 2002) although the combination of high fecundity and high mortality rates in early life stages (type III survivorship curves) alone does not seem enough to seriously limit the N_e/N ratio. Apparently some extrinsic mechanism that enhances the variance in reproductive success must be present (Turner 2006). If siblings are non random distributed and have a tendency to co-occur in time and space, then predation, food availability or exploitation could induce high variance in mortality rates between families or groups (Waples 2002) even beyond the passive drifting stage. This type of behavior is expected especially for species which create schools, and indications for population- and kin- associated schooling beyond the juvenile stage, have been found in schooling species (Fraser et al. 2005). But many other organisms show signs of non random distribution in time and space. An interesting feature is that sampling individuals from different cohorts of species with long generation times and overlapping generations, could lead to elevated estimates of the within area inbreeding coefficient F_{is} , although the effect may be difficult to measure and would require surveillance of individual cohorts. Sweepstake reproductive success could therefore be an explanation of a partial inbreeding (found by for instance Hoarau et al. 2005) in species with large populations and dispersing larvae (Hedgecock 2007). Also the effect may be more pronounced at the boundary of a species distribution range, since the number of individuals should be smaller, fecundity lower and recruitment more sporadic (see Bridle and Vines 2006) in these areas. Many other factors than variance in reproductive output, can however influence the effective population size.

Overlapping generations

Waples 1990 found the effective population size per generation to be equivalent to the effective number of breeders per year N_b times the average age at spawning or generation length g ($N_e = g * N_b$). Since Greenland halibut is a long lived species this should further increase the N_e/N ratio, compared to species with short generation time and the same number of breeders.

Random mating

Even though marine organisms often spawn in schools there are indications that the assumption of “random mating” is not always valid. Atlantic cod have been shown to perform mate choice and lekking behavior which results in a high variance in male reproductive success and thereby contribute to a low N_e/N ratio (Bekkevold 2002). Rowe et al. (2007) proposed that if the majority of males achieve fertilizations by adopting a satellite (sneaking) spawning tactic in which male reproductive success is highest when males are small and this could lead to a frequency distribution of mate size dissimilarity in which males are small relative to the spawning female. The sexual dimorphism in Greenland halibut, where males are much smaller and with a shorter generation time than females, indicates an assortative mating strategy with less variance in individual male reproductive success and therefore also less reduction in the N_e/N ratio. But even in extreme mating systems with highly skewed sex ratios like polygyny (very few males obtaining most of the matings) N_e increases with generation time, because the variance in male reproductive success decreases with increasing generation time (Nunney 1993). In general, unsuccessful individuals in one generation have more opportunities to be successful in the following generations regardless of mating system, and therefore generation time also increases the effective population size (Nunney 1993).

Fluctuating population size

Pertoldi et al. (2007) have recently argued that populations with large census size are subject to higher fluctuations in population size, thus lowering the harmonic mean of the population size over time and therefore also lowering N_e , compared to populations with moderate population sizes. Indeed even marine fish species may vary considerably in the census population sizes, but again long lived species with overlapping generations could limit the effect. But more importantly, Darwin on “the origin of species” suggested that in the northern hemisphere species

are limited by competition at their southern border and by abiotic factors at their northern (Bridle and Vines 2006). Therefore, due to the lower number of species in the arctic environment and the very stable arctic sea climate, arctic species may show much lower fluctuations in population sizes than their temperate counterparts. Loss of genetic diversity and substantial genetic drift (Bottleneck) due to overexploitation have however been observed in restricted marine areas (Hauser et al. 2002). Greenland halibut populations have only recently been heavily exploited in Greenland and therefore the anthropogenic effect on the N_e/N ratio is likely to be negligible. Isolated populations of arctic cod have also shown to maintain high heterozygosities and high N_e/N ratios, despite isolation at the extreme of the species distribution range (Hardie et al. 2006). Hardie et al. (2006) explained this by absence of fishing pressure, low predation and low environmental stochasticity minimizing individual variance in reproductive success.

N_e/N ratio in relation to Greenland halibut.

Several features about Greenland halibut suggests that this species may not be subject to a large reduction in the N_e/N ratio, at least in the offshore areas. In Iteroparous species, which presumably includes the Greenland halibut, family correlated mortality rates in the early life stages, are expected to have a smaller effect on the N_e/N ratio, since failure of a family to survive in one cohort might be compensated by successful reproduction by the same parents in the following years (Waples 2002). Also, Greenland halibut may be a batch spawner, since the eggs seem to mature in cohorts, and if mating takes place several times per season, this should further reduce variance in reproductive success per individual. For these and the previously mentioned reasons, it seems highly likely that Greenland halibut populations are large and very stable (at least over historical times) leading to corresponding low rates of genetic drift.

Problems related to genetic investigations in the marine environment.

Non-random migration patterns

Migratory species may exhibit periods of geographic overlap, in which individuals from multiple populations mix at feeding habitats or during migratory phases (Bowen et al. 2005) and correct population structure may be difficult to identify due to sampling scheme (Latch and Rhodes 2006). For instance, Latch and Rhodes (2006) found significant genetic structuring among localized flocks of wild turkeys during the winter, but no evidence of genetic structure among sampling locations during the spring although it is possible that such a pattern stems from winter flocks being partially related (Latch & Rhodes 2006). But even marine fishes have been found to perform nonrandom migration patterns where populations overlap in time and space and then segregate back into genetic stocks. Buresch 2006 found offshore longfin squid populations to segregate in to distinct genetic populations inshore during the summer months, although this study can be criticized since all markers show signs of null alleles. Atlantic Bluefin Tuna undertake transoceanic movements where two putative stocks are mixed on the feeding grounds. Sampling of Atlantic Bluefin Tuna recruits and larvae have however demonstrated the existence of both a western Atlantic and several Mediterranean stocks (Carlsson et al. 2007). Normally sampling individuals from different populations in the same area would lead to an excess of homozygote individuals. However, a Wahlund effect may not always be detectable by traditional Hardy-Weinberg analysis in marine organisms (Nielsen et al. 2003).

Different results using different genetic markers

The unpredictable and sometimes cryptic migration patterns displayed by marine organisms may sometimes lead to conflicting results revealed by different types of genetic markers. In a study of the Patagonian toothfish Shaw et al. (2004) found discordance between the results of mtDNA and microsatellites in their ability to resolve the genetic structure in the southwest Atlantic Ocean. Data from mtDNA indicated a sharp genetic division with near fixation for different haplotypes on either side of the Antarctic polar front whereas microsatellites showed much less distinct structuring and a slightly different picture. Shaw et al. (2004) subscribed this to male mediated migration across the Antarctic polar front and absence of both female and larval dispersal but were also concerned with both homoplasy and that mtDNA differences could reflect historical

divergence between populations whereas microsatellites patterns could reflect reduced drift effects on allele frequencies that have yet to reach migration-drift equilibrium. The reverse situation, where microsatellites and allozymes are useful in resolving population structure and mtDNA virtually uninformative is also sometimes seen (see Bernal-Ramírez and Adcock 2003). Another example is the somewhat cryptic migration pattern of the Loggerhead turtles. Whereas female Loggerhead turtles faithfully return to their natal nesting colony, males seem to provide gene flow between regional colonies, probably via opportunistic mating (Bowen et al. 2005). As a result of this sex-biased gene flow, nuclear DNA markers (microsatellites) reveal low levels of genetic differentiation between nesting colonies, but the maternally inherited mtDNA indicates multiple isolated populations (Bowen et al. 2005). Obviously, management plans for turtles based solely on nuclear DNA markers would erroneously suggest that local populations were non-existing.

Panmixia

Finding panmixia (the complete lack of any genetic differentiation across the entire range of a species), or at least non-significant genetic structuring within the entire study area, is not unusual in highly migratory and common species and examples are known from fishes, birds, insects and plants. Microsatellite analysis of Atlantic halibut (*Hippoglossus hippoglossus*), which is related to Greenland halibut and having a partially overlapping geographical distribution, have also revealed absence of population structure in the north-west Atlantic Ocean (Reid et al 2005). But lack of genetic structuring among populations can be found in many other organisms. For instance, in a study of Dawson's burrowing bee (large, fast-flying and solitary nesting in large aggregations), Beveridge and Simmons (2006) found the species to be panmictic over its entire western Australian range. This species has one flight season per year where the northern most populations emerge first, and there is no temporal overlap in flight season with the populations in the southern end of the distribution more than 700 km away. Further more, the mating and nesting behavior of this bee, suggests that gene flow should be limited by monoandry and the fact that almost 90% of the females mate immediately upon emergence (Beveridge and Simmons 2006). However, since there seems to be sufficient gene flow to maintain panmixia, it is possible that gene flow depends on special environmental extremes or certain types of metapopulation structures (Beveridge and Simmons 2006). Another example is the 10 million Adélie penguins

breeding in large disjunct colonies on ice free areas around the Antarctic continent. Roeder et al. 2001 in a comparison of all colonies found overall F_{st} values of 0.0007 and, as in the present study, assignment tests were relatively ineffective in assigning individuals to their respective collection site, despite substantial levels of genetic variation. Even seals in the arctic regions have been found to comprise a single large panmictic population (Coltman 2007).

Source-sink meta-population structure of Greenland Halibut

Gaggiotti and Smouse (1996) investigated the effects that demographic parameters and different migration patterns have on the maintenance of genetic variability in sink populations and found that the relative importance of migration, genetic drift and mutation are all a function of sink population decay. When population decay is rapid (as must be expected for Greenland halibut within the fjords, since spawning is negligible within the fjords) migration into the sink is the dominating factor (Gaggiotti and Smouse, 1996) and the genetic constitution of the sink will closely mimic the source population. Therefore, the absence of genetic differentiation between the offshore and West Greenlandic fjords is in concordance with the current view that fjord populations consist mainly of recruitment from offshore areas. Also, if sink populations are sufficiently poor that they produce almost no emigrants, they will contribute almost nothing to the evolutionary future of the species and the differentiation among sink populations can be much greater than that among source populations, if there is a higher rate of population turnover among the sinks (Gaggiotti and Smouse 1996; Whitlock and McCauley 1998). Indeed, although non-significant, the highest estimates of F_{st} are found when comparing the suspected sink populations within the West Greenlandic fjords, and the lowest F_{st} estimates between the presumably offshore source populations.

Additional remarks

This study also demonstrates some of the difficulties of working on species with potential huge effective population sizes. The sample size seems to be far too small for most analysis because of the large number of low frequency or rare alleles (see for instance the TMVP1p infile). That microsatellite mutation patterns and restrictions may have influenced the allele distributions also indicates that microsatellites are not always ideal genetic markers to study population genetic properties of this widely distributed (Arctic part of the North Atlantic) species with potentially

enormous effective population size. For this type of species, genetic markers which to a higher degree resemble the infinite alleles model (SNPs) should be more powerful. It seems safe to conclude that gene flow between populations is high, but the data does not inform us about the variance or direction of gene flow or if gene flow is common enough to rescue harvested sink populations. Merely it is high enough to prevent local populations from existing.

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Part II

Paper draft

High levels of gene flow and lack of genetic structure between fjord- and offshore- populations of Greenland halibut *Reinhardtius hippoglossoides* in Greenland waters revealed by microsatellite DNA analysis.

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Keywords: *Reinhardtius hippoglossoides*, genetic structure, panmixia, microsatellite, Greenland.

Abstract

Thorough knowledge of the genetic composition of a population is essential, not only because genetic differentiation may indicate adaptation to local environmental conditions, but also to secure proper management and conservation. This especially applies to marine fishes which are harvested directly from wild populations. In this study we investigated the genetic composition of Greenland Halibut *Reinhardtius Hippoglossoides* populations in Greenland Waters from the Denmark Strait to the Baffin Bay and inside three major North - Western Greenlandic fjords. Available microsatellite markers from related flatfish species were tested for cross-species amplification, and finally 10 loci were selected. The results revealed insignificant and very low genetic differentiation between all areas, suggesting high migration rates and effective interbreeding across the entire study area. This however does not imply that Greenland halibut should be managed as one population. Rather it emphasizes the importance of protecting the offshore Davis Strait and Iceland population, since they inhabit the only known major spawning areas and all remaining inshore- and fjord- populations may be highly dependent on recruitment from these populations.

