# Neolithic Transitions

## Can genetic data help us understand a major demographic event in human prehistory?

Ana Rita Rodrigues Rasteiro de Campos

Dissertation presented to obtain the Ph.D degree in Biology

Instituto de Tecnologia Química e Biológica | Universidade Nova de Lisboa

Oeiras,
February, 2012

INSTITUTO
DE TECNOLOGIA
QUÍMICA E BIOLÓGICA
/UNL

Knowledge Creation

# Neolithic Transitions:

## Can genetic data help us understand a major demographic event in human prehistory?

Ana Rita Rodrigues Rasteiro de Campos

Dissertation presented to obtain the Ph.D degree in Biology

Instituto de Tecnologia Química e Biológica | Universidade Nova de Lisboa

Research work coordinated by:

FUNDAÇÃO CALOUSTE GULBENKIAN
Instituto Gulbenkian de Ciência

Oeiras,
February, 2012

INSTITUTO
DE TECNOLOGIA
QUÍMICA E BIOLÓGICA
/UNL

Knowledge Creation

FCT Fundação para a Ciência e a Tecnologia
MINISTÉRIO DA EDUCAÇÃO E CIÊNCIA

Esta tese obteve o apoio financeiro da FCT e do FSE no âmbito do Quadro Comunitário de Apoio, BD nº SFRH/BD/30821/2006.

*À minha família*

# Abstract

The Neolithic transition is probably the most important cultural, economic and demographic revolution in human prehistory. It profoundly modified the distribution of human genes, languages and cultures worldwide. However, the study of the transition from hunting and gathering to farming societies has generated major controversies among archaeologists and geneticists alike, with one side favouring demic diffusion models and the other the cultural diffusion models. As a first approximation two alternative demographic scenarios can be considered. Under the cultural diffusion models the transition to agriculture is regarded essentially as a cultural phenomenon, involving the movement of ideas and practices, rather than people. In the demic diffusion models, a movement of people is involved. It can be shown that both models can be seen as special cases of an admixture model between Palaeolithic/Mesolithic and Neolithic populations. In this thesis, I used non-equilibrium and spatial admixture model approaches to help answer this long-standing controversy. I showed that demic diffusion models better explain the patterns of genetic diversity found in today's European and Japanese populations, but I do not rule out the role of cultural processes locally.

In the first part of my thesis, we tried to address the transition to farming in the Japanese islands. The first inhabitants of Japan, the hunter-gatherers Jomon, had their culture completely replaced by that of the Yayoi farmers, who arrived later in the archipelago. Exactly how this cultural replacement occurred is still controversial today. Surprisingly, this

issue was never been addressed from an admixture point of view before, even though this is probably the only point on which most studies agree, i.e. that there was admixture between the two groups of humans. We used Y-chromosome data and an admixture approach to quantify the level of admixture across the Japanese archipelago. The method used, which accounts for genetic drift since the admixture event, clearly points to a demic diffusion process, similar to the process that was suggested for Europe also using Y-chromosome data.

In the second part of my thesis, we integrated Y-chromosome and mitochondrial DNA (mtDNA) data into the same admixture approach to study the European Neolithic transition. We found that contrary to several statements claiming the opposite, both contemporary Y-chromosome and mtDNA data clearly favour a demic diffusion process, i.e. both males and females underwent the same admixture history. However, key differences in the female and male demographic histories were also identified, most likely related with sex-related differences in effective size and migration patterns. Additionally, using an Approximate Bayesian Computation approach and one of the largest ancient DNA dataset available, we compared several demographic models with and without population structure and admixture. Our results show that demic diffusion is again favoured, but population structure and differential growth between farmers and hunter-gatherers are necessary to explain the process.

Finally, a new multi-population spatial simulation framework was developed and applied to study the consequence of sex-biased migration in the genetic make-up of populations, during the European Neolithic transition. Archaeological and anthropological data suggest that changes in post-marital residence rules between males and females took place as a consequence of sedentism and new rules of land control by men. Our results show that the Neolithic transition must have left its mark in the genome of Europeans and confirm that farming was accompanied by

reduced male migration and a movement of females to their husband's birthplace.

All together, the studies presented in this thesis allow us to draw a coherent picture of the Neolithic transition in Europe (and to some extent in Japan), which not only provides an explanation for the patterns of genetic diversity found today and in our past, but also for the apparent contradiction between phylogeographic and model-based studies.

# Resumo

A transição Neolítica é provavelmente a revolução cultural, económica e demográfica mais importante da Pré-História Humana. Esta mudou profundamente a distribuição de genes, de linguagens e culturas no mundo. No entanto, o estudo da transição de sociedades caçadoras-recolectoras para sociedades agricultoras tem gerado muita polémica tanto entre geneticistas, como entre arqueólogos, com um lado favorecendo modelos de difusão démica e o outro modelos de difusão cultural. Nos modelos de difusão cultural, a transição para a agricultura é vista essencialmente como um fenómeno cultural, que envolve o movimento de ideias e práticas, ao invés de pessoas. Pelo contrário, nos modelos de difusão démica, um movimento de pessoas está envolvido. Ambos os modelos podem ser vistos como casos especiais de miscigenação/mistura entre populações do Paleolítico/Mesolítico e Neolítico. Nesta tese foram usados métodos aplicados a modelos de miscigenação, quer em situações de não-equilíbrio, quer distribuídos no espaço, para ajudar a responder a esta controvérsia. Os nossos resultados mostram que modelos de difusão démica explicam melhor os padrões de diversidade genética encontrados actualmente, em populações europeias e japonesas, mas ao mesmo tempo não descartam o papel de processos culturais locais.

Na primeira parte da tese, procurou-se abordar a transição para a produção agrícola nas ilhas japonesas. Os primeiros habitantes do Japão, os caçadores-recoletores Jomon, tiveram a sua cultura completamente substituída pela dos agricultores Yayoi, que chegaram mais tarde no

arquipélago. O modo exacto como ocorreu esta substituição cultural é ainda controverso nos dias de hoje. Surpreendentemente, esta questão nunca foi abordada do ponto de vista de miscigenação, ainda que este seja o único ponto em que a maioria dos estudos concorda, ou seja, que houve um processo de miscigenação entre os dois grupos de seres humanos. Usámos dados do cromossoma Y e uma abordagem de miscigenação para quantificar o nível de mistura em todo o arquipélago japonês. O método utilizado, que tem em conta a deriva genética desde o evento de mistura, aponta claramente para um processo de difusão démica, similar ao processo que foi sugerido para a Europa, também usando dados do cromossoma Y.

Na segunda parte desta tese, integrámos dados de ADN mitocondrial (ADNmt) e de cromossoma Y na mesma abordagem de miscigenação, para estudar a transição Neolítica europeia. Mostrámos que, ao contrário de várias várias declarações afirmando o contrário, tanto os dados de cromossoma Y e como os de ADNmt contemporâneos favorecem claramente um processo de difusão démica, ou seja, tanto homens como mulheres foram submetidos à mesma história de miscigenação. No entanto, diferenças importantes nas histórias demográficas femininas e masculinas também foram identificadas, provavelmente relacionadas com diferenças sexuais no tamanho efectivo das populações e padrões de migração. Além disso, utilizando a abordagem "Approximate Bayesian Computation" e um dos maiores conjunto de dados de ADN antigo disponível, foram comparados vários modelos demográficos com e sem estrutura populacional e mistura. Estes resultados favorecem novamente a difusão démica, mas também apontam a necessidade de introduzir estrutura populacional e crescimento diferencial, entre os agricultores e caçadores-recolectores, para explicar o processo.

Finalmente, desenvolvemos um novo programa computacional capaz de simular diferentes populações no espaço. Este foi aplicado para es-

tudar a consequência da migração enviesada a favor de um sexo na constituição genética das populações, durante a transição Neolítica europeia. Dados arqueológicos e antropológicos sugerem que mudanças nas regras de residência pós-marital, entre homens e mulheres, ocorreram como consequência do sedentarismo e de novas regras de controlo da terra pelos homens. Os nossos resultados mostram que a transição Neolítica deve ter deixado sua marca no genoma europeu e confirmam que a agricultura foi acompanhada por uma redução da migração masculina e pelo movimento de mulheres para o local de nascimento do seu marido.

Ao todo, os estudos apresentados nesta tese permitiu-nos desenhar um quadro coerente da transição Neolítica na Europa (e até certo ponto também no Japão), que não só fornece uma explicação para os padrões de diversidade genética quer encontrados hoje, como no passado, mas também para a aparente contradição entre estudos filogeográficos e aqueles baseados em modelos.

# Acknowledgements

The completion of this thesis would not have been possible without the help and encouragement of many people. Their support has taken many forms, and I want to show here my deepest gratitude to them.

Lounès Chikhi was both the instigator and the supervisor of the research presented in this thesis. The quality of my work owes much to his dynamic and motivating leadership at its high competence and scientific rigor. I also thanks for the friendship he has shown in me during these years.

Instituto Gulbenkian de Ciência (IGC) and Instituto de Tecnologia Química e Biológica (ITQB) for providing me a platform for my PhD work.

Isabel Gordo and Élio Sucena, who kindly have agreed to be members of my thesis committee.

Giorgio Bertorelle, António Amorim, Nuno Ferrand and Gabriela Gomes for agreeing to be part of my PhD defense jury.

The IT services of IGC for showing a great willingness to help me solve the computer problems I encountered during my work.

Pedro Fernandes, responsible for the High Performance Comput-

ing Centre (HERMES) at IGC, for all the support.

Vítor Sousa for introducing me to the wonderful world of R and High Performance Computing. Thank you for all the help and for our many discussions.

Bárbara Parreira for all the discussions, especially when the Population and Conservation Genetics Group (PCG) was composed with just both of us, Vítor and Lounès.

To Pierre-Antoine Bouttier and Damien Mounier, INSA (Toulouse) students, who worked alongside us in the development of SINS.

To all members of PCG, past and current, for making possible to work on a pleasant, motivating and dynamic group. João, Isabel, Jordi, Cristina, Isa, Célia, Sam, Cécile, Fabrice, among others.

Special thanks to Reeta Sharma, for all the discussions, kindness and friendship.

The continued support of all my family and friends for encourage higher education, especially that of my parents and sister.

# Contents

# List of Figures

## LIST OF FIGURES

# List of Tables

# List of Algorithms

# 1. General Introduction

## 1.1 The use of genetic data to characterize human populations

The study of human variation has a long and controversial history. During centuries, human variation was classified only by phenotypic traits and was the root of social inequalities among different populations [Marks, 2007].

At the turn of the 20$^{th}$ century, the immunological characterization of the ABO blood group system and its mode of inheritance provided the first genetic marker to measure human variation. However, it was at the end of World War I that the first study in human population genetics came about. Hirschfeld and Hirschfeld [1919] analysed blood samples from soldiers and locals assembled in the Macedonian front and demonstrated that there was variability in the frequency of the ABO blood groups, in the so-called 'racial groups'. Other blood groups systems were discovered and the same type of results were found [Boyd, 1950; Mourant, 1949]. Later, in the 1950s, it was already possible to ascertain the degree of population genetic variation in the serum proteins [Connell & Smithies, 1959; Smithies, 1959].

However, it was from the 1960s onwards, with the molecularization on Biology, that the question of how to infer and interpret the genetic patterns of human diversity started to be more emphasised. Luigi Luca Cavalli-Sforza was one of the main drivers of this movement. He used classical genetic markers to infer human Prehistory, culminating in the publication of 'The History and Geography of Human Genes' in 1994. In this work, Cavalli-Sforza and colleagues [1994] performed Principal Component analyses and presented it as synthetic maps (Fig. 1.1). They found genetic clines across populations and argued that if variations in many genes

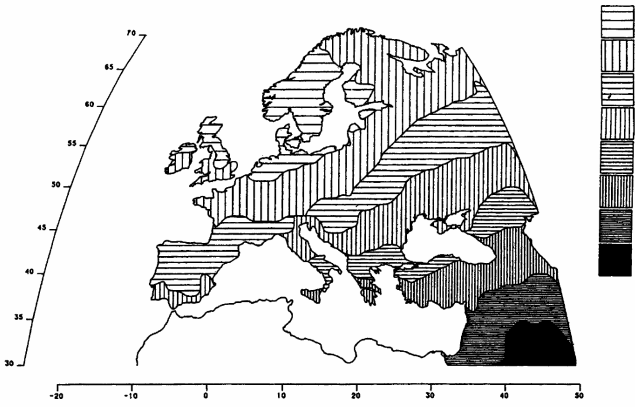



**Figure 1.1: SE-NW gradients in Europe** - Synthetic map of the first principal component of variation found by Cavalli-Sforza and colleagues [1994]). For the authors, this genetic cline represents the spread of agriculture from the Near East, during the Neolithic transition.

between populations are investigated simultaneously, they often correspond to population migrations due to, for example, new sources of food, improved transportation, or shifts in political power. In fact, the genetic clines found in Europe can be connected to the demographic past of the European populations, based on archaeological and linguistic data [Cavalli-Sforza *et al.*, 1994]. Thus, human variation can be seen as continuous, as opposite to discrete, and is not compatible with racial classifications [Marks, 2007]. It was in this context that archaeogenetics emerged. Having been coined independently by Colin Renfrew [2001] and António Amorim [1999], it refers to the application of techniques of molecular population genetics to the study of the human past.

The description of human populations genetic structure has evolved since the days of ABO blood groups typing. More and more genetic data are available for many present-day human populations and different types of genetic markers [Belle & Barbujani, 2007; Liu *et al.*, 2006; Pritchard *et al.*, 1999; Quintana-Murci *et al.*, 2008; Richards *et al.*, 2000] or whole genomes [Gronau *et al.*, 2011; Laval *et al.*, 2010]

are gradually being used to reconstruct the demographic history and prehistory of human populations. Studies using mitochondrial DNA (mtDNA) and the non-recombining portion of the Y-chromosome (NRY) are particularly useful for genetic anthropology and archaeogenetics. Both mtDNA and NRY are inherited almost unaltered by the female and male lineages, respectively and are thus good markers to study sex-biased processes in human evolution [Wilkins, 2006; Wilkins & Marlowe, 2006].