Introduction

Greenland halibut *Reinhardtius Hippoglossoides* (Walbaum, 1792) is large benthic-pelagic boreal flatfish circum polar distributed in both the North Atlantic and North Pacific Oceans, at depths 200 m to more than 2000 m. The annual biomass and abundance estimates of this very common species have fluctuated between 260-512 mill individuals and 13000 - 40000 tons, from 1992 to 2005 in West Greenland waters alone (Sünksen et al. 2005) and since the major collapse in the cod fisheries in the 90'ies, it has constituted Greenland's second most economic valuable fisheries resource. Even today a substantial proportion of Greenland halibut is caught using longlines from small open boats or dog sledges directly from the sea ice in the innermost parts of the ice-fjords, at depths between 500 and 800 meters and for this reason, the Greenland halibut constitutes an important microeconomic component and therefore an important factor in upholding the traditional Greenlandic Inuit way of life.

The main spawning area of Greenland halibut in Greenlandic waters has been identified as the Davis strait (Jensen 1935, Schmidt 1969 Jørgensen 1997a, Simonsen and Gundersen 2005),

although ripe females have also been found in East Greenland near Kap Bille (Gundersen et al. 2001) the Baffin Bay (Gundersen et al. 2004) and inside the deep Ummannaq fjord in West Greenland (Schmidt 1969). Spawning takes place during the ice covered winter months and metamorphosed larvae settle almost a year later in the autumn at a size of 65-70mm (Jensen 1935, Schmidt 1969) and during the long pelagic stage larvae can be transported via the Irminger Current from Icelandic waters to the Davis Strait or from the Davis Strait to Canadian waters and the Baffin Bay (Stenberg et al. in prep). Tagging experiments have demonstrated high fidelity to tagging sites, especially within the fjord areas, where around 99% of the tagged individuals were recaptured within the release area, but offshore individuals seem to be exchanged between areas at a higher rate (Boje 2002). Also, the general long distance migration pattern is in the opposite direction of the passive transport of eggs and larvae and this observation have led Stenberg et al. (in prep.) to suggest some level of genetic structure. The existing view is however that the Greenland halibut within the large West Greenlandic fjord systems function as sink populations in a metapopulation community, where recruitment is dependent on larval transport from offshore areas, and spawning within the fjords is negligible (Riget and Boje 1989; Boje and Jørgensen 1990). Therefore it is presumed that the fisheries for Greenland halibut within the fjords have no negative effect on the overall recruitment and persistence of the populations (Boje and Jørgensen 1990).

A variety of methods has been utilized to reveal the population structure of Greenland Halibut (e.g., allozymes (Fairbairn 1981; Iglund and Nævdal 1986; Riget , Boje and Simonsen 1992), parasites (Khan et al. 1982; Arthur and Albert 1993; Boje, Riget and Koie 1997), morphometrics (Bowering 1988), meristics (Riget and Boje 1989) and mtDNA (Vis et al. 1997). In general these investigations suggest that Greenland halibut comprise a single panmictic stock across the entire North Atlantic Ocean, although some studies indicated the presence of isolated stocks in the West Greenlandic fjords (Riget , Boje and Simonsen 1992) and the Gulf of St. Lawrence (Fairbairn 1981) . The ability of these methods to reveal population structure should however in general be lower than for the highly variable microsatellite markers. For instance, Rasmussen et al. (1999) concluded that the number of vertebrae was an inconclusive method in revealing population structure of Greenland halibut, since they found large deviations between identical areas sampled in different years. Also, the studies based on allozymes were based on relatively few loci with a few dominating alleles. In a recent study, Knutsen et al. (2007) used 9 microsatellite markers to

study Greenland halibut across the North Atlantic Ocean and found indications of partially subdivided populations across the entire North Atlantic with some evidence of isolation by distance following the ocean currents. Their study did however not include the Western Greenlandic fjords or temporally separated samples. In this study, we attempted to clarify the population genetic composition of Greenland halibut by using ten cross-species amplified microsatellite markers. Unlike any preceding genetic studies on Greenland halibut, we have included temporally separated samples and samples from inside three deep North-western Greenlandic fjords and the Baffin Bay.

Materials and methods

Sampling

Samples were collected by the Greenland institute of natural resources onboard their research vessels AD Jensen and Pâmiut during their annual survey programs (fig. 1). Sampling included two temporally separated periods (1995 and 2004-2006) in the large Uummannaq fjords, the fjords around Upernavik, the Baffin Bay and the Davis Strait. The study also included samples from the Denmark Strait (between East Greenland and Island) and the Disco bay, although no temporal samples were available from these areas. Most samples were taken from sub-adult individuals, as spawning areas are poorly known and ripe individuals are rarely seen.

Molecular analysis

Samples from 1995 consisted of muscle tissue whereas gill filament was used in more recent samples. All samples were stored in 96% ethanol. DNA extraction was performed using the EZNA Tissue DNA kit (Omega Bio-tek, inc.). As no microsatellite markers have been developed exclusively for Greenland halibut, available markers from related flatfish species were tested for cross species amplification. Ten microsatellite markers were selected for the genetic analysis. These comprised markers developed for European Flounder, StPf1001 [AJ315970] (Dixon, Taggart, and George, unpublished data), and Atlantic halibut, Hhi3 [AJ270780], Hhi55 [AJ270784], Hhi59 [AJ270787] (Coughlan et al. 2000), HhiC17 [AF133244], HhiA44 [AF133243] (McGonowan and Reith, 1999), Hhi58IMB [AY752692], Hhi111IMB [AY752697], Hhi113IMB [AY752698], Hhi120IMB [AY752700] (Reid et al., 2005). PCR conditions were

optimized with regard to annealing temperature and $MgCl_2$ concentration, and in some instances an alternative primer was designed or a pigtail was added to the reverse primer to avoid double peaks. Electrophoresis of PCR products was performed in two multiplexes on an ABI3130 DNA sequencer and alleles were auto scored and manually checked.

Microsatellite data analysis

Tests for Hardy-Weinberg equilibrium with heterozygote excess and deficiency as alternative hypotheses were performed in GENEPOP 3.4 (Raymond and Rousset 1995) using a Markov chain method to estimate the exact P-values per locus and population (Guo and Thompson, 1992). Sequential Bonferoni adjustment were performed to adjust for multiple tests according to Rice (1989) ($k=10$, because deviations were calculated in all loci for each population). All loci were tested for errors due to stuttering, large allele dropout and null alleles, using MICRO-CHECKER version 2.2.3 <http://www.microchecker.hull.ac.uk/> (Van Oosterhout et al. 2004), and null-allele frequencies were estimated according to Chakraborty et al. (1992). Statistical independence of loci, i.e. genotypic linkage disequilibrium LD, was tested using GENEPOP 3.4. FSTAT 2.9.3 (Goudet 1995) was used to calculate the population inbreeding coefficient (F_{is}), allelic richness (AR) and pairwise θ (F_{st}) values (Weir and Cockerham, 1984).

As geographical areas may contain mixtures of genetically differentiated populations, especially in marine organisms, the Bayesian algorithm implemented in Structure 2.2, was used to search for alternative clustering of individuals. Independent simulations were performed for values of $K = 1-10$, with 200.000 iterations and a burn-in period of 100.000. We used the admixture model with correlated allele frequencies between populations and let the degree of admixture, α , be inferred from the data. An α value close to 0 means that most individuals belongs to separate subpopulation, whereas values above 1 indicates that most individuals are admixed (Falush 2003). “Log probability of the data” $\ln P(D)$ was used to infer the number of subpopulations K , since $K=1$ showed the highest probability, and it is impossible to calculate the better performing ΔK (Evano 2005) for $K=1$.

Results

Exact test for deviations from Hardy-Weinberg expectations in GENPOP revealed significant heterozygote deficiency in locus Hhi55 and Hhi113IMB in 9 of ten populations after sequential

bonferroni correction for both loci (Tabel 1). Significant deficiency of heterozygotes was also found in Hhi111IMB population **B** ($P = 0.0014$) and excess of heterozygotes in Hhi58IMB in population **C** ($P = 0.0047$). The MICRO-CHECKER analysis indicated that the heterozygote deficiency was due to a high frequency of null alleles at Hhi55 and Hhi113Imb (16 and 6% weighted average over populations). MICRO-CHECKER also identified a significant frequency of null alleles in Hhi111IMB in population **B** (4%). Otherwise, MICRO-CHECKER did not identify any technical problems such as stutter or large allele dropout in any of the remaining marker loci. All pairwise estimates of θ (F_{st}) were non-significant, even prior to correction for multiple comparisons (Fig 2). Since both Hhi55 and Hhi113IMB showed signs of technical problems all preceding analyses were performed both including and excluding these loci. In general, excluding these loci had only minor effects on the results. The STRUCTURE analysis revealed the highest Likelihood Ln P(D) value for $K=1$, with higher numbers of subpopulations (K) always giving lower likelihoods and higher variances. (Fig 3). Also, the alpha value inferred by STRUCTURE was above 1 and close to the maximum value of 10 for $K>2$, indicating that essentially all individuals are admixed. No significant LD was found between any pair of marker loci prior to sequential Bonferroni correction.

Discussion

The high level of genetic homogeneity among Greenland Halibut in Greenlandic waters suggests effective interbreeding across the entire area. However, other factors could cause the genetic homogeneity observed in this study, and the results therefore do not exclude the possibility that isolated populations may exist. Since the spawning areas of Greenland halibut are virtually unknown, the presumed populations may constitute mixtures of individuals from multiple related populations sampled at or near feeding grounds during migratory phases (Bowen et al. 2005, Carlsson et al. 2007). This however does not seem to be the case, since STRUCTURE was unable to cluster individuals in alternative groups with a higher likelihood. A more worrying problem is related to the potentially enormous effective population size which renders genetic drift an extremely slow process and in this case mutation and selection, which can normally be ignored for the presumably selectively neutral microsatellites, may suddenly be important. Normally, the effect of mutation in homogenizing subpopulations is very small except in the unlikely case where the mutation rate (u) is similar in magnitude to both the migration rate m and

$1/N_e$ (Whitlock and McCauley 1999; Wang 2004) but at least the second parameter may often be fulfilled in oceanic organisms (large effective population sizes, N_e) and for microsatellite markers (high mutation rates). For selection to have a significant effect, the intensity of selection needs to be on the order of m or $1/N_e$ and this is unlikely for the majority of loci in small populations (Wang 2004). However, even a minimal selective disadvantage of some loci in very large populations may significantly influence allele frequency distributions of microsatellite loci. If this selective disadvantage increases with increasing repeat number this could seriously influence the mutation process and therefore the estimate of genetic divergence by increasing the level of homoplasy. In general, size homoplasy seems to be problematic in situations where the mutation rate is high together with strong allele size constraints in large populations and the effect is to limit estimates of population differentiation and the inference of recent population history (Estoup et al. 2002). That size homoplasy can pose a problem for estimates of genetic differentiation measures in marine fishes have been demonstrated. For instance, O'Reilly et al. (2004) found single locus estimates of F_{st} in the marine fish walleye Pollock (well below the theoretical maximum F_{st} values for the given locus) to decline with locus polymorphism and allelic richness, and subscribed this to the particular mutational properties of the microsatellites and size homoplasy. Although the vast majority of microsatellites seem to follow the classical stepwise mutation model, the mutation process is highly heterogeneous and show distinct differences between species, repeat type, loci and even within alleles (Ellegren 2000). Studying human tetranucleotide repeat loci, Xu et al. (2000) found the rate of expansions to be constant and independent of repeat number, whereas the rate of repeat contractions increases exponentially over repeat length. The result is that at a certain critical repeat number, the rate of expansion and contraction mutations are equal. A mutation process like this creates a "bell shaped" distribution of alleles at equilibrium, with a mode at some intermediate level, since short alleles are likely to expand and long alleles are more likely to contract (Xu et al. 2000). The tetranucleotide microsatellite Stpf1001 does show such a distribution and it is possible that such a pattern could be maintained within independent subpopulations by such constraints on the mutation process, even if they had been separated for long periods.