However, how much information can genetic data really give us? It is in this context that this thesis is integrated with special emphasis for a specific human demographic event: the Neolithic transition.

## 1.2 Neolithic transition

The development and spread of farming, referred to as the 'Neolithic transition' is one of the major demographic events of human prehistory. Gordon Childe [1936] named it Neolithic 'Revolution' and is considered by Mithen [2007] as the 'defining event of human history'. The several transformations that occurred during this period: either social, demographic, economic, cultural or nutritional, were linked to a new way of life mostly based on food production and sedendism. This transition took place independently in different regions of the planet (Fig. 1.2), over a few millenia, and led to the domestication of many plants and animals [Abbo *et al.*, 2006; Tresset & Vigne, 2011]. The shift from hunter-gathering to farming economies coincide with an increase of archaeological data in the Near East [Bocquet-Appel, 2009, 2011; Gkiasta *et al.*, 2003] and in other parts of the world [Bellwod & Oxenham, 2008]. This suggest a major demographic growth after this period [Bellwood, 2004; Price, 2000], that was named by Bocquet-Appel [2002] as 'Neolithic Demographic Transition'.

The earliest Neolithic period in the world started in the Near-East, around 11,0000 BP in the region that is known as the Fertile Crescent (see Figure 1.2), and later expanded into Europe and other directions [Ammerman & Cavalli-Sforza, 1984; Bellwood, 2004; Cavalli-Sforza *et al.*, 1994; Diamond & Bellwood, 2003]. Still, if we

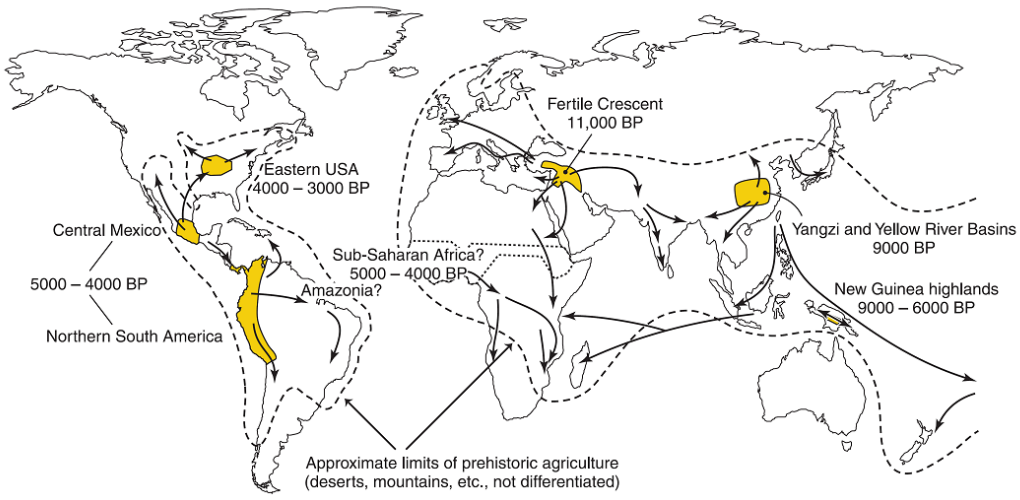



**Figure 1.2: Neolithic transition** - Different independent points of origin, in specific climatic and geographical contexts (adapted from Diamond and Bellwood [2003]).

want to understand how agriculture was adopted by human groups, clearly, a global approach should be taken and genetic data should be analysed using similar approaches in different regions.

As a first approximation, it is possible to consider two alternative demographic scenarios to explain the spread of farming technologies: the cultural (CDM) or the demic (DDM) diffusion models (see Fig. 1.3). Under the CDM the transition to agriculture is regarded essentially as a cultural phenomenon, involving the movement of ideas and practices rather than people [Zvelebil & Zvelebil, 1998]. It is expected that the genetic impact of the neighbouring farmers on the local hunter-gatherers (HG) will be thus limited. In the DDM, a movement of people is involved, and the transmission of agriculture technologies is mostly due to a significant arrival of new people [Ammerman & Cavalli-Sforza, 1984].

During two decades, most genetic studies were based on data from Europe and they all seemed to be in better agreement with the DDM than the CDM. In particu-

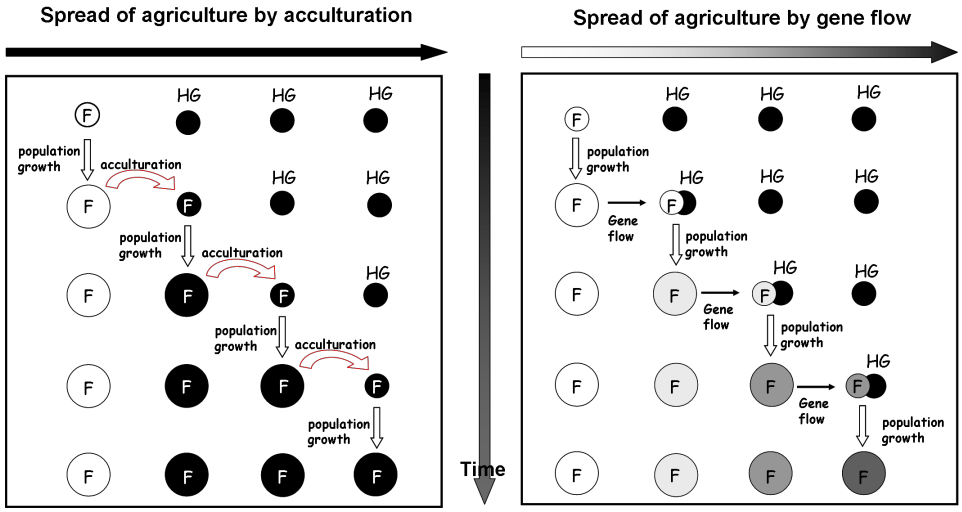



**Figure 1.3: Cultural and Demic diffusion models** - Two different models to explain the spread of agriculture. The CDM (on the left) assumes that the transmission of the farming technologies occurred by an acculturation process, whereas the DDM (on the right) assumes that a movement of individuals was involved and thus a movement of genes. In the CDM, the genetic makeup of present-today populations is expected to be similar to that of the Palaeolithic/Mesolithic HGs, whereas in the DDM, if admixture between populations occurred, it is a "mix" between HGs and farmers. Furthermore, in the DDM it is expected that through successive admixture events, the "Neolithic gene pool" would have suffered a dilution effect since the point of origin and along the axis of expansion (adapted from Jobling and colleagues [2003]).

lar, many studies found very strong correlations between genetic and archaeological maps representing the earliest dates of arrival of agriculture in Europe [Menozzi *et al.*, 1978] or between genetic and linguistic data across different regions [Barbujani & Pilastro, 1993]. However, some authors argued, based on mtDNA data, that the contribution of the early Palaeolithic or Mesolithic HGs was more important than previously thought [Richards, 2003; Richards *et al.*, 1996, 2000, 2002]. The rationale was based on the fact that most haplogroups found in Europe were in general old (>10,000 years) and this was interpreted as an indication that Neolithic haplotypes were a minor contribution. This has generated a major controversy. In particular, it was argued that the age of haplogroups had little to do with the age

of populations and that model-based approaches should be used to infer demographic parameters [Barbujani & Chikhi, 2006; Barbujani *et al.*, 1995; Chikhi, 2009; Chikhi *et al.*, 2002].

Despite the increasing amount of available data, and the numerous studies that have been published in the last decade, a very heated debate between the defenders of the CDM and DDM models was still taking place at the time of start of this thesis [Barbujani *et al.*, 1998; Chikhi *et al.*, 1998, 2002; Dupanloup *et al.*, 2004; Richards, 2003; Richards *et al.*, 2000; Semino *et al.*, 2000]. This clearly suggested that more work was needed to improve our understanding on the processes that took place during the Neolithic transition in different regions of the world. One of the reasons that have led to some controversy is the disagreement revolving around the manner in which genetic data should be analysed. It seemed that any method used to analyse the genetic data should be demonstrated to work on data for which the history is known with certainty. In other words, it should first be applied with success to simulated data. Unfortunately, the methods that have been most used in the literature are based on the interpretation of networks of DNA sequences [Bandelt *et al.*, 1999]. However, despite a very widespread use, these methods have never been tested on simulated data sets. There is no demonstration, so far, that these network-based methods actually provide reliable inference when they are applied to real data, for which we do not know the history. This is why, in this thesis, I favoured model-based approaches, which have the advantages of explicitly stating the assumptions used to make inference, and of being testable using simulated data [Beaumont *et al.*, 2010].

## 1.3 Inference of Human Past Demography using model-based approaches

Natural populations are very different from the ones idealized by the Wright-Fisher model (WFM) [Fisher, 1922; Wright, 1931] in population genetics. Real populations are not constant in size, they could have very complex histories like bottlenecks, expansions or admixture and also could receive immigrants from neighbour-

ing populations. Moreover, real populations are not panmitic. In humans, panmixia could only be achieved if marriages were completely random, independently of geographic boundaries, beliefs, languages, ethnies and social classes. And even then people would choose with whom to mate.

Genetic studies have consistently found differences between human populations [Cavalli-Sforza *et al.*, 1994; Rosenberg *et al.*, 2002] and their actual structure and history is very complex. Therefore, genetic data can help us to infer parameters values for simple [Beaumont *et al.*, 2002] or more complex demographic models [Fagundes *et al.*, 2007]. This is called a parameter-based inference, where parameters are estimated and hypotheses tested to study the distribution of genetic diversity and variation.

The first step for demographic inference is to choose the demographic model(s), which could explain the patterns of genetic diversity that we see in today's populations. As it is clearly impossible to model the full biological complexity of population demography, one should look for the simplest model that captures the relevant features of the known demography of the population. Population Genetics uses several types of demographic models that try to capture the demography of populations and that represent deviations to the Wright-Fisher model (see Fig. 1.4). However, the effects such complications have on population genetic inference, how such deviations can be detected and how it may be possible to estimate some of the important parameters relating to the demographic models are some of the main questions when using demographic inference.

Of course, any real population may well have experienced several of these demographic complexities and to model them we need *a priori* knowledge of the demography of the populations. In the case of human populations, to build our models we use several fonts of information like archaeology, linguistics, anthropology or genetics.

In the next sections, I will discuss some of the different types of demographic models that will be applied to The Neolithic transition in this thesis.

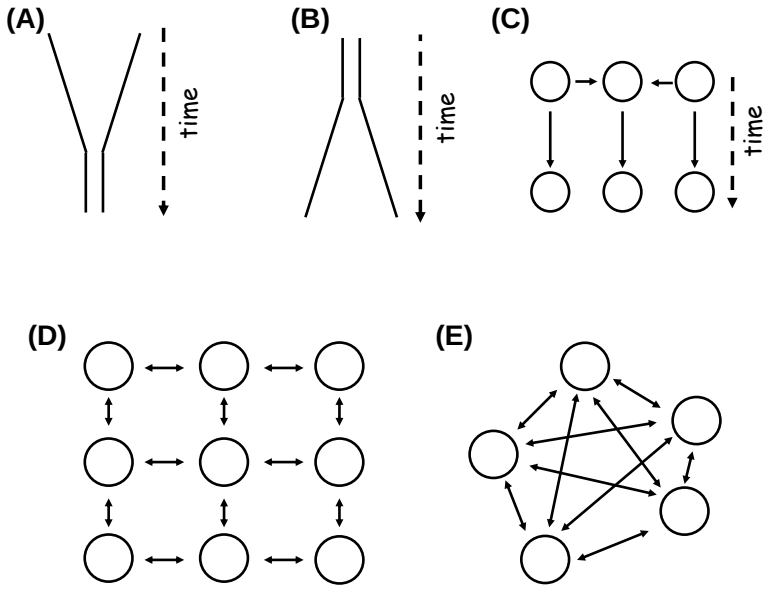



**Figure 1.4: Demographic models used in Population Genetics** - **(A)** Bottleneck, **(B)** expansion and **(C)** admixture represent some of the non-equilibrium models used in Population Genetics. Population structure can also be modelled, with or without the dimension space integrated, like the **(D)** 2D stepping-stone **(E)** or the island models, respectively. In the 2D stepping-stone model [Kimura & Weiss, 1964], individuals from one deme can only migrate to their neighbours, in the four cardinal directions, while in the island model [Wright, 1931] they are able to migrate in any direction.

### 1.3.1 Admixture models

Admixture in human populations is both widespread and important. In admixture, a new population (hybrid) is formed from two or more source populations that come together for a limited period of time. Such models can be applied to many humans populations, like for example in the colonization of America by Europeans [Carvajal-Carmona *et al.*, 2000; Salzano, 2004] or in the Anglo-Saxon transition in the British Isles [Capelli *et al.*, 2003; Weale *et al.*, 2002]. They can also be applied to older events like the the Neolithic transition. We could consider that during the Neolithic transition the Mesolithic populations and the first Neolithic populations come to-

gether and create an hybrid population and that modern populations are the result of this event.
It has been shown that the CDM and DDM can been seen as as extreme cases of an admixture model, whereby two or more parental populations mixed in the past to produce the hybrid ancestors of present-day populations [Chikhi *et al.*, 2002; Currat & Excoffier, 2005]. Thus, in extreme cases of admixture, with no genetic contribution of one of the parental populations (see Fig. 1.6), we would expect that the gene pool of present-day populations is similar to the Mesolithic HGs, in the case of CDM, or to the Neolithic farmers, in the case of DDM. However, it was also shown that the genetic consequences of CDM and DDM models are more complex than is usually believed particularly when spatial processes are considered [Chikhi *et al.*, 2002; Currat & Excoffier, 2005] (see Fig. 1.3 and 1.5). For instance, if we consider a process where the first farmers arrive to a new land, admix with the indigenous populations (HGs) and, as a result, raise the carrying capacity of that same area, due to new ways of exploring the land and food resources. Consequently, the size of the newly admixed population increases until the carrying capacity is reached, forcing part of the individuals to move and repeat the admixture process. This process would lead to a dilution of the "Neolithic genes", through the axis of expansion and was described for the European Neolithic transition by Chikhi *et al.* [2002] (see Fig. 1.5).