If the large Western Greenlandic fjord systems are true sink populations in a metapopulation community, what effect would this have on the genetic variation? Gaggiotti and Smouse (1996) investigated the effects that demographic parameters and different migration patterns have on the

maintenance of genetic variability within sink populations and found that the relative importance of migration, genetic drift and mutation are all a function of the sink population decay. When population decay is rapid (as must be expected for Greenland halibut within the fjords, since spawning in these areas is negligible) migration into the sink is the dominating factor and the genetic constitution of the sink will closely mimic the source population (Gaggiotti and Smouse, 1996). Therefore, the absence of genetic differentiation between the offshore and West Greenlandic fjords is in concordance with the current view that fjord populations consist mostly or exclusively of recruits from offshore areas. Also, if sink populations produce almost no emigrants, they will contribute nothing to the evolutionary future of the species and the differentiation among sink populations can be much greater than that among source populations, if there is a higher rate of population turnover among the sinks (Gaggiotti and Smouse 1996; Whitlock and Mccauley 1998). Indeed, although non-significant, the highest F_{st} estimates are found when comparing the suspected sink populations within the West Greenlandic fjords, and the lowest F_{st} estimates between the presumably source populations offshore.

That microsatellite mutation patterns and restrictions may have influenced the allele distributions also indicates that microsatellites are not always ideal genetic markers to study population genetic properties of this widely distributed (Arctic part of the North Atlantic) species with potentially enormous effective population size. For this type of species, genetic markers which to a higher degree resemble the infinite alleles model (SNPs) should be more powerful. It seems safe to conclude that gene flow between populations is high, but the data does not inform us about the variance or direction of gene flow but merely that it is high enough to prevent local populations from existing.

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4 catch areas of Greenland halibut in waters around Greenland

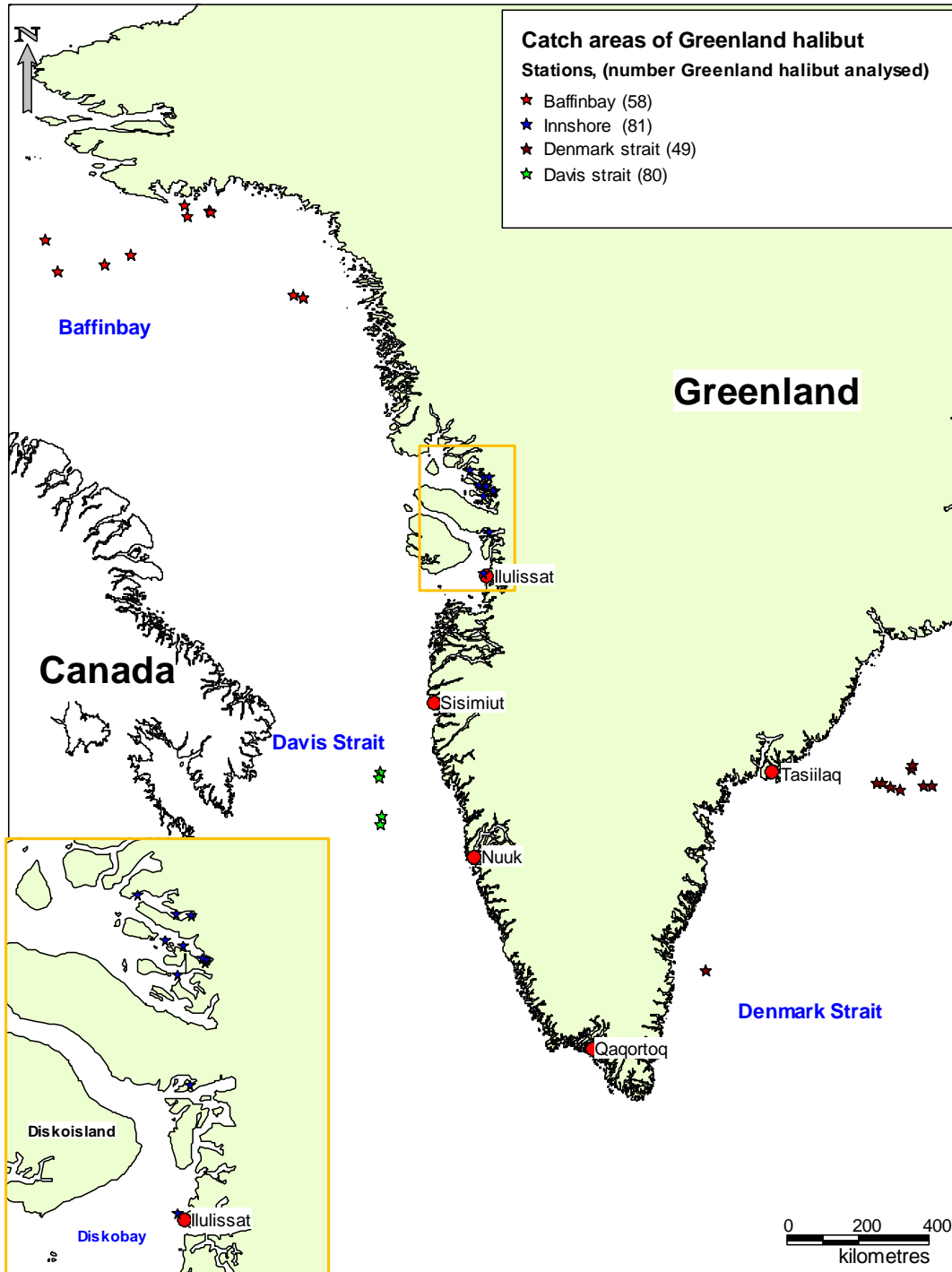


Table 1

Summary table of the genetic data for the ten populations and ten loci investigated, showing number of alleles, allelic richness (AR), observed (H_o) and expected (H_e) heterozygosity, F_{IS} and the estimated null allele frequency (Chackraborty). Asterisks denotes a significant deficiency or excess of heterozygotes estimated by Genpop using the (Guo and Thompson, 1992) method. Null allele frequencies are estimated according to chakraborty et al. (1992).

Locus	Hhi3	HhiC17	HhiA44	Hhi55	Hhi59	Hhi111IMB	Hhi113IMB	Hhi58IMB	Hhi120IMB	Stpf1001
Upernavik 1995 (A) n = 49										
no. of alleles	15	21	17	6	13	28	15	8	3	18
AR	12.43	14.97	11.99	5.66	9.67	21.17	13.13	6.89	2.55	14.97
HO	0.857	0.694	0.792	0.500 *	0.771	0.918	0.500 ***	0.511	0.327	0.915
HE	0.807	0.744	0.804	0.709	0.803	0.935	0.772	0.507	0.279	0.912
Fis	-0.062	0.068	0.016	0.298	0.040	0.018	0.356	-0.007	-0.172	-0.003
null allele freq.	-0.035	0.030	0.003	0.167	0.015	0.004	0.207	-0.009	-0.083	-0.007
Uummannaq 1995 (B) n = 57										
no. of alleles	16	25	17	7	15	33	14	14	3	16
AR	11.15	16.44	11.36	6.67	9.46	23.97	11.02	10.08	2.73	14.11
HO	0.696	0.807	0.667	0.462 ***	0.807	0.859 *	0.611 *	0.545	0.351	0.912
HE	0.787	0.811	0.752	0.733	0.795	0.948	0.673	0.535	0.342	0.903
Fis	0.116	0.004	0.114	0.371	-0.015	0.094	0.093	-0.020	-0.026	-0.011
null allele freq.	0.057	-0.002	0.056	0.222	-0.012	0.045	0.044	-0.014	-0.017	-0.010
Baffin Bay 1995 (C) n = 45										
no. of alleles	14	23	16	7	17	30	13	11	3	15
AR	11.44	17.70	11.56	6.71	13.15	23.27	10.50	8.72	2.60	13.24
HO	0.800	0.844	0.778	0.452 ***	0.844	0.978	0.477 *	0.659 *	0.356	0.955
HE	0.805	0.815	0.738	0.732	0.836	0.947	0.584	0.547	0.314	0.912
Fis	0.006	-0.036	-0.055	0.385	-0.011	-0.033	0.185	-0.208	-0.135	-0.047
null allele freq.	-0.003	-0.023	-0.032	0.231	-0.011	-0.022	0.095	-0.099	-0.068	-0.028
Davis Strait 1995 (D) n = 51										
no. of alleles	13	24	11	8	15	29	15	11	5	18
AR	9.85	15.78	8.39	8.00	10.72	23.73	12.12	8.87	3.96	16.39
HO	0.729	0.706	0.700	0.481 ***	0.714	0.952	0.707 *	0.592	0.412	0.905
HE	0.765	0.733	0.729	0.730	0.791	0.948	0.733	0.603	0.372	0.921
Fis	0.048	0.037	0.040	0.344	0.098	-0.005	0.036	0.018	-0.109	0.017
null allele freq.	0.019	0.014	0.015	0.196	0.046	-0.008	0.012	0.004	-0.056	0.003
Denmarks strait 2004 (E) n = 63										
no. of alleles	18	24	15	9	19	36	12	12	3	18
AR	12.84	15.15	9.82	6.93	12.57	24.82	7.90	9.35	2.43	14.98
HO	0.794	0.778	0.714	0.540 *	0.778	0.905	0.428 **	0.597	0.333	0.905
HE	0.809	0.765	0.749	0.748	0.831	0.938	0.553	0.608	0.333	0.909
Fis	0.019	-0.017	0.047	0.279	0.065	0.036	0.227	0.018	0.000	0.005
null allele freq.	0.006	-0.012	0.020	0.157	0.029	0.014	0.123	0.005	-0.004	-0.002

Table 1 continued....

Summary table of the genetic data for the ten populations and ten loci investigated, showing number of alleles, allelic richness (AR), observed (Ho) and expected (He) heterozygosity, F_{IS} and the estimated null allele frequency (Chackraborty). Asterisks denotes a significant deficiency or excess of heterozygotes estimated by Genpop using the (Guo and Thompson, 1992) method. Null allele frequencies are estimated according to chakraborty et al. (1992).

Locus	Hhi3	HhiC17	HhiA44	Hhi55	Hhi59	Hhi111IMB	Hhi113IMB	Hhi58IMB	Hhi120IMB	Stpf1001
Baffin Bay 2004 (F) n = 54										
no. of alleles	15	28	13	7	17	35	18	13	2	14
AR	11.08	17.88	10.73	6.53	11.38	25.06	13.01	8.96	2.00	12.16
HO	0.778	0.741	0.741	0.490 **	0.852	0.963	0.698 *	0.547	0.352	0.906
HE	0.784	0.791	0.768	0.732	0.818	0.949	0.683	0.573	0.316	0.905
Fis	0.008	0.064	0.036	0.333	-0.042	-0.015	-0.023	0.046	-0.114	-0.001
null allele freq.	-0.001	0.028	0.014	0.193	-0.025	-0.012	-0.016	0.019	-0.058	-0.005
Davis Strait 2004 (S) n = 58										
no. of alleles	17	24	16	10	15	34	14	14	2	20
AR	11.79	16.93	11.70	8.27	10.13	24.04	11.05	10.07	2.00	15.46
HO	0.776	0.741	0.845	0.545 ***	0.707	0.897	0.637 *	0.569	0.259	0.931
HE	0.800	0.754	0.783	0.774	0.787	0.944	0.690	0.549	0.252	0.921
Fis	0.031	0.017	-0.080	0.297	0.103	0.051	0.077	-0.037	-0.025	-0.011
null allele freq.	0.011	0.004	-0.043	0.169	0.050	0.022	0.035	-0.022	-0.017	-0.010
Illulissat 2005 (I) n = 36										
no. of alleles	13	19	10	6	5	30	13	9	2	16
AR	11.37	16.73	9.16	5.50	4.96	25.80	11.84	8.29	2.00	14.86
HO	0.750	0.833	0.778	0.639	0.706	0.972	0.583 *	0.529	0.167	0.944
HE	0.814	0.856	0.744	0.728	0.741	0.954	0.665	0.493	0.155	0.923
Fis	0.079	0.027	-0.046	0.124	0.048	-0.020	0.124	-0.074	-0.077	-0.023
null allele freq.	0.034	0.006	-0.029	0.058	0.017	-0.017	0.059	-0.043	-0.044	-0.018
Uummannaq 2005 (U) n = 45										
no. of alleles	14	23	11	8	12	34	13	15	2	16
AR	11.63	18.06	9.15	7.30	9.53	26.34	10.57	11.18	2.00	14.54
HO	0.733	0.778	0.778	0.659 *	0.711	0.953	0.628	0.711	0.289	0.867
HE	0.772	0.790	0.736	0.745	0.785	0.952	0.662	0.690	0.281	0.914
Fis	0.051	0.015	-0.058	0.116	0.095	-0.001	0.051	-0.031	-0.029	0.052
null allele freq.	0.020	0.002	-0.034	0.055	0.044	-0.007	0.020	-0.021	-0.020	0.021
Upernavik 2006 (P) n = 51										
no. of alleles	18	23	15	7	15	34	11	13	2	18
AR	12.77	16.45	10.76	6.24	10.17	24.79	9.35	9.80	2.00	15.16
HO	0.863	0.816	0.765	0.500 *	0.843	0.980	0.562 *	0.510	0.294	0.920
HE	0.833	0.822	0.705	0.734	0.795	0.948	0.589	0.496	0.253	0.919
Fis	-0.036	0.007	-0.085	0.321	-0.062	-0.033	0.046	-0.028	-0.163	-0.001
null allele freq.	-0.022	-0.002	-0.045	0.185	-0.035	-0.021	0.018	-0.019	-0.079	-0.005

Figure 1

FST values for all areas using all 10 markers below diagonal and corresponding P values above.