#### *1.3.1.1 Thus, how can one go about detecting admixture?*

In 1931, Bernstein (see [Bertorelle & Excoffier, 1998]) was the first to describe how genetic data could be used to estimate the contribution of two parental populations to a hybrid one. Traditionally, over the last 60 years, the estimation of the degree of admixture relied on the comparison of allele frequencies of each parental and hybrid populations [Chakraborty & Weiss, 1986, 1988; Long, 1991].
Recently, several methods were developed that differ either on the type of information available for the putative parental populations or on the assumptions related to the time of admixture. On on hand,For example, if there is no *a priori* choice for specific source populations and as long as the admixture event is recent, clustering

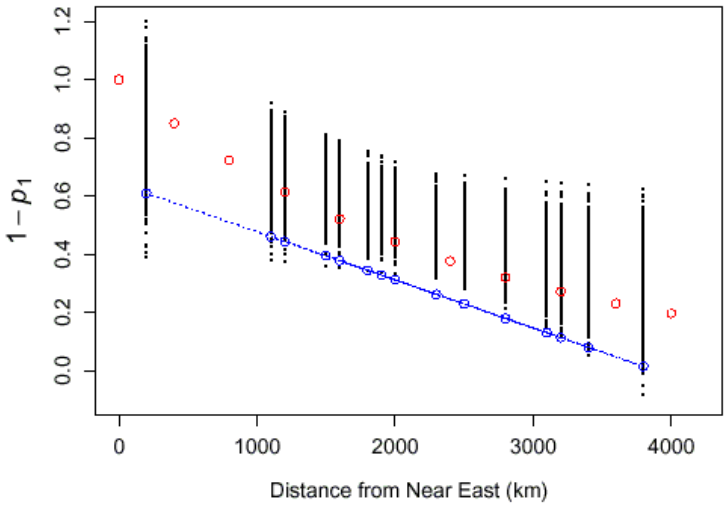



**Figure 1.5: Neolithic contribution across Europe** - In 2002, Chikhi and colleagues analysed a large published Y-chromosome dataset [Semino *et al.*, 2000], using an admixture approach (see Fig. 1.6) described in chapters 2 and 3. Their results revealed a significantly larger genetic contribution from Neolithic farmers than did previous indirect approaches based on the distribution of haplotypes. In this figure (taken from Chikhi and colleagues [2002]) is represented the linear regression of Neolithic contribution ($1\text{-}p_1$) against the geographic distance from the Near East, where they detected a significant decrease in admixture across the entire range between the Near East and Western Europe, supporting the DDM.

algorithms can be used to group similar classes of individuals within a population and identify individuals that are admixed [Pritchard *et al.*, 2000]. In contrast, if there is information from putative source populations, even old admixture events can be detected and the relative contribution of source populations estimated [Chikhi *et al.*, 2001; Sousa *et al.*, 2009; Wang, 2003]. Hence, admixture can be estimated by incorporating information on the molecular diversity present in the admixed and in parental populations [Bertorelle & Excoffier, 1998; Dupanloup & Bertorelle, 2001] and also by explicitly taking into account the genetic drift of allele frequencies since the admixture event [Chikhi *et al.*, 2001; Sousa *et al.*, 2009; Wang, 2003].

Several different approaches have been developed to calculate the relative genetic

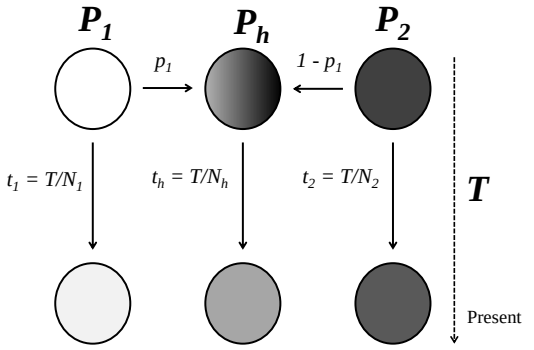



**Figure 1.6: Admixture model** - In this model, two populations join together sometime in the past to create an hybrid population. After the admixture event, the three populations evolve independently under pure genetic drift, with no mutation, no selection and no migration involved. This is the model used by the Chikhi *et al.* [2001] method.

contribution of each parental population in single admixture event scenarios, including Bayesian [Chikhi *et al.*, 2001] (see Fig. 1.6) and maximum-likelihood [Wang, 2003] methods. While these approaches are computationally intensive, they have been shown to produce good estimates with smaller variances across independent simulations [Chikhi *et al.*, 2001; Choisy *et al.*, 2004; Wang, 2003]. More recently, a new Approximate Bayesian Computation (ABC) approach was developed to estimate admixture parameters [Bray *et al.*, 2010; Sousa *et al.*, 2009]. This method is considerable faster than the others and is able to model more complex scenarios, like with one or two admixture events and with two or three parental populations.

### 1.3.2 Spatial models

The admixture models described before just account for time and not space. However, if we want to study the effect of geographical space in the patterns of genetic diversity, we have to use models that specifically add the dimension space. Below, I will focus on one type of spatial model used to study spatial expansions: the

stepping-stone model [Kimura & Weiss, 1964], where demes can only change individuals with their neighbours.
Recently, several studies have used this kind of models coupled with geographic information to study the consequences of spatial range expansions on genetic diversity. Some studies used one-dimensional (1D) stepping-stone modelling, i.e. individuals can only migrate in two directions, to simulate the colonization of the world through a serial founder effect [Deshpande *et al.*, 2009; Estoup *et al.*, 2004; Liu *et al.*, 2006; Prugnolle *et al.*, 2005; Ramachandran *et al.*, 2005].

Other methods were developed, not only to study the consequence range expansions, but also the consequence of the interaction between different cultural groups (like admixture) on genetic diversity. These methods use a more realistic geographic modelling approach, the two-dimensional (2D) stepping-stone model, where individuals can migrate in the four cardinal directions. Itan *et al.* [2009] applied this model to study of lactase persistence in Europe and found an association between the lactase persistence expansion and the dissemination of the Neolithic culture in Central Europe. In this work, they modelled space using one layer (i.e. one 2D stepping-stone lattice), where each deme had associated different cultural groups (HG and dairying farmers) that could interact and have different demographic parameters associated to them.

A similar, yet different approach, was developed the by Laurent Excoffier lab and is partially incorporated in SPLATCHE [Currat *et al.*, 2004], recently upgraded to SPLATCHE2 [Ray *et al.*, 2010]. Contrary to the Itan et al. [2009] study, each cultural group is associated with a layer (i.e. a different 2D stepping-stone lattice). In turn, each layer can have different demographic parameters and different layers can interact either by admixture or competition. In addition, environmental information obtained from Geographical Information Systems (GIS) can be added to constrain migration and deme densities. They applied this framework to several questions on human evolution, like the colonization of the world by early modern humans [Ray *et al.*, 2005] and to the Neanderthal and the modern humans cohabitation/hybridization problematic [Currat & Excoffier, 2004, 2011]. It was also applied

to study: i) gender-related asymmetries on gene flow [Hamilton *et al.*, 2005b]; ii) recent migration rates estimates after a spatial expansion [Hamilton *et al.*, 2005a]; iii) intra-deme molecular diversity in expanding populations [Ray *et al.*, 2003]; iv) the fate of mutations that are on the edge of a range expansion, commonly know as "surfing" phenomenon [Klopfstein *et al.*, 2006] and recently, v) the importance of the Gibraltar Strait on the Iberian peninsula [Currat *et al.*, 2010]. Finally, as one of the most important issues on human evolution, the Neolithic transition was also addressed by using this approach in Currat *et al.* [2005]. In this study, they estimate the contribution of HG and farmer populations to the genetic diversity of modern Europeans. Their results show that even a very limited HG contribution can lead to situations where the current human European gene pool could be traced to the Palaeolithic. The Neolithic contribution was also found to decrease very quickly along the axis of colonization, from the Neolithic point of origin. In fact, the allele frequency clines often found after a range expansion [Barbujani *et al.*, 1995; Chikhi *et al.*, 2002; Currat & Excoffier, 2005; Hallatschek & Nelson, 2008; Klopfstein *et al.*, 2006; Liu *et al.*, 2006; Long, 1991] can be explained by demographic events like "surfing" phenomena [Edmonds *et al.*, 2004; Hallatschek & Nelson, 2008; Klopfstein *et al.*, 2006; Long, 1991]. Surfing describes the geographic spread of an allele that rides on the front of the wave of advance of a spatial expansion and is favoured when populations at the wave front grow rapidly and exchange genes with their neighbours [Hallatschek & Nelson, 2008]. Regarding the Neolithic transition, surfing alleles can explain the "dilution" of the Neolithic lineages along the axis of expansion (see also section 1.3.1).

All the frameworks presented above use coalescent theory to generate the genetic diversity of the populations. Thus, the whole population does not need to be simulated. While this has several advantages in efficiency and computing time, as only the sampled genealogies are simulated, there are some disadvantages as well. For example, with this kind of approach more complex scenarios that take into account certain aspects of cultural practices, like sex-biased migration, cannot be model. In addition, at the time of start of this thesis, SPLATCHE2 [Ray *et al.*, 2010] was not available to the public and thus it was not possible to simulate different cultural

groups, in our case HG and farmers. This is why we developed and tested a new simulation framework, based on forward simulations, that enable us to model different cultural groups and to address more realistic scenarios in human populations (see chapters 4 and 5).

## 1.4 Aims

Despite the progress made in the last decade, both in terms of the increasing number of datasets, and the new model-based approaches that have been developed, (i) the debate between the two opposite models (CDM and DDM), is still going on and (ii) more work is needed to model the Neolithic transition in different regions of the world. Thus the work presented here aims to:

- Study the spread of farming using genetic data, taking into account anthropological, linguistic and archaeological data
- Model the consequence of admixture between the Palaeolithic populations and the Neolithic ones, using contemporary and ancient DNA.
- Use model-based approaches to infer if the processes that can be inferred in Europe are also found in other regions of the world.
- Ascertain if different patterns of genetic differentiation and diversity are encountered between present-day mtDNA and Y-chromosome data. Infer if these differences are due to different demographic histories for both females and males.

Chapters 2 and 3 of this thesis look at two case studies of admixture in human populations, with particular emphasis on migration, population size, and cultural behaviours. In particular, I applied a model-based admixture analysis to the Neolithic transition in Japan (Chapter 2) and Europe (Chapter 3). I also applied Approximate Bayesian Computation (ABC) to analyse a Central European Mesolithic and Neolithic aDNA dataset (Chapter 3).

The results of the analysis of Chapter 3 led us to believe that there were differences between the demographics histories of females and males. Chapters 4 and 5 explore the development and application to the European Neolithic of a spatial expansion simulation framework that allows the study of sex-biased processes.

## 1.5 References

ABBO, S., GOPHER, A., PELEG, Z., SARANGA, Y., FAHIMA, T., SALAMINI, F. & LEV-YADUN, S. (2006). The ripples of "the Big (agricultural) Bang": the spread of early wheat cultivation. *Genome*, **49**, 861–3.

AMMERMAN, A.J. & CAVALLI-SFORZA, L.L. (1984). *The Neolithic transition and the genetics of populations in Europe*. Princeton University Press, Princeton.

AMORIM, A. (1999). Archaeogenetics. *Journal of Iberian Archaeology*, **1**, 15–25.

BANDELT, H.J., FORSTER, P. & ROHL, A. (1999). Median-joining networks for inferring intraspecific phylogenies. *Mol Biol Evol*, **16**, 37–48.

BARBUJANI, G. & CHIKHI, L. (2006). Population genetics: DNAs from the European Neolithic. *Heredity*, **97**, 84–85.

BARBUJANI, G. & PILASTRO, A. (1993). Genetic evidence on origin and dispersal of human populations speaking languages of the Nostratic macrofamily. *Proc Natl Acad Sci U S A*, **90**, 4670–3.

BARBUJANI, G., SOKAL, R.R. & ODEN, N.L. (1995). Indo-European origins: a computer-simulation test of five hypotheses. *Am J Phys Anthropol*, **96**, 109–32.

BARBUJANI, G., BERTORELLE, G. & CHIKHI, L. (1998). Evidence for Paleolithic and Neolithic gene flow in Europe. *Am J Hum Genet*, **62**, 488–492.

BEAUMONT, M.A., ZHANG, W. & BALDING, D.J. (2002). Approximate Bayesian Computation in population genetics. *Genetics*, **162**, 2025–35.

BEAUMONT, M.A., NIELSEN, R., ROBERT, C., HEY, J., GAGGIOTTI, O., KNOWLES, L., ESTOUP, A., PANCHAL, M., CORANDER, J., HICKERSON, M., SISSON, S.A., FAGUNDES, N., CHIKHI, L., BEERLI, P., VITALIS, R., CORNUET, J.M., HUELSENBECK, J., FOLL, M., YANG, Z., ROUSSET, F., BALDING, D. & EXCOFFIER, L. (2010). In defence of model-based inference in phylogeography. *Mol Ecol*, **19**, 436–446.

BELLE, E.M.S. & BARBUJANI, G. (2007). Worldwide analysis of multiple microsatellites: language diversity has a detectable influence on DNA diversity. *Am J Phys Anthropol*, **133**, 1137–46.