	UPE95	UUM95	BAF95	DAV95	UPE06	UUM05	BAF04	DAV04	ILL05	DKS04
UPE95		0.046	0.452	0.650	0.540	0.036	0.262	0.719	0.169	0.017
UUM95	0.0035		0.662	0.547	0.507	0.773	0.867	0.602	0.404	0.423
BAF95	0.0002	-0.0014		0.544	0.716	0.090	0.567	0.834	0.086	0.056
DAV95	0.0009	0.0011	0.0001		0.495	0.618	0.645	0.832	0.035	0.217
UPE06	0.0027	-0.0001	-0.0033	0.0024		0.317	0.080	0.782	0.445	0.602
UUM05	0.0061	-0.0017	0.0011	0.0009	0.0041		0.841	0.278	0.019	0.212
BAF04	0.0017	-0.0034	-0.0028	-0.0011	0.0006	-0.0025		0.903	0.474	0.137
DAV04	0.0007	0.0002	-0.001	-0.0008	-0.0004	0.0014	-0.0018		0.696	0.201
ILL05	0.0025	-0.0007	-0.0013	0.0040	-0.0014	0.0057	-0.0006	-0.0007		0.041
DKS04	0.0060	-0.0012	-0.0008	0.0000	0.0014	-0.0004	-0.0020	0.0003	0.0018	

P-values obtained after : 45000 permutations

Indicative adjusted nominal level (5%) for multiple comparisons is : 0.001111

FST values for all areas excluding Hhi55 and Hhi113IMB below diagonal and corresponding P values above.

	UPE95	UUM95	BAF95	DAV95	UPE06	UUM05	BAF04	DAV04	ILL05	DKS04
UPE95		0.068	0.170	0.317	0.585	0.116	0.281	0.789	0.192	0.340
UUM95	0.0016		0.680	0.461	0.582	0.662	0.881	0.726	0.619	0.486
BAF95	-0.0005	-0.0026		0.259	0.746	0.186	0.635	0.886	0.164	0.207
DAV95	0.0027	-0.0004	0.0001		0.472	0.279	0.305	0.735	0.021	0.337
UPE06	0.0016	-0.0016	-0.0027	0.0028		0.333	0.166	0.891	0.596	0.557
UUM05	0.0042	0.0000	-0.0002	-0.0003	0.0042		0.747	0.425	0.053	0.265
BAF04	0.0012	-0.0033	-0.0030	-0.0011	0.0009	-0.0019		0.818	0.656	0.211
DAV04	0.0010	-0.0016	-0.0015	-0.0004	0.0004	0.0005	-0.0017		0.840	0.503
ILL05	0.0028	-0.0002	-0.0004	0.0040	-0.0010	0.0072	0.0006	-0.0006		0.198
DKS04	0.0028	-0.0020	-0.0002	-0.0014	0.0030	-0.0006	-0.0022	-0.0006	0.0028	

P-values obtained after : 45000 permutations

Indicative adjusted nominal level (5%) for multiple comparisons is : 0.001111

FST \ P-values for all areas with temporal samples pooled using all 10 markers.

	Uumm	Baffin	Davis st.	DK. st.	Upernavik	Illulissat
Uumm		0.345	0.084	0.093	0.004	0.040
Baffin	-0.0006		0.733	0.042	0.140	0.167
Davis st.	0.0014	-0.0001		0.151	0.425	0.156
DK. st.	-0.0005	-0.0007	0.0003		0.166	0.043
Upernavik	0.0028	-0.0001	0.0004	0.0027		0.356
Illulissat	0.0024	-0.0003	0.0014	0.0018	-0.0002	

P-values obtained after : 15000 permutations

Indicative adjusted nominal level (5%) for multiple comparisons is : 0.003333

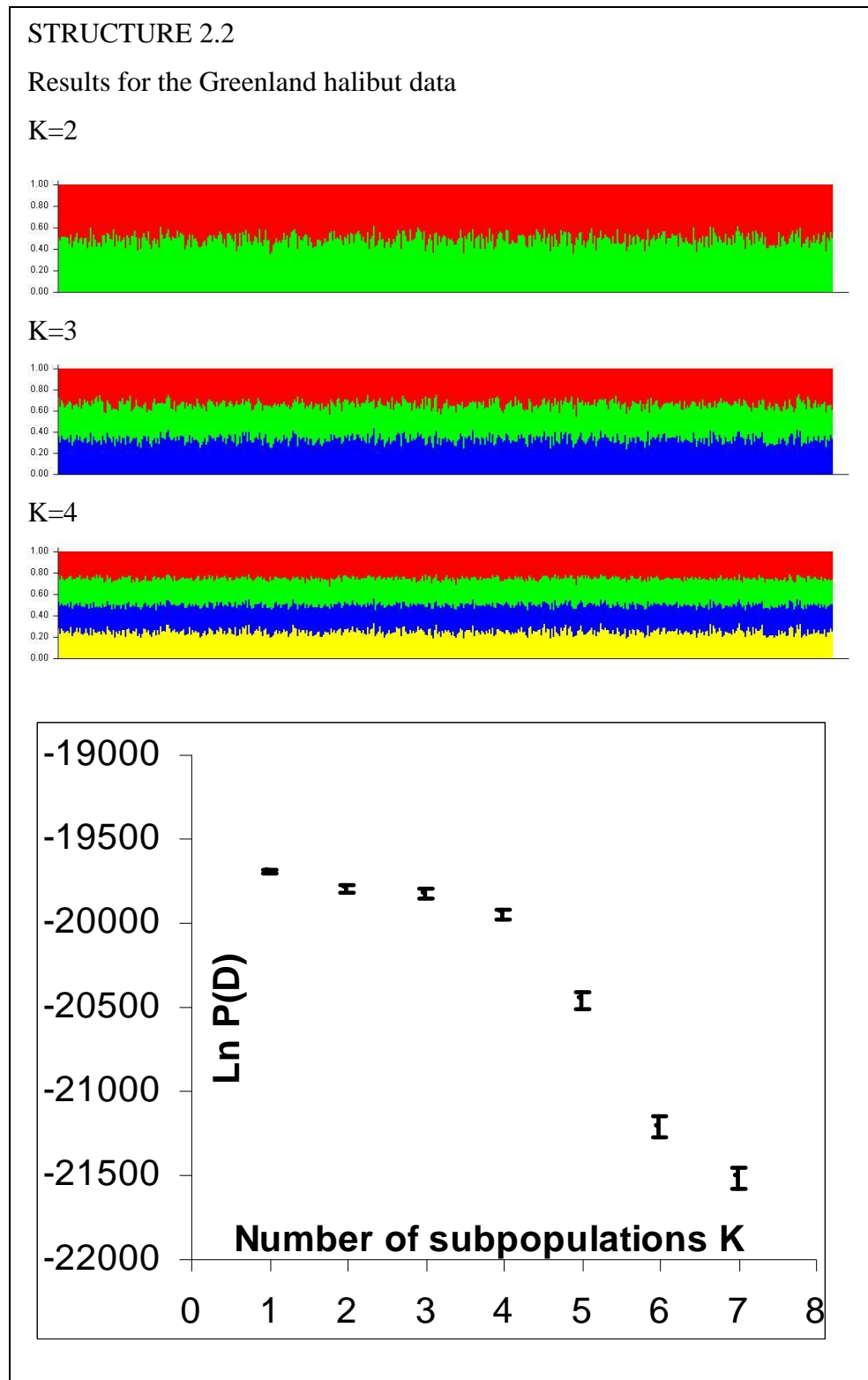
FST \ P-values for all areas with temporal samples pooled excluding Hhi55 and Hhi113IMB.

	Uumm	Baffin	Davis st.	DK. st.	Upernavik	Illulissat
Uumm		0.323	0.094	0.095	0.029	0.034
Baffin	-0.0008		0.623	0.038	0.105	0.133
Davis st.	-0.0004	-0.0001		0.081	0.491	0.101
DK. st.	-0.0006	0	0.0006		0.187	0.023
Upernavik	0.0014	0.0002	0.0009	0.0035		0.342
Illulissat	0.0034	0.0007	0.0014	0.0033	0.0001	

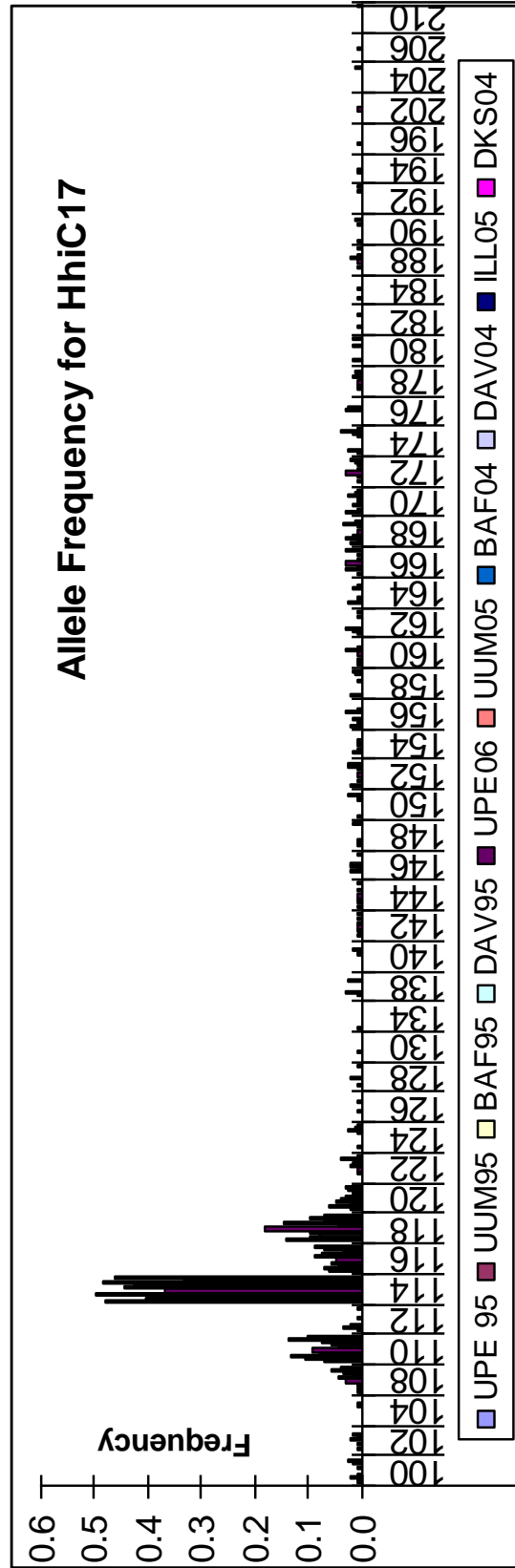
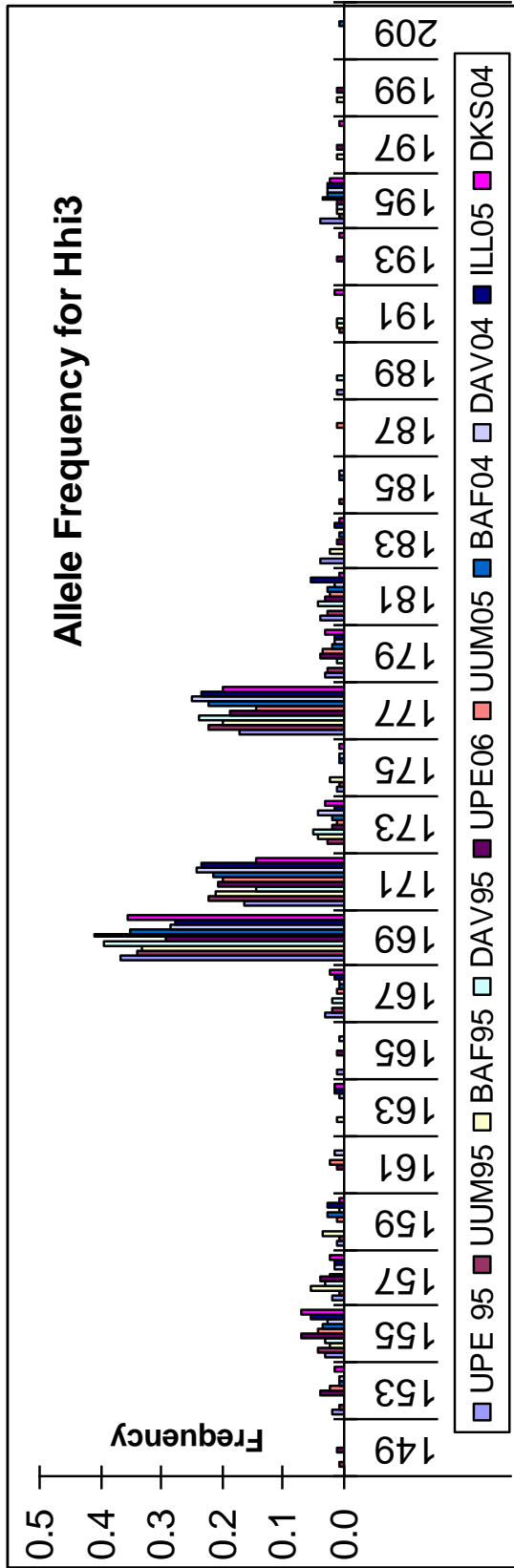
P-values obtained after : 15000 permutations