BELLWOD, P. & OXENHAM, M. (2008). *The expansions of farming societies and the role of*

*the Neolithic Demographic Transition*, 13–34. Springer.

BELLWOOD, P. (2004). *First Farmers: the origins of agricultural societies*. Blackwell Publishing, Oxford.

BERTORELLE, G. & EXCOFFIER, L. (1998). Inferring admixture proportions from molecular data. *Mol Biol Evol*, **15**, 1298–1311.

BOCQUET-APPEL, J.P. (2002). Paleoanthropological traces of a Neolithic demographic transition. *Curr Anthropol*, **43**, 637–650.

BOCQUET-APPEL, J.P. (2009). The demographic impact of the agricultural system in human history. *Curr Anthropol*, **50**, 657–660.

BOCQUET-APPEL, J.P. (2011). When the world's population took off: the springboard of the Neolithic Demographic Transition. *Science*, **333**, 560–561.

BOYD, W.C. (1950). Use of blood groups in human classification. *Science*, **112**, 187–96.

BRAY, T., SOUSA, V., PARREIRA, B., BRUFORD, M. & CHIKHI, L. (2010). 2BAD an application to estimate the parental contributions during two independent admixture events. *Mol Ecol Resour*, **10**, 538–541.

CAPELLI, C., REDHEAD, N., ABERNETHY, J.K., GRATRIX, F., WILSON, J.F., MOEN, T., HERVIG, T., RICHARDS, M., STUMPF, M.P.H., UNDERHILL, P.A., BRADSHAW, P., SHAHA, A., THOMAS, M.G., BRADMAN, N. & GOLDSTEIN, D.B. (2003). A Y chromosome census of the British Isles. *Curr Biol*, **13**, 979–84.

CARVAJAL-CARMONA, L.G., SOTO, I.D., PINEDA, N., ORTÍZ-BARRIENTOS, D., DUQUE, C., OSPINA-DUQUE, J., MONTOYA, M.M.P., ALVAREZ, V.M., BEDOYA, G. & RUIZ-LINARES, A. (2000). Strong Amerind/White sex bias and a possible sephardic contribution among the founders of a population in Northwest Colombia. *Am J Hum Genet*, **67**, 1287–1295.

CAVALLI-SFORZA, L.L., MENOZZI, P. & PIAZZA, A. (1994). *The History and Geography of Human Genes*. Princeton University Press, Princeton.

CHAKRABORTY, R. & WEISS, K.M. (1986). Frequencies of complex diseases in hybrid populations. *Am J Phys Anthropol*, **70**, 489–503.

CHAKRABORTY, R. & WEISS, K.M. (1988). Admixture as a tool for finding linked genes and detecting that difference from allelic association between loci. *Proc Natl Acad Sci U S A*, **85**, 9119–9123.

CHIKHI, L. (2009). Update to Chikhi et al.'s "Clinal variation in the nuclear DNA of europeans" (1998): genetic data and storytelling–from archaeogenetics to astrologenetics? *Hum Biol*, **81**, 639–643.

CHIKHI, L., DESTRO-BISOL, G., PASCALI, V., BARAVELLI, V., DOBOSZ, M. & BARBUJANI,

G. (1998). Clinal variation in the nuclear DNA of Europeans. *Hum Biol*, **70**, 643–657.

CHIKHI, L., BRUFORD, M.W. & BEAUMONT, M.A. (2001). Estimation of admixture proportions: a likelihood-based approach using Markov chain Monte Carlo. *Genetics*, **158**, 1347–1362.

CHIKHI, L., NICHOLS, R.A., BARBUJANI, G. & BEAUMONT, M.A. (2002). Y genetic data support the Neolithic demic diffusion model. *Proc Natl Acad Sci U S A*, **99**, 11008–11013.

CHILDE, V.G. (1936). *Man Makes Himself*. Oxford University Press, Oxford.

CHOISY, M., FRANCK, P. & CORNUET, J.M. (2004). Estimating admixture proportions with microsatellites: comparison of methods based on simulated data. *Mol Ecol*, **13**, 955–968.

CONNELL, G.E. & SMITHIES, O. (1959). Human haptoglobins: estimation and purification. *Biochem J*, **72**, 115–121.

CURRAT, M. & EXCOFFIER, L. (2004). Modern humans did not admix with Neanderthals during their range expansion into Europe. *PLoS Biol*, **2**, e421.

CURRAT, M. & EXCOFFIER, L. (2005). The effect of the Neolithic expansion on European molecular diversity. *Proc R Soc B*, **272**, 679–688.

CURRAT, M. & EXCOFFIER, L. (2011). Strong reproductive isolation between humans and Neanderthals inferred from observed patterns of introgression. *Proc Natl Acad Sci U S A*, **108**, 15129–15134.

CURRAT, M., RAY, N. & EXCOFIER, L. (2004). SPLATCHE: a program to simulate genetic diversity taking into account environmental heterogeneity. *Mol Ecol Notes*, **4**, 139–142.

CURRAT, M., POLONI, E.S. & SANCHEZ-MAZAS, A. (2010). Human genetic differentiation across the Strait of Gibraltar. *BMC Evol Biol*, **10**, 237.

DESHPANDE, O., BATZOGLOU, S., FELDMAN, M.W. & CAVALLI-SFORZA, L.L. (2009). A serial founder effect model for human settlement out of Africa. *Proc Biol Sci*, **276**, 291–300.

DIAMOND, J. & BELLWOOD, P. (2003). Farmers and their languages: the first expansions. *Science*, **300**, 597–603.

DUPANLOUP, I. & BERTORELLE, G. (2001). Inferring admixture proportions from molecular data: extension to any number of parental populations. *Mol Biol Evol*, **18**, 672–675.

DUPANLOUP, I., BERTORELLE, G., CHIKHI, L. & BARBUJANI, G. (2004). Estimating the impact of prehistoric admixture on the genome of Europeans. *Mol Biol Evol*, **21**, 1361–1372.

EDMONDS, C.A., LILLIE, A.S. & CAVALLI-SFORZA, L.L. (2004). Mutations arising in the wave front of an expanding population. *Proc Natl Acad Sci*, **101**, 975–9.

ESTOUP, A., BEAUMONT, M., SENNEDOT, F., MORITZ, C. & CORNUET, J.M. (2004). Genetic analysis of complex demographic scenarios: spatially expanding populations of the cane toad, Bufo marinus. *Evolution*, **58**, 2021–2036.

FAGUNDES, N.J.R., RAY, N., BEAUMONT, M., NEUENSCHWANDER, S., SALZANO, F.M., BONATTO, S.L. & EXCOFFIER, L. (2007). Statistical evaluation of alternative models of human evolution. *Proc Natl Acad Sci U S A*, **104**, 17614–9.

FISHER, R.A. (1922). On the dominance ratio. *Proc R Soc Edin*, **42**, 321–341.

GKIASTA, M., RUSSELL, T., SHENNAN, S. & STEELE, J. (2003). Neolithic transition in Europe: the radiocarbon revisited. *Antiquity*, **77**, 45–62.

GRONAU, I., HUBISZ, M.J., GULKO, B., DANKO, C.G. & SIEPEL, A. (2011). Bayesian inference of ancient human demography from individual genome sequences. *Nature Genetics*, **43**, 1031–1034.

HALLATSCHEK, O. & NELSON, D.R. (2008). Gene surfing in expanding populations. *Theor Popul Biol*, **73**, 158–170.

HAMILTON, G., CURRAT, M., RAY, N., HECKEL, G., BEAUMONT, M. & EXCOFFIER, L. (2005a). Bayesian estimation of recent migration rates after a spatial expansion. *Genetics*, **170**, 409–417.

HAMILTON, G., STONEKING, M. & EXCOFFIER, L. (2005b). Molecular analysis reveals tighter social regulation of immigration in patrilocal populations than in matrilocal populations. *Proc Natl Acad Sci U S A*, **102**, 7476–80.

HIRSZFELD, L. & HIRSZFELD, H. (1919). Serological differences between the blood of different races. *Lancet*, **194**, 675–679.

ITAN, Y., POWELL, A., BEAUMONT, M.A., BURGER, J. & THOMAS, M.G. (2009). The origins of lactase persistence in Europe. *PLoS Comput Biol*, **5**, e1000491.

JOBLING, M.A., HURLES, M. & TYLER-SMITH, C. (2003). *Human Evolutionary Genetics: Origins, Peoples and Disease*. Garland Science, New York.

KIMURA, M. & WEISS, G.H. (1964). The stepping stone model of population structure and the decrease of genetic correlation with distance. *Genetics*, **49**, 561–76.

KLOPFSTEIN, S., CURRAT, M. & EXCOFFIER, L. (2006). The fate of mutations surfing on the wave of a range expansion. *Mol Biol Evol*, **23**, 482–490.

LAVAL, G., PATIN, E., BARREIRO, L.B. & QUINTANA-MURCI, L. (2010). Formulating a Historical and Demographic Model of Recent Human Evolution Based on Resequencing Data from Noncoding Regions. *PLoS One*, **5**, e10284.

LIU, H., PRUGNOLLE, F., MANICA, A. & BALLOUX, F. (2006). A geographically explicit genetic model of worldwide human-settlement history. *Am J Hum Genet*, **79**, 230–237.

LONG, J.C. (1991). The genetic structure of admixed populations. *Genetics*, **127**, 417–428.

MARKS, J. (2007). Long shadow of Linnaeus's human taxonomy. *Nature*, **447**, 28.

MENOZZI, P., PIAZZA, A. & CAVALLI-SFORZA, L. (1978). Synthetic maps of human gene frequencies in Europeans. *Science*, **201**, 786–792.

MITHEN, S. (2007). Did farming arise from a misapplication of social intelligence? *Philos Trans R Soc Lond B Biol Sci*, **362**, 705–718.

MOURANT, A.E. (1949). The ethnological distribution of the Rh and MN blood-groups. *Adv Sci*, **5**, 313.

PRICE, T.D. (2000). *Europe's First Farmers: an introduction*, chap. 1, 1–18. Cambridge University Press, Cambridge.

PRITCHARD, J.K., SEIELSTAD, M.T., PEREZ-LEZAUN, A. & FELDMAN, M.W. (1999). Population growth of human Y chromosomes: a study of Y chromosome microsatellites. *Mol Biol Evol*, **16**, 1791–1798.

PRITCHARD, J.K., STEPHENS, M. & DONNELLY, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.

PRUGNOLLE, F., MANICA, A. & BALLOUX, F. (2005). Geography predicts neutral genetic diversity of human populations. *Curr Biol*, **15**, R159–R160.

QUINTANA-MURCI, L., QUACH, H., HARMANT, C., LUCA, F., MASSONNET, B., PATIN, E., SICA, L., MOUGUIAMA-DAOUDA, P., COMAS, D., TZUR, S., BALANOVSKY, O., KIDD, K.K., KIDD, J.R., VAN DER VEEN, L., HOMBERT, J.M., GESSAIN, A., VERDU, P., FROMENT, A., BAHUCHET, S., HEYER, E., DAUSSET, J., SALAS, A. & BEHAR, D.M. (2008). Maternal traces of deep common ancestry and asymmetric gene flow between Pygmy hunter-gatherers and Bantu-speaking farmers. *Proc Natl Acad Sci U S A*, **105**, 1596–601.

RAMACHANDRAN, S., DESHPANDE, O., ROSEMAN, C.C., ROSENBERG, N.A., FELDMAN, M.W. & CAVALLI-SFORZA, L.L. (2005). Support from the relationship of genetic and geographic distance in human populations for a serial founder effect originating in Africa. *Proc Natl Acad Sci U S A*, **102**, 15942–15947.

RAY, N., CURRAT, M. & EXCOFFIER, L. (2003). Intra-deme molecular diversity in spatially expanding populations. *Mol Biol Evol*, **20**, 76–86.

RAY, N., CURRAT, M., BERTHIER, P. & EXCOFFIER, L. (2005). Recovering the geographic origin of early modern humans by realistic and spatially explicit simulations. *Genome Res*, **15**, 1161–1167.

RAY, N., CURRAT, M., FOLL, M. & EXCOFFIER, L. (2010). SPLATCHE2: a spatially explicit simulation framework for complex demography, genetic admixture and recombination.

*Bioinformatics*, **26**, 2993–2994.

RENFREW, C. (2001). From molecular genetics to archaeogenetics. *Proc Natl Acad Sci U S A*, **98**, 4830–4832.

RICHARDS, M. (2003). The neolithic invasion of Europe. *Annu Rev Anthropol*, **32**, 135–162.

RICHARDS, M., CÔRTE-REAL, H., FORSTER, P., MACAULAY, V., WILKINSON-HERBOTS, H., DEMAINE, A., PAPIHA, S., HEDGES, R., BANDELT, H.J. & SYKES, B. (1996). Paleolithic and neolithic lineages in the European mitochondrial gene pool. *Am J Hum Genet*, **59**, 185–203.

RICHARDS, M., MACAULAY, V., HICKEY, E., VEGA, E., SYKES, B., GUIDA, V., RENGO, C., SELLITTO, D., CRUCIANI, F., KIVISILD, T., VILLEMS, R., THOMAS, M., RYCHKOV, S., RYCHKOV, O., RYCHKOV, Y., GÖLGE, M., DIMITROV, D., HILL, E., BRADLEY, D., ROMANO, V., CALÌ, F., VONA, G., DEMAINE, A., PAPIHA, S., TRIANTAPHYLLIDIS, C., STEFANESCU, G., HATINA, J., BELLEDI, M., RIENZO, A.D., NOVELLETTO, A., OPPENHEIM, A., NØRBY, S., AL-ZAHERI, N., SANTACHIARA-BENERECETTI, S., SCOZARI, R., TORRONI, A. & BANDELT, H.J. (2000). Tracing european founder lineages in the Near Eastern mtDNA pool. *Am J Hum Genet*, **67**, 1251–1276.