Indicative adjusted nominal level (5%) for multiple comparisons is : 0.003333

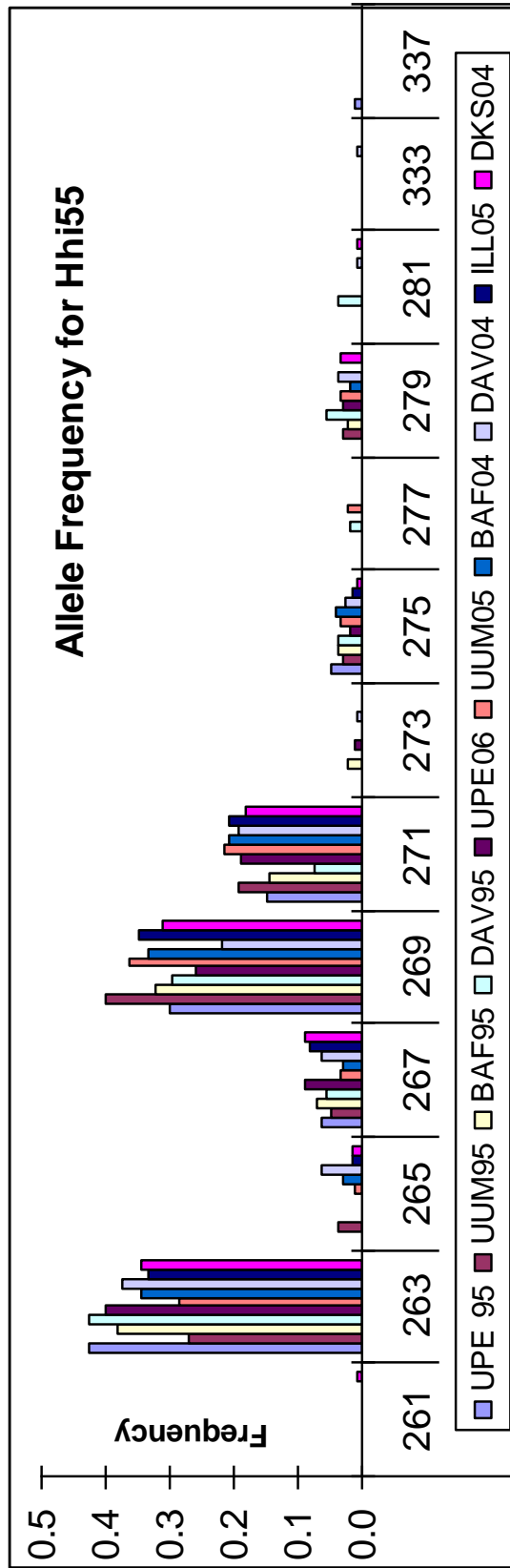
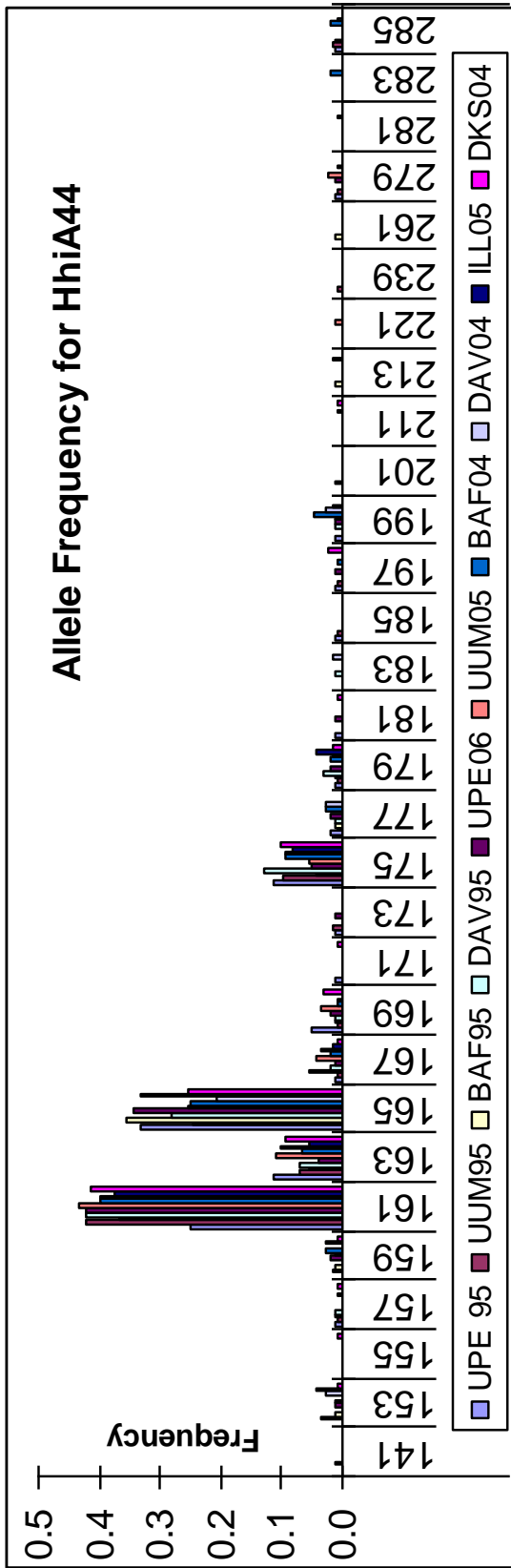
Figure 2



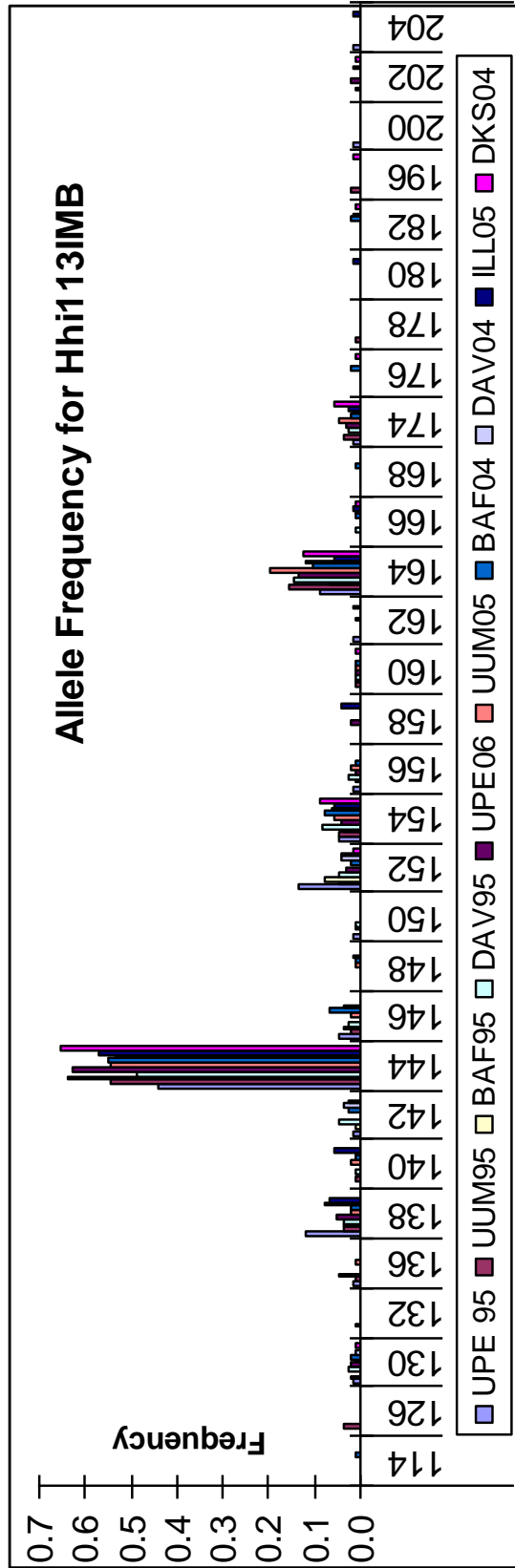
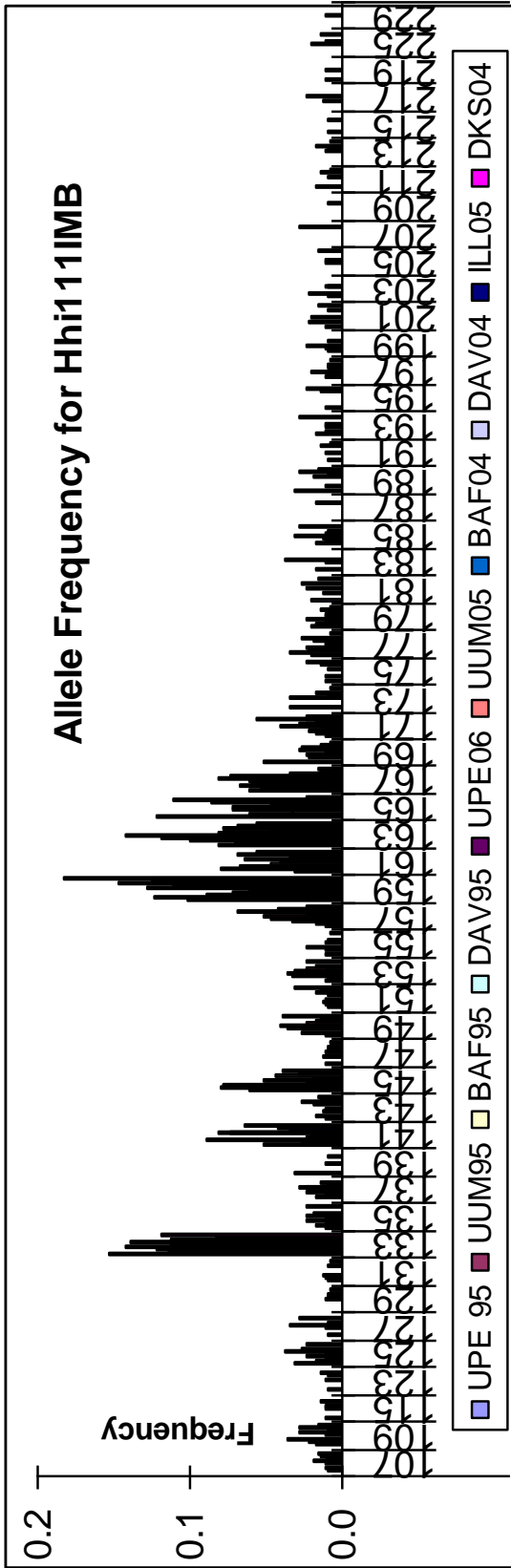
Appendix I