RICHARDS, M., MACAULAY, V., TORRONI, A. & BANDELT, H.J. (2002). In search of geographical patterns in European mitochondrial DNA. *Am J Hum Genet*, **71**, 1168–1174.

ROSENBERG, N.A., PRITCHARD, J.K. & FELDMAN, M.W. (2002). Genetic Structure of Human Populations. *Science*, **298**, 2381–2385.

SALZANO, F.M. (2004). Interethnic variability and admixture in latin America–social implications. *Rev Biol Trop*, **52**, 405–15.

SEMINO, O., PASSARINO, G., OEFNER, P.J., LIN, A.A., ARBUZOVA, S., BECKMAN, L.E., BENEDICTIS, G.D., FRANCALACCI, P., KOUVATSI, A., LIMBORSKA, S., MARCIKIAE, M., MIKA, A., MIKA, B., PRIMORAC, D., SANTACHIARA-BENERECETTI, A.S., CAVALLI-SFORZA, L.L. & UNDERHILL, P.A. (2000). The genetic legacy of Paleolithic Homo sapiens sapiens in extant Europeans: a Y chromosome perspective. *Science*, **290**, 1155–1159.

SMITHIES, O. (1959). An improved procedure for starch-gel electrophoresis: further variations in the serum proteins of normal individuals. *Biochem J*, **71**, 585–587.

SOUSA, V.C., FRITZ, M., BEAUMONT, M.A. & CHIKHI, L. (2009). Approximate bayesian computation without summary statistics: the case of admixture. *Genetics*, **181**, 1507–1519.

TRESSET, A. & VIGNE, J.D. (2011). Last hunter-gatherers and first farmers of Europe. *C R Biol*, **334**, 182–189.

WANG, J. (2003). Maximum-likelihood estimation of admixture proportions from genetic data. *Genetics*, **164**, 747–765.

WEALE, M.E., WEISS, D.A., JAGER, R.F., BRADMAN, N. & THOMAS, M.G. (2002). Y chromosome evidence for Anglo-Saxon mass migration. *Mol Biol Evol*, **19**, 1008–21.

WILKINS, J.F. (2006). Unraveling male and female histories from human genetic data. *Curr Opin Genet Dev*, **16**, 611–7.

WILKINS, J.F. & MARLOWE, F.W. (2006). Sex-biased migration in humans: what should we expect from genetic data? *Bioessays*, **28**, 290–300.

WRIGHT, S. (1931). Evolution in Mendelian Populations. *Genetics*, **16**, 97–159.

ZVELEBIL, M. & ZVELEBIL, K. (1998). Agricultural transition and Indo-European dispersals. *Antiquity*, **62**, 574–583.

# 2. Revisiting the peopling of Japan: an admixture perspective

Rita Rasteiro[1] and Lounès Chikhi[1,2,3]

[1]Instituto Gulbenkian de Ciência, Rua da Quinta Grande, 6, 2780-156 Oeiras,Portugal; [2]CNRS, Laboratoire Évolution et Diversité Biologique (EDB), Bât. 4R3 b2, 118 Route de Narbonne, 31062 Toulouse cédex 9, France; [3]Université de Toulouse, UPS, EDB, Bât. 4R3 b2, 118 Route de Narbonne, 31062 Toulouse cédex 9, France

**Data collection:** R Rasteiro
**Analysis:** R Rasteiro
**Manuscript:** R Rasteiro and L Chikhi
**Citation:** Rasteiro R, Chikhi L (2009) Revisiting the peopling of Japan: an admixture perspective. *J Hum Genet* 54: 349-54

---

## 2.1 Abstract

The first inhabitants of Japan, the Jomon hunter-gatherers, had their culture significantly modified by that of the Yayoi farmers, who arrived at a later stage from mainland Asia. How this change took place is still debated, but it has been suggested that modern Japanese are the product of admixture between these two populations. Here, we applied for the first time an admixture approach to study the Jomon-Yayoi transition, using previously published Y-chromosomal data.

Our results suggest that the Neolithic transition, in this part of the world, probably took place by a process of demic diffusion. We also show that for two populations

that could not have contributed to this process, our approach is able to detect inconsistencies when they are used as parental populations. However, despite these promising results, we could not locate precisely the geographical origin of the Yayoi in mainland Asia, as different potential sources gave similarly good results. This suggests that more loci would be required for a better understanding of the peopling of Japan.

**Keywords:** Japan/Neolithic/Jomon/Yayoi/admixture/Y-chromosome

## 2.2 Introduction

The development and spread of farming, referred to as the Neolithic transition was one of the major demographic events of human prehistory [Bellwood, 2004]. This process took place independently in different geographic areas, each one most likely associated with different demographic changes and with different domesticated animals and plants. In principle, each of these changes can be described as a process by which at least two human groups (Palaeolithic hunter-gatherers [HG] and Neolithic farmers) admixed to different extents. These processes can be seen as admixture models and although they have been used to study the Neolithic transition in Europe [Chikhi *et al.*, 2002; Currat & Excoffier, 2005; Dupanloup *et al.*, 2004], this has not been the case for Asia. Here, we focus on Eastern Asia, where the transition to agriculture has long been controversial, specifically regarding the prehistory of Japan [Cavalli-Sforza *et al.*, 1994; Hanihara, 1991; Matsumura, 2001; Mizoguchi, 1986].

Archaeological data suggest that there were probably two migratory waves of incoming people, both from the Asian continent to Japan. The first migration took place *c.* 38,000 - 37,000 BP, before the Pleistocene land bridges were submerged [Pope & Terrell, 2008], and later gave rise to the Jomon culture ($\geq$ 12,000 BP) [Ono *et al.*, 2002]. Although they were a HG society, the Jomon were the holders of one of the oldest pottery cultures known in the world and probably also led a sedentary or semi-sedentary life, well before showing any clear evidence of having devel-


**Figure 2.1: Map of the Japanese Islands** - Approximate geographical locations of the Japanese populations analysed in the present study. The other samples used as parental are not represented on the map.

oped agriculture [Bellwood, 2004; Highman, 2005]. A long time after this period, *c.* 2,300 BP, a second wave of people, together with a 'wet rice culture', weaving and metalwork, entered the southern Kyushu island (Figure 2.1), through the Korean Peninsula [Jin *et al.*, 2003], and then spread northeastward, starting the Yayoi period. The transformation and the replacement models represent the two opposite extremes of the demographic models that have been proposed to explain

the peopling of Japan and the contribution of both Jomon and Yayoi populations to modern Japanese. While the latter model claims that modern Japanese should be descendants of the incoming Yayoi who replaced completely the Jomon people [Cavalli-Sforza *et al.*, 1994], the former entails a movement of the Yayoi culture and ideas rather than people, with consequently no genetic contribution of the Yayoi to modern Japanese [Mizoguchi, 1986]. However, reality must have been less extreme and currently, it is widely accepted that modern Japanese are the result of admixture between the two populations that produced both the Jomon and Yayoi cultures. This was suggested by Hanihara [1991] and Matsumura [2001] based on dental and cranial characteristics, and more recently by a number of authors who used genetic data [Hammer & Horai, 1995; Hammer *et al.*, 2006; Horai *et al.*, 1996; Omoto & Saitou, 1997; Sokal & Thomson, 1998], including ancient DNA [Horai *et al.*, 1991; Oota *et al.*, 1995].

Since, one of the few points on which all studies agree is that at least two human groups admixed at some point in the past, a simplified way to explain the data is the use of an admixture approach. However, one of the limitations, of most of admixture models, is that they usually ignore genetic drift since the admixture event. This is why we used an approach [Chikhi *et al.*, 2001] that has already been applied to address the Neolithic transition in Europe [Belle *et al.*, 2006; Chikhi, 2003; Chikhi *et al.*, 2002] and where drift is explicitly accounted for. We expect that the admixture process varied geographically, as the incomers (early farmers) were meeting and admixing with the local populations and their descendants were themselves mixing with other populations. While the admixture process must have been complex, we can predict that a correlation should exist between the admixture level at a particular location, measured by the contribution of one parental population, and the geographic distance from that parental population, as has been shown in Europe [Chikhi *et al.*, 2002]. We also expect that this relationship should not hold, if the same analysis was performed using parental populations that could not have contributed. We note here that in order to carry out the admixture analysis, two modern populations are chosen to approximate the haplotype frequencies of the original parental populations (Jomon and Yayoi). The choice of these parental pop-

ulations is based on archaeological evidence and is described in the Material and Methods section.
Thus, the aim of this work was to determine whether an admixture approach could be fruitful to study the Neolithic transition in Japan. To do this we analysed Y-chromosomal data from the literature, using different 'parental' populations, in order to test different hypotheses. In a first set of analyses, the parental populations were chosen among a set of Asian populations (see below for details). The data were also analysed by using, as a negative test, populations that were unlikely to have contributed to the gene pool of modern Japanese, namely a European (Sardinia) and a geographically closer (Oceania) population, and for which comparable Y-chromosomal data was available. Altogether, we show that admixture models can provide indeed interesting insights in the peopling of Japan. In particular, our results strongly suggest that the Yayoi immigrants spread by a process similar to the demic diffusion, first proposed for Europe by Ammerman and Cavalli-Sforza [1984].

## 2.3 Material and Methods

### 2.3.1 Populations used

The analyses presented in this work were based on published non recombining Y-chromosome (NRY) data of Japanese and other Asian populations. A total of 275 individuals, representing each of the Japanese islands (Figure 2.1), were analysed: Ainu (20), Aomori (26), Shizuoka (61), Tokushima (70), Kyushu (53) and Okinawa (45). All the Japanese data were published by Hammer and colleagues [2006], except the Ainu data that were pooled with data from Tajima et al. [2004]. Mainland Asian data [Hammer *et al.*, 2006] were obtained for populations from Northeast (441), Southeast (683) and Central (419) Asia and also a sample from Korea (43) [Xue *et al.*, 2006]. We also used two additional populations, Sardinia (77) [Semino *et al.*, 2000] and Oceania (209) [Hammer *et al.*, 2006], as parental populations in the admixture model used (see below). Y-chromosome binary haplogroups, were defined by the analysis of the binary polymorphisms described in Hammer et al. [Hammer *et al.*, 2006]. The Y-chromosome lineages from Japan, mainland

Asia, Korea, Oceania and Sardinia followed the haplogroups nomenclature of the Y Chromosome Consortium [2002].

### 2.3.2 The Admixture Model

The admixture method used assumes that an 'admixed' or 'hybrid' population ($H$), of size $N_h$, is the result of the admixture of two independent parental populations, $P_1$ and $P_2$, of size $N_1$ and $N_2$, $T$ generations ago, with respective contributions $p_1$ and $p_2$ ($p_2 = 1 - p_1$). After the admixture event, the three populations are isolated and assumed to evolve independently under pure genetic drift (Fig. 1.6). The advantage of this model, and of the associated inference methods, is that (i) the three populations have different $N_i$ (where $i$ can be *1, 2 or h*) and (ii) drift and admixture are separated. It is important to note that, by explicitly accounting for drift after the admixture event, the method allows for present-day samples from parental populations to have drifted significantly from the original unknown parental populations. Also, the method does not fix the original parental allele frequencies. Instead, they were allowed to vary and this uncertainty is explicitly taken into account. A Bayesian full-likelihood method based on this model was developed by Chikhi and colleagues [2001], implemented in the LEA (Likelihood-based Estimation of Admixture) software [Langella *et al.*, 2001]. LEA implements a Monte Carlo Markov Chain algorithm to jointly infer all the parameters of the admixture model, including the ancestral allelic configurations that are compatible with the present, observed allelic frequencies. For each analysis, LEA was run for 300 000 steps, as it has been shown that it is enough to reach equilibrium for Y-chromosomal data [Chikhi *et al.*, 2001, 2002].

### 2.3.3 Choice of parental populations

For simplicity and consistency, the $P_1$ population was always used to represent the HG or Jomon, whereas the population $P_2$ was used to represent the farmers of the Yayoi period. Hence, the parameter $p_1$ represents the 'Jomon' contribution, at the moment of admixture, whereas $p_2$ would represent the 'Yayoi' contribution.

However, like all admixture methods, it requires that these parental populations be defined. While it is unlikely that today's populations are direct descendants from any of the original groups, we can use current archaeological and anthropological data to identify populations that are likely to be less admixed, and use them as descendants from the original parental populations. It is noteworthy that if there has been a lot of admixture in these parental populations, the general effect should be to blur the original signal, and make it less clear. Therefore, any signal observed today should be an indication that some information is still present in the data. Although the Jomon culture has almost been replaced across Japan, there are some indigenous minority ethnic groups who live in the peripheral areas of Japan, which are considered descendants of this ancient culture [Hanihara, 1991; Horai *et al.*, 1996; Omoto & Saitou, 1997; Tajima *et al.*, 2004]. Those are the Ainu people, in the northern part of the Hokkaido Island, and the Ryukyuans, in the southern Ryukyu Islands. Moreover, the Ainu lived in relative isolation until the end of the 19$^{th}$ century [Hudson, 1994], and show unique physical characteristics such as hairiness, wavy hair and deep-set eyes, which are different from those of most Japanese. On the other hand, the Ryukyuan kingdom had past-relations with mainland Japan since medieval, with possibly frequent gene flow [Haneji *et al.*, 2007; Toma *et al.*, 2007], but it is thought to have nevertheless maintained genetic differentiation from mainland Japan [Yamaguchi-Kabata *et al.*, 2008]. For these reasons the admixture analyses were performed using either the Ainu or the Ryukyuans, the latter represented by the Okinawa sample, as descendants of the $P_1$population, in the different analyses. For the descendants of the Yayoi (considered the $P_2$ population), different parental populations from mainland Asia were also used, namely NEA (North East Asia), SEA (South East Asia), CAS (Central Asia), and Korea.