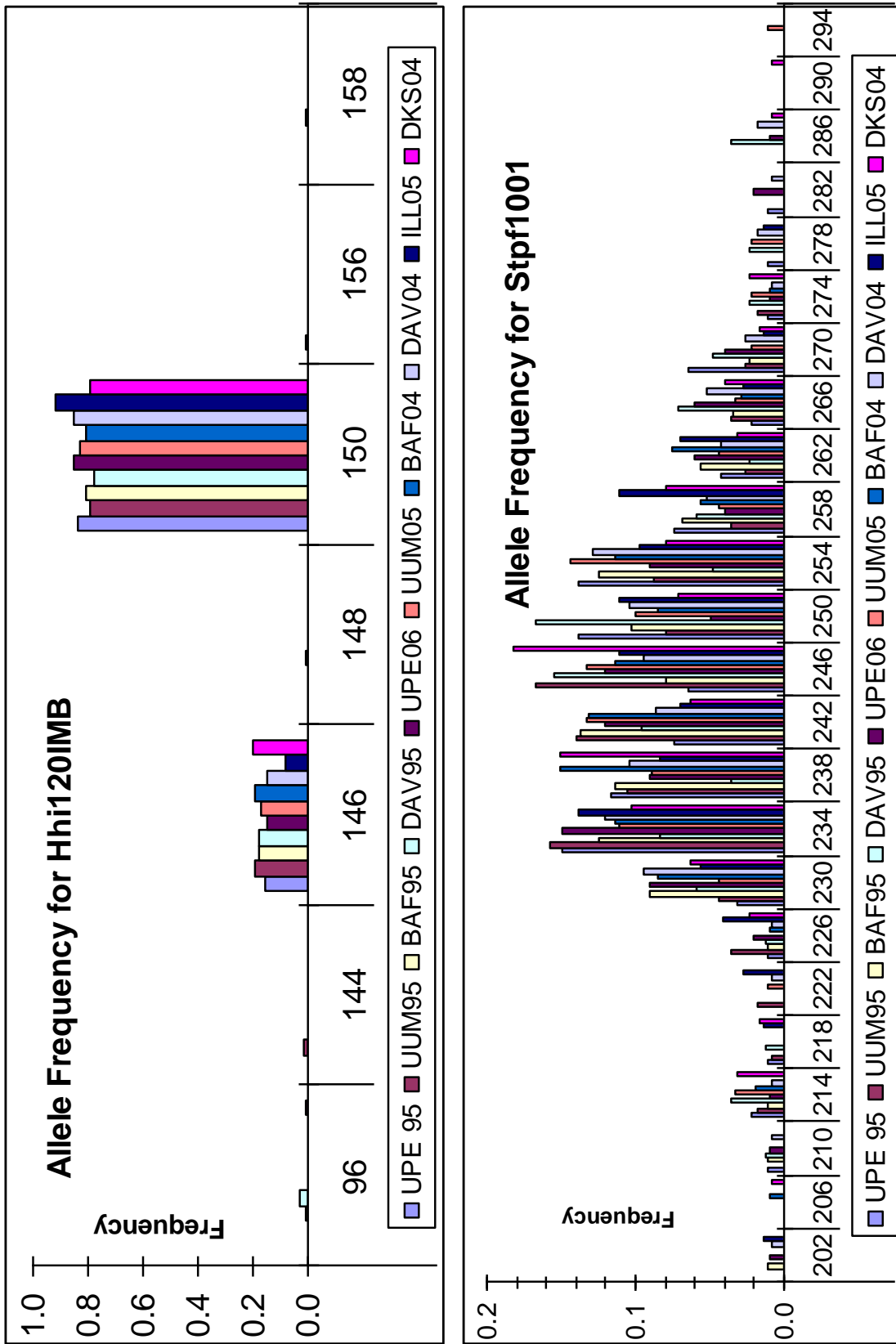
Appendix I continued...



Appendix I continued...



Appendix I continued...



Appendix II

TMVP1p infile

10

2 7

0.00 1 0 99 0 514 0 0

2.00 4 2 71 1 324 1 1

2 26

0.00 1 10 31 12 8 5 4 2 7 203 125 15 3 127 16 15 4 2 1 0 2 2 15 2 1 1

2.00 1 3 13 11 5 0 1 1 7 142 74 12 4 83 7 11 6 1 0 2 3 0 7 1 1 0

2 49

0.00 5 5 2 22 51 2 260 41 62 17 9 6 1 3 0 0 3 4 4 3 5 2 4 7 3 4 4 5 2 3 9 7 8 9 7 6 5 4 1 1 5 2 2 1 0 1 1 1 1

2.00 5 2 0 4 39 7 180 24 42 16 1 1 1 1 1 1 4 0 2 3 3 3 1 3 3 6 2 3 6 5 5 8 6 2 4 0 2 2 1 1 1 1 0 1 1 0 0 0 0

2 30

0.00 0 9 1 2 9 250 49 165 13 11 1 1 50 8 11 2 2 0 5 11 0 2 1 1 0 0 4 1 2 3

2.00 1 5 0 4 3 147 31 120 9 8 1 3 39 5 6 1 1 2 2 2 1 0 1 0 1 1 2 0 0 4

2 13

0.00 1 207 14 39 179 117 2 14 2 16 2 1 0

2.00 0 118 4 19 110 49 2 12 1 8 2 0 1

2 25

0.00 1 0 2 1 2 12 5 387 25 20 14 2 7 15 3 2 1 1 0 66 35 0 2 0 3

2.00 0 1 0 0 1 11 2 257 13 11 8 4 0 6 0 2 0 0 1 44 23 1 1 1 3

2 54

0.00 8 9 3 3 13 7 4 2 69 8 7 2 32 9 20 4 14 9 11 3 23 78 28 47 36 28 10 18 7 5 8 5 7 5 7 2 7 3 4 4 5 1 5 2 4 2 1 3 5 1 2 1 4 1

2.00 2 7 1 1 8 1 0 3 51 6 5 3 18 4 24 2 7 3 8 4 10 38 22 35 28 23 9 6 4 2 10 7 3 2 4 0 4 1 3 1 1 4 3 3 0 0 0 2 0 1 1 1 0 0

2 30

0.00 1 0 7 0 1 23 8 9 350 13 4 0 15 39 4 5 4 2 75 4 1 20 3 0 1 5 2 0 5 1

2.00 0 4 5 1 6 18 2 6 185 10 0 3 25 18 4 0 3 1 41 1 0 7 0 1 0 0 2 1 1 1

2 41

0.00 0 7 0 0 0 0 195 1 137 101 13 92 2 1 8 4 1 4 0 0 4 0 1 1 1 1 2 2 4 1 4 7 3 5 2 0 1 1 2 0 2

2.00 1 2 2 1 1 1 116 2 98 70 7 55 2 0 8 1 0 1 1 1 2 1 1 0 1 0 0 0 4 1 2 2 1 3 2 4 3 0 0 1 0

2 24

0.00 3 2 2 11 3 4 10 45 74 70 61 78 52 66 38 32 25 12 8 5 3 4 1 1

2.00 1 0 3 8 3 2 7 21 50 36 43 45 45 38 22 14 15 15 5 3 1 3 0 0

Note: All 509 individuals have been pooled into two separated periods 2 generations apart. Although this raises the sample size considerably, most loci still contain a large proportion of alleles which are observed less than 10 times resulting in a poor estimate of the individual allele frequency.

Appendix II continued...

Eksample of TMVP1p outfile visualized using the R package

Parameters:

Maxit: 1000

Thining interval: 10

Sd of parameter update: 0.1

Statefile:

17868 20000 20000 -1.725268e+003 2 10000

2249.969160 2249.969160

(no. of successful updates) (no. updates) (max. no. of updates) (log likelihood) (ploidy) (the max. pop. size)

Gelman and Rubin's convergence diagnostic

Variable:log(loglike)

Potential scale reduction factors:

Point est. 97.5% quantile

[1,] 1.16 1.48

Variable:mean_Ne

Potential scale reduction factors:

Point est. 97.5% quantile

[1,] 1.03 1.14

*****For the following, prob is set at = 0.1 *****

[1] "log difference from max is "

[1] 2.62251

***** mode for log_like = -1724.868 *****

** Limits will occur in pairs if there is more than one mode.**

** Choose the appropriate set that encloses the reported mode.*****

[1] -1725.177 -1724.735

*****For the following, prob is set at = 0.1 *****

[1] "log difference from max is "

[1] 0.7045285

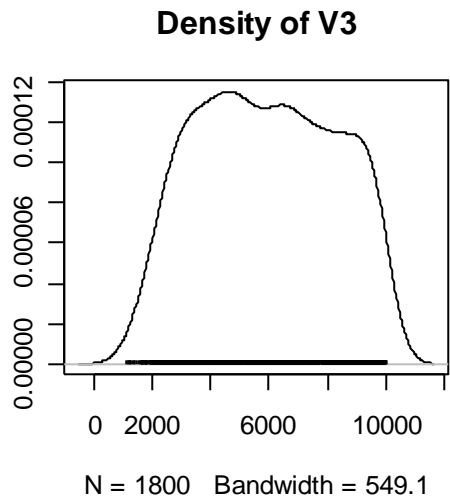
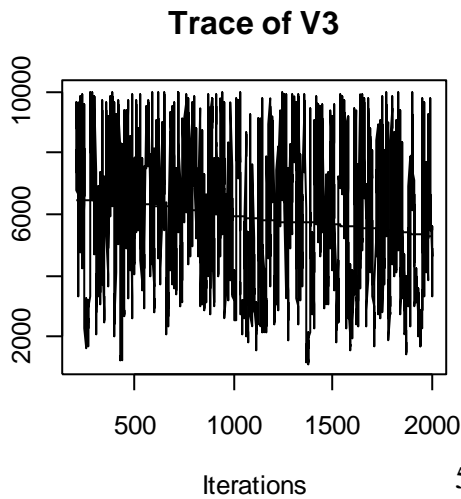
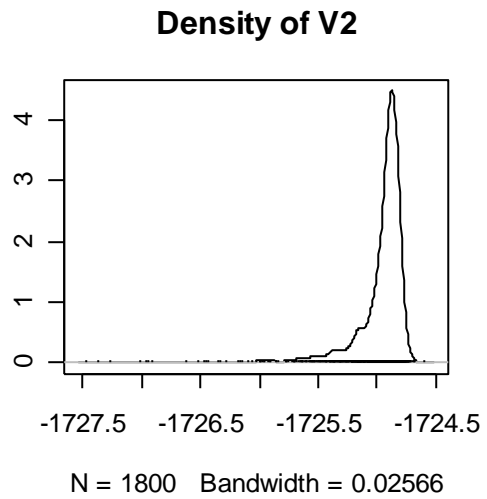
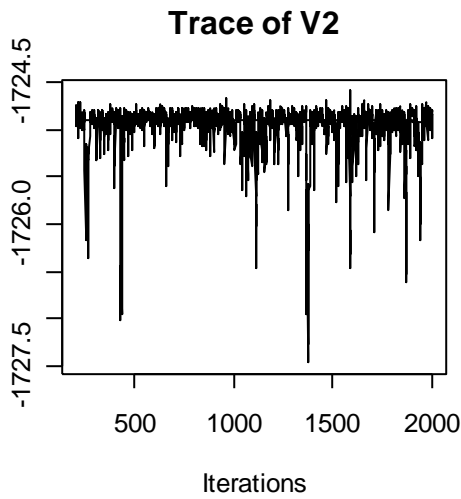
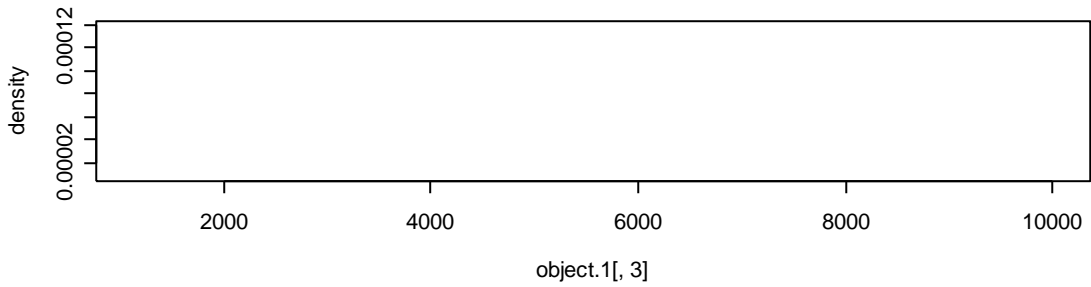
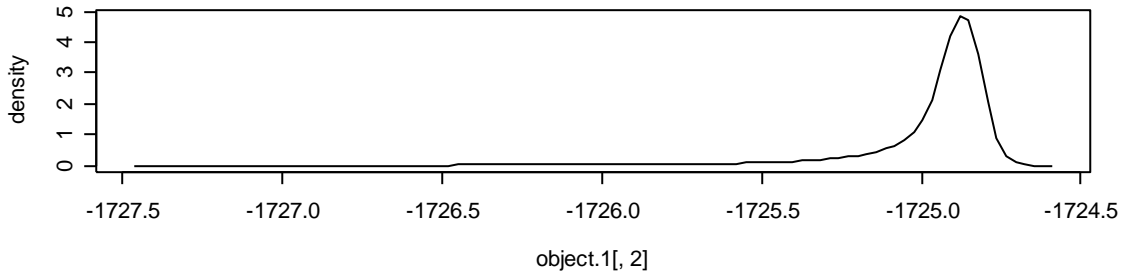
***** mode for mean Ne = 5440.942 *****