To determine whether our approach was robust to incorrect specification of the parental populations, we also used as $P_2$ two populations that are unlikely to have contributed to the gene pool of the Japanese: one from Europe (Sardinia) and the other from a closer geographical area, Oceania. We expected that there should be no correlation (or at least much less) between admixture and geographical distances in these cases. Altogether, each of the four Japanese 'admixed' populations

(Aomori, Shizuoka, Tokushima, Kyushu) were analysed using two populations for $P_1$ (Ainu and Okinawa), six populations for $P_2$ (including Sardinia and Oceania), making a total of 12 different sets of admixture analyses. In addition, for each admixture analysis, the parental populations were also considered as 'admixed' populations. For example, we used the Ainu as $P_1$, as $H$, against the six different $P_2$ populations. This kind of analysis allowed us to quantify the uncertainty around the estimation of$p_1$, since the hybrid and one parental (here $P_1$) are exactly identical. Thus, the $p_1$ posteriors should always have a mode equal or very close to one, with a variance related to both the sample size and drift since the admixture event. Of course, when the Ainu and Okinawa are used as 'pseudo-hybrids' the corresponding posteriors were not used in the regression analysis described below.

### 2.3.4 Calculating Drift

The LEA software also allowed us to estimate genetic drift since the admixture event in the three populations, through the parameters $t_i = T/N_i$, where $i$ corresponds to *1* (Jomon parental population), *2* (Yayoi parental population) or $h$ (Japanese hybrid population). Populations that have developed agriculture earlier would have increased in size earlier and would thus exhibit lower amounts of drift since the admixture event. Consequently, if the admixture model is consistent, the *ttextsubscript1* values should in general be higher than the $t_2$ values, whereas $t_h$ values should be more variable across populations.

### 2.3.5 Spatial variation of admixture: regression analysis

To detect, quantify, and assess the significance of any geographical trend in admixture proportions across Japan, we used a linear regression approach similar to that used by Chikhi et al. [2002]. The idea is to determine whether there is a correlation between the 'Yayoi contribution', measured by $p_2$, and the geographic distance from the population used for $P_2$. For each location sampled in Japan, we computed a geographic distance from the sample used as $P_2$ and then estimated a linear regression between this distance and $p_2$. To account for the uncertainty around $p_2$,

we followed the resampling approach used by Chikhi et al. [2002]. For each of the Japanese samples, one $p_2$ value was randomly sampled from the corresponding posterior distribution. This process was repeated 1,000 times to obtain the empirical distribution of regression lines. This was done independently, for each set of admixture analyses performed, using a particular pair of parental populations. A similar approach was used for $t_h$, to determine whether drift in the admixed populations was also correlated with geographic distance. The geographic distance was calculated as a straight line from the central point of the area corresponding to the population used as $P_2$(e.g. close to Seoul for Korea), taking in account an entering in Japan from Korea, through Kyushu. It is worth noting that the spatial points used are necessarily non-independent, because of local gene flow, as was for instance noted by Sokal and colleagues [1989]. As a consequence, allele or haplotype frequencies are spatially autocorrelated, and hence violate the assumption of independence necessary to calculate the significance of a linear regression, using classical approaches. This is why we did not perform such tests, and used the values to represent the relationship between the parameter of interest and geographical distance.

### 2.3.6 $F_{ST}$ analysis

The genetic structure of the populations was also described using $F_{ST}$ values. These values were computed with the equation $F_{\mathrm{ST}} = (\bar{H}_{\mathrm{T}} - \bar{H}_{\mathrm{S}}) \,/\, \bar{H}_{\mathrm{T}}$ [Nei, 1977], using the Okinawans and Ainu against all other Asian populations.

## 2.4 Results

### 2.4.1 Admixture proportions

Figure 2.2 shows, for the different pairs of parentals tested, the mode of the $p_1$ posterior distributions (represented in Figure 2.3a), where $p_1$ represents the HG contribution to the Japanese populations. The modes represent the most probable values, but since the $p_1$ distributions are wide they should be interpreted with cau-

tion. In fact, previous simulation results suggest that any point estimate (median, mean or mode) should be interpreted with care [Chikhi *et al.*, 2001] and we should rather focus on spatial trends, if any, across the populations [Chikhi *et al.*, 2002]. Although it was difficult to infer precise contributions, this figure shows a clear difference in the results, whether Asian or non-Asian populations are used as $P_2$: i) with Sardinia or Oceania, the $p_1$ modes are equal to one, indicating no contribution of these populations to the Japanese populations and ii) with Asian populations, the modal $p_1$ values are widespread and are most of the time higher than 0.2, suggesting a variable but significant contribution of the HG. There is one exception, when we use the CAS population as $P_2$ the $p_1$ modes for the Ainu are very low, which is counterintuitive. A closer look at these posteriors shows that they are very flat and hence that there is little information to infer $p_1$.

**Table 2.1: Spatial variation of admixture and drift**

| $P_2$ | $P_1$ | | | |
|---|---|---|---|---|
| | *Ainu* | | *Okinawa* | |
| | $r\ p_2$ | $r\ t_h$ | $r\ p_2$ | $r\ t_h$ |
| NEA | -0.08730 | 0.03413 | -0.24240 | 0.64510 |
| CAS | -0.04634 | 0.06180 | -0.18400 | 0.48580 |
| SEA | -0.17550 | 0.06690 | -0.11460 | 0.65360 |
| Korea | -0.13770 | 0.06445 | -0.19810 | 0.66640 |
| Oceania | 0.16190 | 0.03664 | 0.24639 | 0.62980 |
| Sardinia | 0.15670 | -0.02782 | -0.00095 | 0.56270 |

**Abbreviations:** NEA, Northeast Asia; CAS, Central Asia; SEA, Southeast Asia.
Correlation values ($r$), for the $p_2$ and th regression analyses described in section 2.3.5, for the parental population pairs used (represented by $P_1$ and $P_2$).

Even though there is an uncertainty on $p_1$ values for specific populations (see Figure 2.3a), a geographical trend in the 'Yayoi' contribution ($p_2$) is found (Figure 2.3b). The randomization approach applied to test this trend is summarized in Table 2.1 (and Figure 2.4a), where the correlation coefficients obtained, from the linear regressions, are represented. Although the values are small, this figure shows again a clear signal. First, when the Sardinian or Oceanian samples are used as $P_2$,

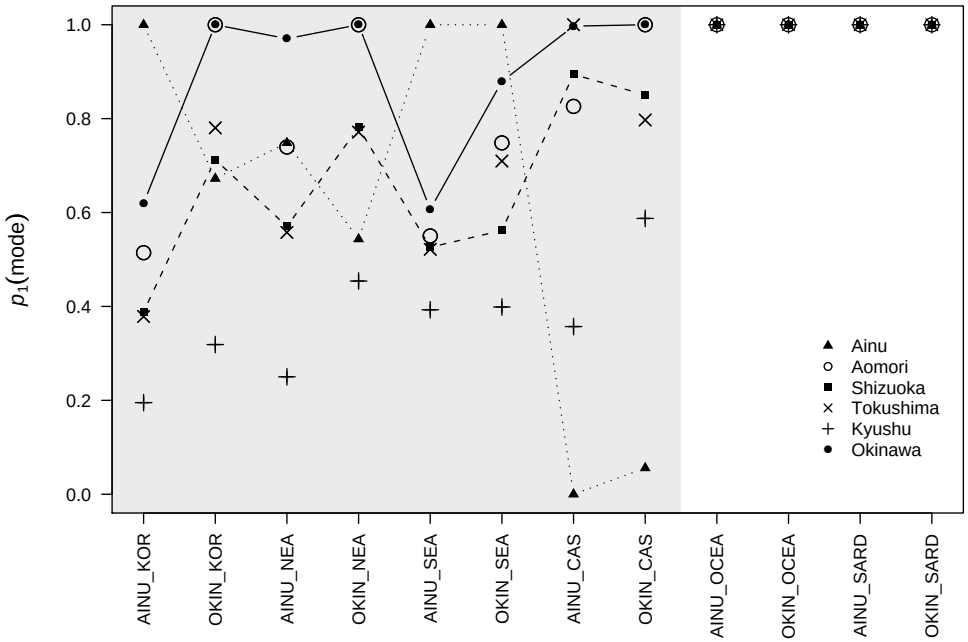



**Figure 2.2: Jomon contribution, across Japan** - Mode of the $p_1$ posterior distributions, for all the Japanese populations analysed, with $p_1$ representing the hunter-gatherers Jomon contribution to modern Japanese. In the x axis are represented the parental populations used ($P_1$ followed by $P_2$). The letter codes are as follows: AINU – Ainu, OKIN – Okinawa, KOR – Korea, NEA – Northeast Asia, SEA – Southeast Asia, CAS – Central Asia, OCEA – Oceania and SARD – Sardinia.

which are unlikely to have contributed to the Japanese gene pool, there is a positive or no correlation, suggesting that the contribution of these two populations increases (or stays constant) with geographical distance from their current location. Second, in all the other analyses (namely when the $P_2$ populations are Korea, NEA, SEA and CAS), the correlation coefficients values are negative, that is, $p_2$ logically

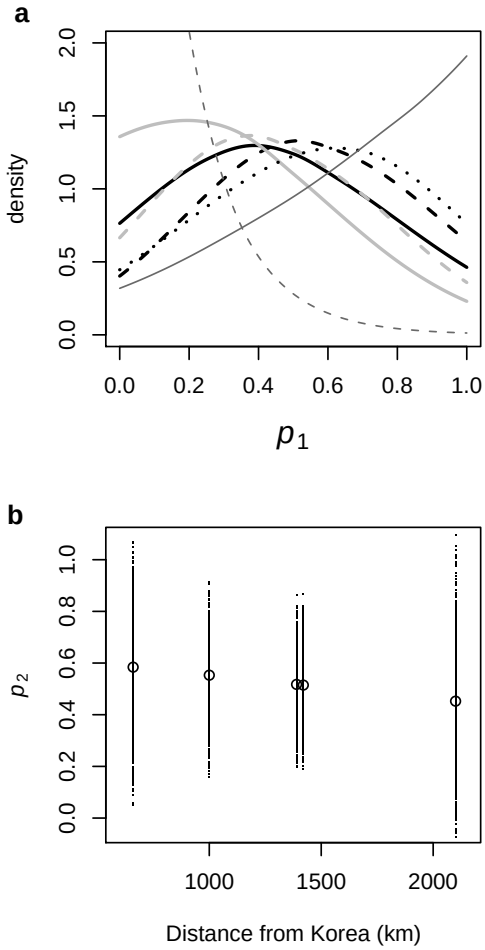



**Figure 2.3: Jomon and Yayoi contributions, across Japan** - **(a)** Posterior distributions of $p_1$ for all Japanese populations (Kyushu – grey, Tokushima – dashed grey, Shizuoka – black, Aomori – dashed black and Okinawa – dotted black) and parental populations (thinner lines: Ainu – dark grey and Korea – dashed dark grey) used . Each curve corresponds to the analysis of a specific hybrid population. **(b)** Linear regression of $p_2$ against geographical distance from $P_2$ (Korea). The circles represent the mean value for each population. These analyses were done using the Ainu and Korean populations as $P_1$ and $P_2$, respectively.

decreases with geographic distance from the population used as $P_2$. Also, with the exception of SEA as $P_2$, when comparing the analyses of Ainu *vs.* Okinawa as $P_1$, the most negative correlation values seem to be associated with the Ainu. This trend is, however, only close to significance ($p = 0.058$).

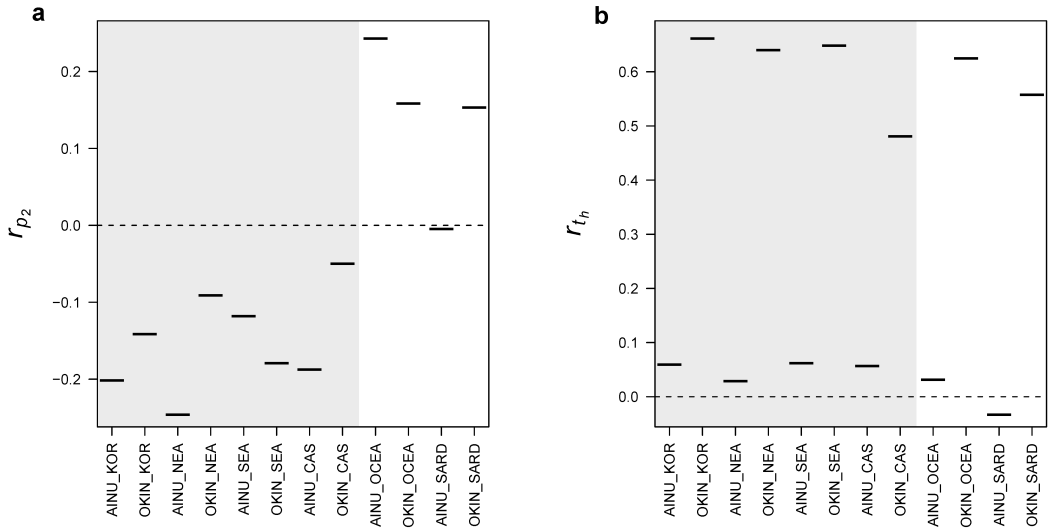



**Figure 2.4: Spatial variation of admixture and drift** - Correlation values ($r$), for the **(a)** $p_2$ and **(b)** $t_h$ regression analyses described in the Material and Methods. In the x axis are represented the parental populations used ($P_1$ followed by $P_2$). The letter codes are as follows: AINU – Ainu, OKIN – Okinawa, KOR – Korea, NEA – Northeast Asia, SEA – Southeast Asia, CAS – Central Asia, OCEA – Oceania and SARD – Sardinia.

### 2.4.2 Drift

In Figures 2.5a and 2.5b, the amount of drift between the present-day samples of the populations used as $P_1$ or$P_2$ ,and the ancestral populations, is represented through $t_1$ and $t_2$,respectively. Comparing the $t_1$ and $t_2$ distributions clearly indicates that the two estimates are extremely different. In fact, in all the analyses performed, the $t_1$ modal values, of all the Japanese populations studied, were always greater

than $t_2$ and, at the same time, were higher if we used the Ainu sample as $P_1$, instead of Okinawa (data not shown). Moreover, the $t_1$ posteriors were wider and had more variable modal values than $t_2$, especially when using the Ainu as $P_1$, but nevertheless were similar to each other.

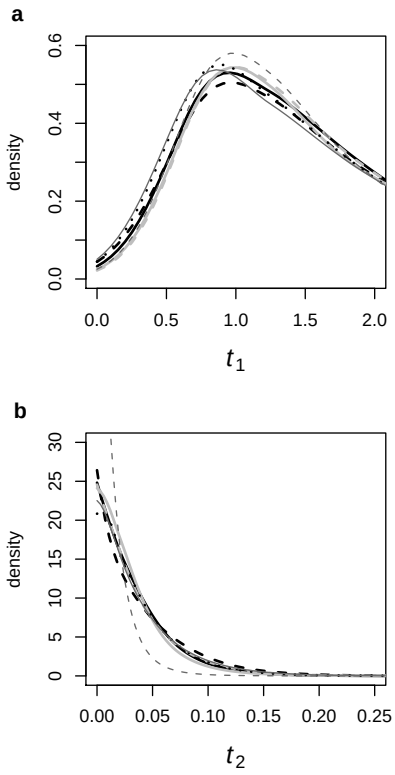



**Figure 2.5: Distributions of the $t_i$'s for all Japanese populations** - **(a)** Posterior distributions of $t_1$. The different curves represent the amount of genetic drift, since the admixture event, between the present sample of Ainu and the ancestral populations of HG (Jomon) that interbred with the incoming farmers (Yayoi). **(b)**Posterior distributions of $t_2$. As in a, but for the drift between the Korean and Yayoi populations instead. The colour codes are as in Figure 2.3

When we analysed the $t_h$ estimates, they appeared to be highly variable (data not

shown) and to display a geographical trend. As for $p_2$, we applied a regression of these estimates against the geographical distance, from the population used as $P_2$, to all the possible combinations of parentals. The correlation coefficients obtained from these linear regressions, are represented in Table 2.1 (and Figure 2.4b). All the correlation coefficients are positive (except with Ainu *vs.* Sardinia), showing that the $t_h$ values increase as geographical distance increases, but at the same time are much smaller when we use the Ainu as $P_1$, on the order of 0.034 to 0.065 *vs.* the 0.486 to 0.666 for the Okinawans.

### 2.4.3 $F_{ST}$

Table 2.2 (see also Figure 2.6) shows higher $F_{ST}$ values in the pairwise comparisons involving the Ainu, compared to those involving the Okinawans. It also shows that for both sets of pairwise comparisons, the $F_{ST}$ values increase when the geographic distance increases in a southwestern direction from the northern tip of Honshu (Aomori) towards Kyushu. This is particularly interesting since the Ainu and Okinawan are located on opposite sides of the Japanese archipelago and hence of this axis. We also note that this trend of $F_{ST}$ values shows a clear and sudden increase when the samples are taken from the Asian continent, starting with Korea. This is particularly clear despite the fact that, when we consider only the Japanese populations, the $F_{ST}$ values involving Okinawans and Ainu are on different scales, the first set of values being all below 0.1 and the others all above 0.1. In fact, the $F_{ST}$ between the Ainu and Okinawans (0.096) is the smallest $F_{ST}$ value among the pairs involving the Ainu (which vary from 0.096 to 0.219 in Kyushu), but it is the largest among the pairs involving the Okinawans (which vary from 0.018 in Aomori to 0.096 against the Ainu). Another consequence of this cline is that the Okinawan population seems genetically close to the northernmost populations of Honshu, but strangely not to the Ainu, the only sampled population north of Honshu. Indeed, the $F_{ST}$ with the latter is higher than the $F_{ST}$ values between Okinawans and all other Japanese populations. Thus, the Okinawans appear to be the Japanese that are genetically closest to the Ainu from the Ainu viewpoint, whereas it is exactly the

opposite from the Okinawans viewpoint. It is as if the Okinawans were 'virtually' located in northern Honshu, and the Ainu were genetically close to them but had been submitted to significant drift.

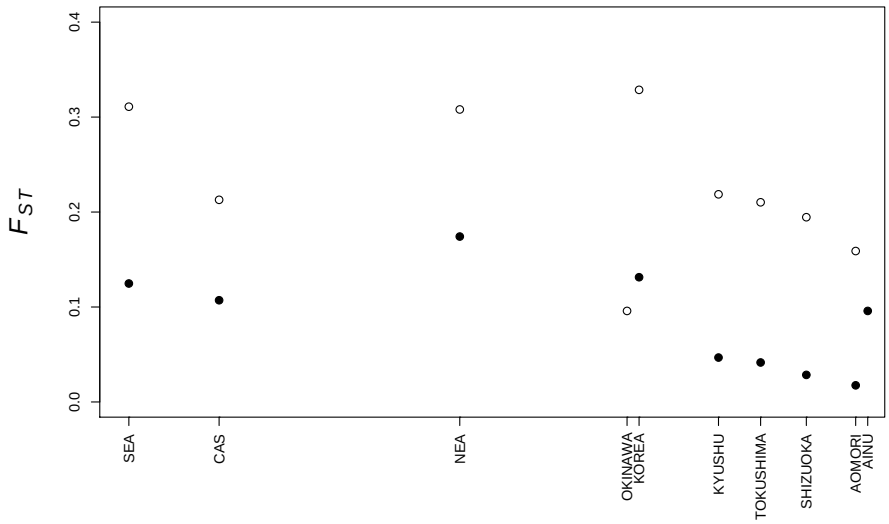



**Figure 2.6: Population differentiation with Ainu and Okinawa populations** - $F_{ST}$ values, for Ainu (open circles) or Okinawans (filled circles), against the other Asian populations. As a simplified geographical representation, we plotted these $F_{ST}$ values, by taking distances from a ' central point' which we took to be Kyushu, the island through which the Yayoi farmers are thought to have entered Japan. Thus, positive distances correspond to distances between Kyushu and populations located northeast (the Japanese samples) of Kyushu and negative distance values correspond to those located west (Continental samples) or southwest (Okinawa) of Kyushu.

**Table 2.2: Population differentiation with Ainu and Okinawans populations.**

| | $F_{ST}$ | |
|---|---|---|
| | Ainu | Okinawa |
| Ainu | 0.000 | 0.096 |
| Aomori | 0.159 | 0.018 |
| Shizuoka | 0.195 | 0.029 |
| Tokushima | 0.210 | 0.042 |
| Kyushu | 0.219 | 0.047 |
| Okinawa | 0.096 | 0.000 |
| Korea | 0.329 | 0.131 |
| NEA | 0.213 | 0.107 |
| CAS | 0.308 | 0.174 |
| SEA | 0.311 | 0.125 |

**Abbreviations:** NEA, Northeast Asia; CAS, Central Asia; SEA,Southeast Asia.

## 2.5 Discussion

### 2.5.1 Dual origins of Japanese

While our results may only reflect the paternal history of the Japanese, they confirm the idea that a significant admixture took place and thus do not support either the replacement or the transformation models between the incoming Yayoi and the local Jomon. Indeed, in the replacement model the estimate of $p_1$ should be equal to zero (or at least very close, due to statistical uncertainty), whereas in the transformation model $p_1$ would be close or equal to 1. This is clearly not what we observe. Moreover, our results show a decreasing geographical trend in the Yayoi contribution across Japan, when populations are sampled in a southwest-northeast direction (Figures 2.3a and 2.3b). These results agree with a model in which the first farmers entered in Japan from Korea, through the closest island (Kyushu), and then spread across most of Japan moving to the northeast (until the geographical limits of the Honshu Island). During the expansion of farmers, it is expected that the rise in population density, due to food production, should lead to a more limited drift (since populations were larger). This can be seen in the gradient observed with the $t_h$ estimates, which suggests that drift is higher in the northernmost populations

(with a maximum observed in the Ainu), where the archaeological record suggests a later arrival of agriculture [Highman, 2005]. Also, the differences encountered between $t_1$ and $t_2$ estimates ($t_1 >> t_2$) are consistent with a model of an expanding population who dispersed to a less populated area, i.e., the $P_2$ populations (Yayoi) increased in size earlier in time, having suffered a lower amount of drift. Interestingly, the $t_1$ values are higher than the ones found in Europe [Chikhi *et al.*, 2002], which could be due to an earlier introduction of agriculture in Europe.

During the admixture process, it is important to note that the indigenous populations, the Jomon, who admixed with the Yayoi, were probably genetically differentiated from each other across the Japanese islands. How differentiated they were, some 2,000 years ago, is difficult to say, but this pre-admixture differentiation should have some implication in the analyses, and their interpretation. The fact that all $t_1$ posteriors were very similar to each other suggests that even if there was differentiation between HG populations, prior to the arrival of the Yayoi, they were not dramatically different, compared with the amount of drift that occurred since the admixture event. This indirectly shows that our model, despite its simplicity, captures important aspects of the 'Neolithic transition' in the Japanese archipelago. We note however that, depending on whether we use the Ainu or Okinawans as $P_1$, the $t_1$ estimates are rather different, suggesting much higher drift when the Ainu are used. This, together with the fact that the Ainu have much higher $F_{ST}$ than the Okinawans against all other populations, suggests that the Ainu have probably had a much lower effective size than the Okinawans. It could be due to a greater isolation, a later and more limited influence of agriculture or a combination of both.

### 2.5.2 The continental origin of the Yayoi farmers

Several hypotheses have been suggested regarding the geographic origin of the Asian populations, which gave rise to the Yayoi, even though it is usually accepted that they probably entered Japan through South Korea. Nevertheless, skull and teeth morphology inference [Hanihara, 1991] and classical markers [Omoto & Saitou, 1997; Sokal & Thomson, 1998] support a NEA origin.

More recently, Hammer and colleagues [2006] placed the Yayoi farmers as having originated in SEA. However, with the same data as Hammer at al. [2006] (with the exception of the Korean data), the admixture model we used could not establish with so much accuracy the continental origins of these populations, but our results suggest that they entered in Japan through the Korean Peninsula. It may be important to note that our approach is model-based and has been tested on simulated data [Chikhi *et al.*, 2001], whereas the conclusion reached by Hammer et al. [2006] were based on visual patterns of allele or haplogroup frequencies and were neither justified by any statistical test, nor by analyses of simulated data. Thus, our approach is not 'just' confirming established results, but rather adding more solid results to conclusions whose statistical validity was not determined. Also, when we found that the exact location of the Yayoi cannot be ascertained with certainty whereas Hammer et al. [2006] assert that they arrived from SEA, one should question the strength of the latter statement. This should not be taken as a criticism of Hammer et al. [2006] study, which provided both new results and hypotheses to test. Rather, what our results show is that it might be possible, using an admixture approach, to test different hypotheses, something that has not been done so far. Indeed, our method was able to identify populations that clearly could not have contributed to the modern Japanese gene pool at that time (namely Sayearparrdinia and Oceania). If some of the Asian parental populations analysed had generated results similar to those of Sardinia and Oceania, they could have been identified as unlikely parentals. This type of results was not observed, which suggests that these data do not contain enough information to clearly identify the most likely descendent of one of the parental populations of modern Japanese, namely the Yayoi. This is not necessarily surprising since the Y-chromosome represents only one set of linked markers. We believe thus that until more *loci* are obtained this question may not be easily answered, and should remain open. If we have contributed to make this statement, we feel that a significant step will have been done.

In summary, our results support at least one admixture event in the peopling of Japan, namely the spread of Yayoi farmers by a process of demic diffusion, similar to the one in Europe during the Neolithic [Ammerman & Cavalli-Sforza, 1984;

Barbujani *et al.*, 1995; Chikhi *et al.*, 2002]. We suggest that when the Yayoi males entered Japan, and brought with them agriculture and new technologies, they also raised the carrying capacity of the area first colonized, leading to an increase in size of the newly admixed populations. When this area could no longer support the increased population, their descendants expanded into new territories, repeating the admixture process. By the time the geographic limits of Japan were reached (northeastward until the Hokkaido Island and southwestward in the Ryukyu Islands), there was a gradual dilution of the Yayoi's gene pool. However, in spite of having detected the presence of Jomon and Yayoi contributions in Japanese populations, the method we used was not capable of locate precisely the area of origin of the ancestral populations, and different populations seemed to produce similarly consistent results. Nevertheless, the general approach appears to provide interesting and promising results, which should open new avenues for research.

**Acknowledgements**

R.R. was supported by a Fundação para a Ciência e Tecnologia (FCT) grant (ref. SFRH/BD/30821/2006). L.C. was partly funded by the CNRS, the FCT grant PTDC/BIA-BDE/71299/2006, and the Institut Français de la Biodiversité, Programme Biodiversité de l'Océan Indien (CD-AOOI-07-003). L.C. travels, between Toulouse and Lisbon, were partly funded by the Programme d'Actions Universitaires Intégrées Luso-françaises 2007/2008, and were made possible thanks to Prof. A. Coutinho and B. Crouau-Roy. LEA calculations were performed, with the help of P. Fernandes, using the High Performance Computing Centre (HERMES, FCT grant H200741/re-equip/2005). We are grateful to V. Sousa and to anonymous reviewers for helpful comments.