** Limits will occur in pairs if there is more than one mode.**

** Choose the appropriate set that encloses the reported mode.*****

[1] 2419.683 9601.056

Appendix II continued...



Appendix II continued...

Parameters:

Maxit: 1000

Thinning interval: 10

Sd of parameter update: 0.1

Statefile:

18529 20000 20000 -1.724913e+003 2 1000000

975241.100830 975241.100830

(no. of successful updates) (no. updates) (max. no. of updates) (log likelihood) (ploidy) (the max. pop. size)

Gelman and Rubin's convergence diagnostic

Variable:log(loglike)

Potential scale reduction factors:

Point est. 97.5% quantile

[1,] 1.01 1.01

Variable:mean_Ne

Potential scale reduction factors:

Point est. 97.5% quantile

[1,] 1.01 1.01

*****For the following, prob is set at = 0.1 *****

[1] "log difference from max is "

[1] 2.798664

***** mode for log_like = -1724.916 *****

** Limits will occur in pairs if there is more than one mode.**

** Choose the appropriate set that encloses the reported mode.*****

[1] -1724.933 -1724.886

*****For the following, prob is set at = 0.1 *****

[1] "log difference from max is "

[1] 0.6366816

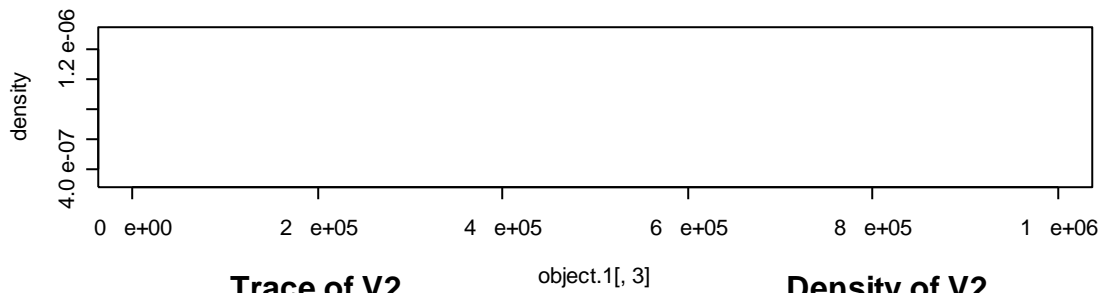
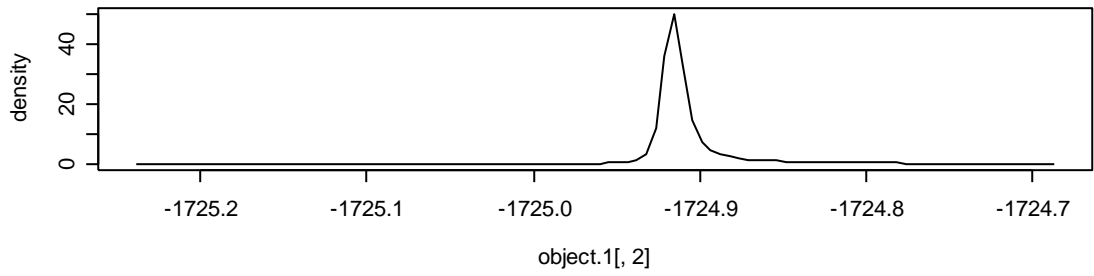
***** mode for mean Ne = 132906.6 *****

** Limits will occur in pairs if there is more than one mode.**

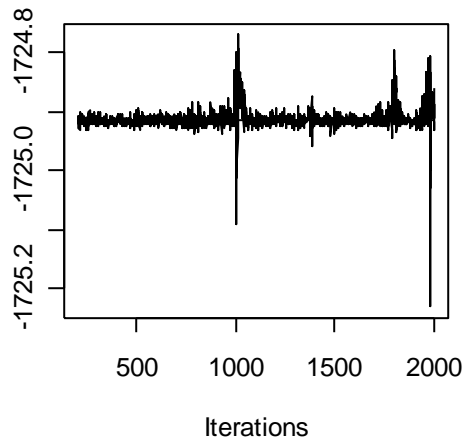
** Choose the appropriate set that encloses the reported mode.*****

[1] 3094.637 893118.785

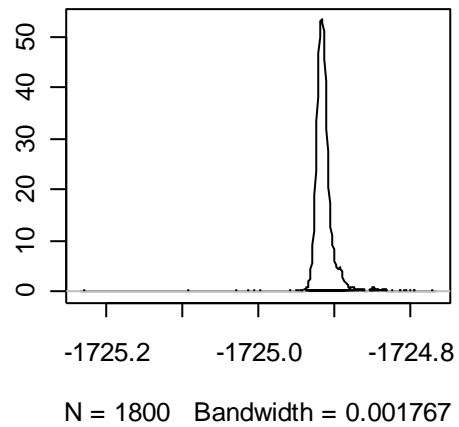
Appendix II continued...



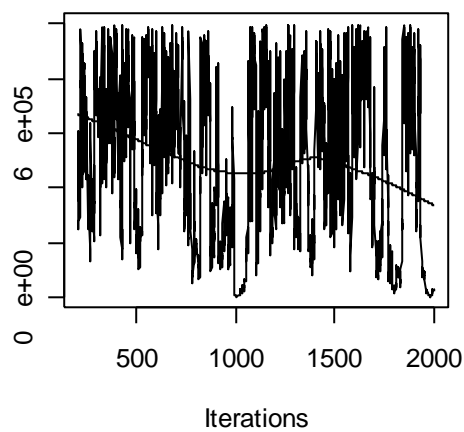
Trace of V2



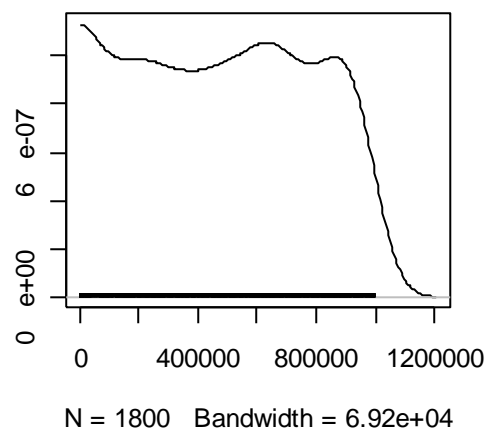
Density of V2



Trace of V3



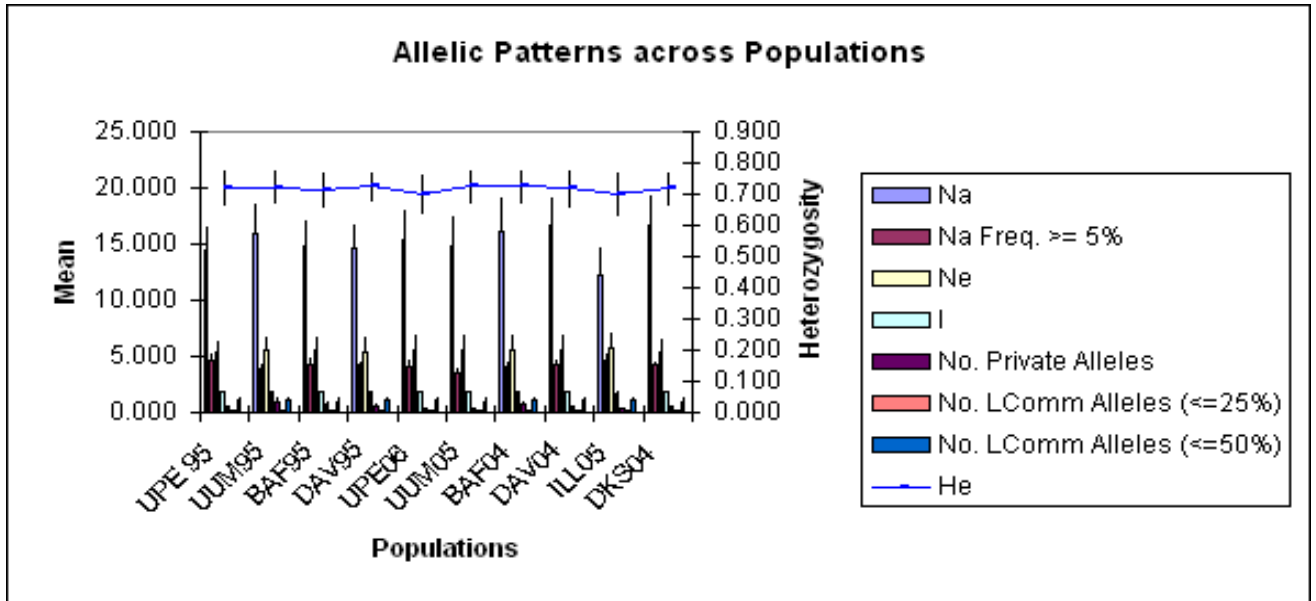
Density of V3



Appendix III (From GenAlEx6)

Allelic Patterns for Codominant Data

No. Loci 10
 No. Samples 509
 No. Pops. 10



Mean Allelic Patterns Across Populations

Mean values

Population	UPE 95	UUM95	BAF95	DAV95	UPE06	UUM05	BAF04	DAV04	ILL05	DKS04
Na	14.400	16.000	14.900	14.600	15.400	14.800	16.200	16.600	12.300	16.600
Na Freq. >= 5%	4.700	3.800	4.300	4.200	4.100	3.600	4.100	4.200	4.700	4.200
Ne	5.308	5.464	5.506	5.399	5.592	5.546	5.543	5.590	5.651	5.360
I	1.818	1.845	1.833	1.842	1.812	1.846	1.858	1.885	1.747	1.856
No. Private Alleles	0.500	1.000	0.800	0.600	0.300	0.400	0.800	0.600	0.300	0.600
No. LComm Alleles (<=25%)	0.200	0.200	0.200	0.200	0.200	0.100	0.200	0.200	0.200	0.200
No. LComm Alleles (<=50%)	1.100	1.100	1.000	1.100	1.100	1.000	1.100	1.100	1.100	1.000
He	0.719	0.721	0.715	0.724	0.702	0.724	0.725	0.719	0.697	0.719

Standard Error (SE) values

Population	UPE 95	UUM95	BAF95	DAV95	UPE06	UUM05	BAF04	DAV04	ILL05	DKS04
Na	2.339	2.646	2.410	2.232	2.770	2.736	2.999	2.672	2.556	2.845
Na Freq. >= 5%	0.616	0.554	0.667	0.416	0.722	0.400	0.605	0.611	0.597	0.490
Ne	1.174	1.413	1.378	1.404	1.481	1.469	1.424	1.406	1.526	1.271
I	0.216	0.216	0.221	0.203	0.239	0.224	0.222	0.225	0.249	0.225
No. Private Alleles	0.167	0.494	0.359	0.267	0.213	0.163	0.327	0.221	0.153	0.163
No. LComm Alleles (<=25%)	0.133	0.133	0.133	0.133	0.133	0.100	0.133	0.133	0.133	0.133
No. LComm Alleles (<=50%)	0.433	0.433	0.422	0.433	0.433	0.422	0.433	0.433	0.433	0.422
He	0.061	0.056	0.060	0.050	0.066	0.057	0.056	0.063	0.073	0.057

Appendix IV (Fstat293) 10 populations 10 markers

(GHL509all.out)

Weir & Cockerham (1984) estimation of Fit (CapF), Fst (theta) and Fis (smallF).

relat is Relatedness estimated following Queller & Goodnight (1989)

relatc is relatedness inbreeding corrected following Pamilo (1984, 1985)

sig_a, sig_b and sig_w are the component of variance among samples, among individuals within samples and within individuals respectively.

Over all loci

Capf	Theta	Smallf	Relat	Relatc	Sig_a	Sig_b	Sig_w
0.044	0.000	0.044	0.000	0.004	0.002	0.317	6.899

Rst Over all samples estimated following Rousset (1996) and Goodman (1997)

Rst	sigma	sigb	sigw	amean	astdev	
H120	0.008	0.1	0.1	16.1	149.07	4.0368
Hhi3	-0.004	-0.3	0.3	60.0	171.52	7.7423
C17	-0.007	-3.2	7.3	453.8	124.32	21.3958
A44	0.003	0.8	2.6	284.6	167.48	16.9569
H55	-0.007	-0.2	2.7	23.9	267.70	5.1368
H58	0.001	0.1	-24.1	229.9	126.07	14.3426
H111	-0.001	-0.6	-26.1	478.6	156.70	21.2509
H113	-0.001	-0.1	13.9	114.7	149.27	11.3285
H59	-0.002	-0.5	-11.0	236.0	168.20	14.9771
S1001	0.003	0.5	-2.2	198.0	245.75	14.0045

Rst over loci	Weighted	Goodman	Unweighted
Rst:	-0.0016	-0.0009	-0.0009

Jackknifing over populations.

For locus : H120					
Total	Capf	Theta	Smallf	Relat	Means
	-0.088	-0.000	-0.087	-0.001	
	0.022	0.004	0.021	0.009	Std. Err.
For locus : Hhi3					
Total	Capf	Theta	Smallf	Relat	Means
	0.023	-0.002	0.025	-0.004	
	0.016	0.001	0.017	0.000	Std. Err.
For locus : C17					
Total	Capf	Theta	Smallf	Relat	Means
	0.021	0.001	0.019	0.003	
	0.010	0.001	0.010	0.000	Std. Err.
For locus : A44					
Total	Capf	Theta	Smallf	Relat	Means
	-0.001	0.002	-0.002	0.003	
	0.022	0.003	0.023	0.007	Std. Err.
For locus : H55					
Total	Capf	Theta	Smallf	Relat	

Total	0.288	-0.002	0.290	-0.004	Means
	0.027	0.003	0.027	0.005	Std. Err.

For locus : H58

	Capf	Theta	Smallf	Relat	
Total	-0.023	0.002	-0.025	0.004	Means
	0.020	0.004	0.020	0.008	Std. Err.

For locus : H111

	Capf	Theta	Smallf	Relat	
Total	0.014	0.000	0.014	0.000	Means
	0.014	0.001	0.014	0.000	Std. Err.

For locus : H113

	Capf	Theta	Smallf	Relat	
Total	0.114	0.005	0.110	0.008	Means
	0.036	0.004	0.034	0.006	Std. Err.

For locus : H59

	Capf	Theta	Smallf	Relat	
Total	0.035	-0.001	0.036	-0.002	Means
	0.020	0.001	0.020	0.000	Std. Err.

For locus : S1001

	Capf	Theta	Smallf	Relat	
Total	-0.002	-0.000	-0.002	-0.001	Means
	0.008	0.002	0.007	0.004	Std. Err.

Jackknifing over loci.

	Capf	Theta	Smallf	Relat	
total	0.044	0.000	0.044	0.000	Means
	0.030	0.001	0.030	0.001	Std. Err.

Bootstrapping over Loci.

95% Confidence Interval.

	CapF	theta	Smallf	Relat
	0.000	-0.001	-0.000	-0.002
	0.108	0.002	0.109	0.003

99% Confidence Interval.

	CapF	theta	Smallf	Relat
	-0.007	-0.001	-0.008	-0.002
	0.133	0.002	0.133	0.004

Appendix IV (Fstat293) 10 populations 8 markers

(GHL509u55113.out)

Weir & Cockerham (1984) estimation of Fit (CapF), Fst (theta) and Fis (smallF).

relat is Relatedness estimated following Queller & Goodnight (1989)

relatc is relatedness inbreeding corrected following Pamilo (1984, 1985)

sig_a, sig_b and sig_w are the component of variance among samples, among individuals within samples and within individuals respectively.

Over all loci

Capf	Theta	Smallf	Relat	Relatc	Sig_a	Sig_b	Sig_w
0.005	0.000	0.005	0.000	0.004	0.000	0.029	5.790

Rst Over all samples estimated following Rousset (1996) and Goodman (1997)

	Rst	sigma	sigb	sigw	amean	astdev
H120	0.008	0.1	0.1	16.1	149.07	4.0368
Hhi3	-0.004	-0.3	0.3	60.0	171.52	7.7423
C17	-0.007	-3.2	7.3	453.8	124.32	21.3958
A44	0.003	0.8	2.6	284.6	167.48	16.9569
H58	0.001	0.1	-24.1	229.9	126.07	14.3426
H111	-0.001	-0.6	-26.1	478.6	156.70	21.2509
H59	-0.002	-0.5	-11.0	236.0	168.20	14.9771
S1001	0.003	0.5	-2.2	198.0	245.75	14.0045

Rst over loci	Weighted	Goodman	Unweighted
Rst:	-0.0016	-0.0001	-0.0001

Jackknifing over loci.

total	Capf	Theta	Smallf	Relat	Means
	0.006	0.000	0.006	0.000	
	0.007	0.000	0.008	0.001	Std. Err.

Bootstrapping over Loci.

95% Confidence Interval.

CapF	theta	Smallf	Relat
-0.012	-0.001	-0.013	-0.002
0.017	0.001	0.018	0.002

99% Confidence Interval.

CapF	theta	Smallf	Relat
-0.020	-0.001	-0.021	-0.002
0.020	0.001	0.021	0.002

Appendix IV (Fstat293) 6 populations (4 temporal and 2 10 markers

(GHL509temporalesamlet.out)

Weir & Cockerham (1984) estimation of Fit (CapF), Fst (theta) and Fis (smallF).

relat is Relatedness estimated following Queller & Goodnight (1989)

relatc is relatedness inbreeding corrected following Pamilo (1984, 1985)

sig_a, sig_b and sig_w are the component of variance among samples, among individuals within samples and within individuals respectively.

Over all loci

Capf	Theta	Smallf	Relat	Relatc	Sig_a	Sig_b	Sig_w
0.044	0.001	0.044	0.001	0.005	0.005	0.314	6.899

Rst Over all samples estimated following Rousset (1996) and Goodman (1997)

	Rst	sigma	sigb	sigw	amean	astdev
H120	0.002	0.0	0.2	16.1	149.07	4.0368
Hhi3	-0.004	-0.2	0.2	60.0	171.52	7.7423
C17	-0.004	-1.9	6.0	453.8	124.32	21.3958
A44	-0.000	-0.1	3.3	284.6	167.48	16.9569
H55	-0.003	-0.1	2.6	23.9	267.70	5.1368
H58	0.002	0.3	-24.3	229.9	126.07	14.3426
H111	-0.000	-0.2	-26.4	478.6	156.70	21.2509
H113	0.002	0.2	13.6	114.7	149.27	11.3285
H59	0.001	0.2	-11.6	236.0	168.20	14.9771
S1001	0.002	0.4	-2.0	198.0	245.75	14.0045

Rst over loci	Weighted	Goodman	Unweighted
Rst:	-0.0006	-0.0003	-0.0003

Jackknifing over populations.

For locus : H120					
	Capf	Theta	Smallf	Relat	
Total	-0.086	0.001	-0.087	0.001	Means
	0.028	0.005	0.028	0.010	Std. Err.
For locus : Hhi3					
	Capf	Theta	Smallf	Relat	
Total	0.022	-0.002	0.024	-0.004	Means
	0.023	0.001	0.023	0.000	Std. Err.
For locus : C17					
	Capf	Theta	Smallf	Relat	
Total	0.020	0.001	0.019	0.002	Means
	0.008	0.002	0.007	0.003	Std. Err.
For locus : A44					
	Capf	Theta	Smallf	Relat	
Total	-0.001	0.000	-0.002	0.001	Means
	0.013	0.002	0.014	0.004	Std. Err.
For locus : H55					
	Capf	Theta	Smallf	Relat	
Total	0.290	0.002	0.289	0.002	Means
	0.021	0.005	0.022	0.008	Std. Err.
For locus : H58					
	Capf	Theta	Smallf	Relat	
Total	-0.024	0.001	-0.025	0.002	Means

	0.013	0.003	0.012	0.005	Std. Err.
	For locus : H111				
	Capf	Theta	Smallf	Relat	
Total	0.013	0.000	0.013	0.001	Means
	0.014	0.001	0.014	0.000	Std. Err.
	For locus : H113				
	Capf	Theta	Smallf	Relat	
Total	0.113	0.003	0.110	0.005	Means
	0.031	0.002	0.031	0.004	Std. Err.
	For locus : H59				
	Capf	Theta	Smallf	Relat	
Total	0.033	0.000	0.032	0.001	Means
	0.024	0.001	0.024	0.000	Std. Err.
	For locus : S1001				
	Capf	Theta	Smallf	Relat	
Total	-0.002	0.000	-0.002	0.001	Means
	0.007	0.001	0.007	0.000	Std. Err.

Jackknifing over loci.

	Capf	Theta	Smallf	Relat	
total	0.044	0.001	0.044	0.001	Means
	0.030	0.000	0.030	0.001	Std. Err.

Bootstrapping over Loci.

95% Confidence Interval.

	CapF	theta	Smallf	Relat
	0.000	-0.000	-0.000	-0.000
	0.108	0.001	0.107	0.003

99% Confidence Interval.

	CapF	theta	Smallf	Relat
	-0.007	-0.000	-0.008	-0.001
	0.133	0.002	0.132	0.003

Appendix V (Arlequin3.11)

Distance method: No. of different alleles (FST)
 StructureName = "New Edited Structure"

```
NbGroups = 6
Group={ "UUM95" "UUM05" }
Group={ "BAF95" "BAF04" }
Group={ "DAV95" "DAV04" }
Group={ "DAN04" }
Group={ "UPE95" "UPE05" }
Group={ "ILL05" }
```

 AMOVA design and results :

Reference: *Weir, B.S. and Cockerham, C.C. 1984.*
Excoffier, L., Smouse, P., and Quattro, J. 1992.
Weir, B. S., 1996.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among groups	5	13.971	0.00312 Va	0.11
Among populations within groups	4	9.087	-0.00543 Vb	-0.19
Among individuals within populations	499	1409.338	-0.02300 Vc	-0.81
Within individuals	509	1461.000	2.87033 Vd	100.89
Total	1017	2893.396	2.84503	

Fixation Indices

```
FIS : -0.00808
FSC : -0.00191
FCT : 0.00110
FIT : -0.00890
```

 Significance tests (1023 permutations)

```
Vd and FIT : P(rand. value < obs. value) = 0.88856
              P(rand. value = obs. value) = 0.00978
              P-value = 0.89834+-0.00959
```

```
Vc and FIS : P(rand. value > obs. value) = 0.85630
              P(rand. value = obs. value) = 0.01075
```

P-value = 0.86706+-0.01254

Vb and FSC : P(rand. value > obs. value) = 0.95308
P(rand. value = obs. value) = 0.00000
P-value = 0.95308+-0.00641

Va and FCT : P(rand. value > obs. value) = 0.11535
P(rand. value = obs. value) = 0.00000
P-value = 0.11535+-0.00957