## 2.6 References

AMMERMAN, A.J. & CAVALLI-SFORZA, L.L. (1984). *The Neolithic transition and the genetics of populations in Europe*. Princeton University Press, Princeton.

BARBUJANI, G., SOKAL, R.R. & ODEN, N.L. (1995). Indo-European origins: a computer-simulation test of five hypotheses. *Am J Phys Anthropol*, **96**, 109–32.

BELLE, E.M.S., LANDRY, P.A. & BARBUJANI, G. (2006). Origins and evolution of the Europeans' genome: evidence from multiple microsatellite loci. *Proc R Soc B*, **273**, 1595–

1602.

BELLWOOD, P. (2004). *First Farmers: the origins of agricultural societies*. Blackwell Publishing, Oxford.

CAVALLI-SFORZA, L.L., MENOZZI, P. & PIAZZA, A. (1994). *The History and Geography of Human Genes*. Princeton University Press, Princeton.

CHIKHI, L. (2003). *Admixture in Europe: Y chromosome datat support the demic diffusion model*, 435–447. McDonald Institute fo Archaeological Science, Cambridge.

CHIKHI, L., BRUFORD, M.W. & BEAUMONT, M.A. (2001). Estimation of admixture proportions: a likelihood-based approach using Markov chain Monte Carlo. *Genetics*, **158**, 1347–1362.

CHIKHI, L., NICHOLS, R.A., BARBUJANI, G. & BEAUMONT, M.A. (2002). Y genetic data support the Neolithic demic diffusion model. *Proc Natl Acad Sci U S A*, **99**, 11008–11013.

CURRAT, M. & EXCOFFIER, L. (2005). The effect of the Neolithic expansion on European molecular diversity. *Proc R Soc B*, **272**, 679–688.

DUPANLOUP, I., BERTORELLE, G., CHIKHI, L. & BARBUJANI, G. (2004). Estimating the impact of prehistoric admixture on the genome of Europeans. *Mol Biol Evol*, **21**, 1361–1372.

HAMMER, M.F. & HORAI, S. (1995). Y chromosomal DNA variation and the peopling of Japan. *Am J Hum Genet*, **56**, 951–962.

HAMMER, M.F., KARAFET, T.M., PARK, H., OMOTO, K., HARIHARA, S., STONEKING, M. & HORAI, S. (2006). Dual origins of the Japanese: common ground for hunter-gatherer and farmer Y chromosomes. *J Hum Genet*, **51**, 47–58.

HANEJI, K., HANIHARA, T., SUNAKAWA, H., TOMA, T. & ISHIDA, H. (2007). Non-metric dental variation of Sakishima Islanders, Okinawa, Japan: a comparative study among Sakishima and neighboring populations. *Anthropol Sci*, **115**, 35–45.

HANIHARA, K. (1991). Dual structure model for the population history of the Japanese. *Jpn Rev*, **2**, 1–33.

HIGHMAN, C. (2005). *East Asian agriculture and its impact*, 234–263. Thames and Hudson, London.

HORAI, S., KONDO, R., MURAYAMA, K., HAYASHI, S., KOIKE, H. & NAKAI, N. (1991). Phylogenetic affiliation of ancient and contemporary humans inferred from mitochondrial DNA. *Philos Trans R Soc Lond B Biol Sci*, **333**, 409–16; discussion 416–7.

HORAI, S., MURAYAMA, K., HAYASAKA, K., MATSUBAYASHI, S., HATTORI, Y., FUCHAROEN, G., HARIHARA, S., PARK, K.S., OMOTO, K. & PAN, I.H. (1996). mtDNA polymorphism in East Asian populations, with special reference to the peopling of Japan. *Am J Hum*

*Genet*, **59**, 579–590.

HUDSON, M. (1994). The Linguistic Prehistory of Japan: some archaeological speculations. *Anthropol Sci*, **102**, 231–255.

JIN, H.J., KWAK, K.D., HAMMER, M.F., NAKAHORI, Y., SHINKA, T., LEE, J.W., JIN, F., JIA, X., TYLER-SMITH, C. & KIM, W. (2003). Y-chromosomal DNA haplogroups and their implications for the dual origins of the Koreans. *Hum Genet*, **114**, 27–35.

LANGELLA, O., CHIKHI, L. & BEAUMONT, M. (2001). LEA (likelihood-based estimation of admixture) : a program to simultaneously estimate admixture and the time since admixture. *Mol Ecol Notes*, **1**, 357–358.

MATSUMURA, H. (2001). Differentials of Yayoi immigration to Japan as derived from dental metrics. *Homo*, **52**, 135–156.

MIZOGUCHI, Y. (1986). *Contributions of prehistoric far east populations to the population of modern Japan: a Q-mode path analysis based on cranial measurements*, 107–136. University of Tokyo Press, Tokyo.

NEI, M. (1977). F-statistics and analysis of gene diversity in subdivided populations. *Ann Hum Genet*, **41**, 225–233.

OMOTO, K. & SAITOU, N. (1997). Genetic origins of the Japanese: a partial support for the dual structure hypothesis. *Am J Phys Anthropol*, **102**, 437–446.

ONO, A., SATO, H., TSUTSUMI, T. & KUDO, Y. (2002). Radiocarbon dates and archaeology of the late Pleistocene in the Japanese islands. *Radiocarbon*, **44**, 447–494.

OOTA, H., SAITOU, N., MATSUSHITA, T. & UEDA, S. (1995). A genetic study of 2,000-year-old human remains from Japan using mitochondrial DNA sequences. *Am J Phys Anthropol*, **98**, 133–145.

POPE, K.O. & TERRELL, J.E. (2008). Environmental setting of human migrations in the circum-Pacific region. *J Biogeogr*, **35**, 1–21.

SEMINO, O., PASSARINO, G., OEFNER, P.J., LIN, A.A., ARBUZOVA, S., BECKMAN, L.E., BENEDICTIS, G.D., FRANCALACCI, P., KOUVATSI, A., LIMBORSKA, S., MARCIKIAE, M., MIKA, A., MIKA, B., PRIMORAC, D., SANTACHIARA-BENERECETTI, A.S., CAVALLI-SFORZA, L.L. & UNDERHILL, P.A. (2000). The genetic legacy of Paleolithic Homo sapiens sapiens in extant Europeans: a Y chromosome perspective. *Science*, **290**, 1155–1159.

SOKAL, R.R. & THOMSON, B.A. (1998). Spatial genetic structure of human populations in Japan. *Hum Biol*, **70**, 1–22.

SOKAL, R.R., HARDING, R.M. & ODEN, N.L. (1989). Spatial patterns of human gene frequencies in Europe. *Am J Phys Anthropol*, **80**, 267–294.

TAJIMA, A., HAYAMI, M., TOKUNAGA, K., JUJI, T., MATSUO, M., MARZUKI, S., OMOTO, K. & HORAI, S. (2004). Genetic origins of the Ainu inferred from combined DNA analyses of maternal and paternal lineages. *J Hum Genet*, **49**, 187–193.

TOMA, T., TSUNEHIKO, H., HAJIME, S., KUNIAKI, H. & HAJIME, I. (2007). Metric dental diversity of Ryukyu Islanders: a comparative study among Ryukyu and other Asian populations. *Anthropol Sci*, **115**, 119–131.

XUE, Y., ZERJAL, T., BAO, W., ZHU, S., SHU, Q., XU, J., DU, R., FU, S., LI, P., HURLES, M.E., YANG, H. & TYLER-SMITH, C. (2006). Male demography in East Asia: a north-south contrast in human population expansion times. *Genetics*, **172**, 2431–2439.

YAMAGUCHI-KABATA, Y., NAKAZONO, K., TAKAHASHI, A., SAITO, S., HOSONO, N., KUBO, M., NAKAMURA, Y. & KAMATANI, N. (2008). Japanese population structure, based on SNP genotypes from 7003 individuals compared to other ethnic groups: effects on population-based association studies. *Am J Hum Genet*, **83**, 445–456.

YCC (2002). A nomenclature system for the tree of human Y-chromosomal binary haplogroups. *Genome Res*, **12**, 339–348.

# 3. Female and Male Shared Views on the Neolithic Transition in Europe: clues from ancient and modern genetic data

Rita Rasteiro[1] and Lounès Chikhi[1,2,3]

[1]Instituto Gulbenkian de Ciência, Rua da Quinta Grande, 6, 2780-156 Oeiras,Portugal; [2]CNRS, Laboratoire Évolution et Diversité Biologique (EDB), Bât. 4R3 b2, 118 Route de Narbonne, 31062 Toulouse cédex 9, France; [3]Université de Toulouse, UPS, EDB, Bât. 4R3 b2, 118 Route de Narbonne, 31062 Toulouse cédex 9, France

**Data collection:** R Rasteiro
**Admixture Analysis:** R Rasteiro and L Chichi
**aDNA Analysis:** R Rasteiro
**Manuscript:** R Rasteiro and L Chikhi

---

## 3.1 Abstract

The arrival of agriculture into Europe during the Neolithic transition brought a significant shift in human lifestyle and subsistence. However, the conditions under which the spread of the new culture and technologies occurred are still debated. Similarly, the roles played by women and men during the Neolithic transition are not well understood, probably due to the fact that mitochondrial DNA (mtDNA) and

Y-chromosome (NRY) data are usually studied independently rather than within the same statistical framework. Here, we applied an integrative approach, using different model-based inferential techniques, to analyse published datasets from contemporary and ancient European populations. By integrating mtDNA and NRY data into the same admixture approach we show that males and females underwent the same admixture history, hence supporting the Demic Diffusion model of Ammerman and Cavalli-Sforza [1984]. Similarly, the patterns of genetic diversity found in extant and ancient populations demonstrate that both modern and ancient mtDNA support the Demic Diffusion model. They also show that population structure and differential growth between farmers and hunter-gatherers are necessary. However, we also found some differences between male and female markers, suggesting that the female effective population size was larger than that of the males, probably due to different demographic histories. We argue that these differences are probably related to the various shifts in cultural practices and lifestyles that followed the Neolithic Transition, such as sedentism, the shift from polygyny to monogamy and the increase of patrilocal residence systems.

## 3.2 Introduction

Major progress has been made in the use of genetic data to reconstruct the demographic history of human populations and compare alternative models of human origins [Currat & Excoffier, 2005; Fagundes *et al.*, 2007; Goldstein & Chikhi, 2002]. Despite these advances, one of the most important cultural, economic and demographic revolutions in human prehistory, the Neolithic transition [Mithen, 2007], remains the subject of continuing and hotly debated controversies [Barbujani & Chikhi, 2006; Bellwood, 2004; Chikhi, 2009; Chikhi *et al.*, 2002; Goldstein & Chikhi, 2002; Richards, 2003; Richards *et al.*, 2000, 2002]. Even for Europe, where most genetic studies have been carried out, there is a major disagreement among archaeologists and anthropologists [Bellwood, 2004; Bocquet-Appel *et al.*, 2009; Gkiasta *et al.*, 2003; Pinhasi & von Cramon-Taubadel, 2009; Pinhasi *et al.*, 2005] and among geneticists [Chikhi *et al.*, 2002; Dupanloup *et al.*, 2004; Richards *et al.*,

2000; Semino *et al.*, 2000]. Some favour the hypothesis that this process resulted from an active migratory process starting in the Near-East, where the odomestication of Old World animals and plants began [Bellwood, 2004], whereas others believe that it was merely due to cultural contact between hunter-gathering and farming societies. These two extreme alternatives are usually encapsulated in two widely used models assuming either Demic Diffusion (DDM) [Ammerman & Cavalli-Sforza, 1984] or Cultural Diffusion (CDM) [Zvelebil & Zvelebil, 1998]. The CDM predicts that there should be no or very little contribution in Europe from the Near-Eastern populations. The genetic consequences of the DDM are much less straightforward and depend on the details of the spatial processes that took place during the expansion, including the importance of intermarriage (admixture) events between farmers and hunter-gatherers (HG) [Chikhi *et al.*, 1998, 2002; Currat & Excoffier, 2005]. For instance, Chikhi *et al.* [2002] showed that even assuming that farmers represented 90% of all the newly formed farming societies (and with only 10% of HG) as they expanded into Europe, the average contribution of Near-Eastern genes in Europe could be as low as a few per cent, due to a dilution effect along the expansion axis, and close to zero on the western borders of Europe. They stressed a fundamental asymmetry between the two models in terms of genetic patterns and the need to use model-based approaches explicitly accounting for drift and admixture. These points were also stressed by Currat and Excoffier [2005], who used more complex and sophisticated models.

Until now, one of the major limitations in the studies published is the fact that they either use mtDNA or NRY (non-recombinant region of the Y-chromosome) data, which are sometimes claimed to favour opposite models [Balter, 2009], even though they have never been used jointly. For instance, mtDNA data are often claimed to support CDM [Richards, 2003; Richards *et al.*, 2000, 2002] whereas NRY data would support the DDM [Balaresque *et al.*, 2010; Chikhi *et al.*, 2002; Rosser *et al.*, 2000]. It is indeed very tempting to imagine that, during the Neolithic expansion in Europe, male farmers eliminated HG males whereas they integrated HG females in the newly founded farming societies, hence generating an asymmetry between male and female lineages similar to that described between Bantu speakers and

African HG societies [Quintana-Murci *et al.*, 2008] or during the colonization of the Americas by Europeans [Salzano, 2004].
In addition, recent technological advances have allowed the use of ancient DNA (aDNA) from early HG and farmer societies, hence raising new hopes that the long-lasting controversy between the CDM and DDM can be resolved. However, the recent attempts to model the colonization of Europe using ancient and modern DNA jointly [Bramanti *et al.*, 2009; Haak *et al.*, 2005, 2010; Malmström *et al.*, 2009], have assumed very simple models that fail to incorporate crucial aspects of the demographic history of early Europeans including Neolithic farmers. They have also, in most cases, failed to use some recent advances in population genetics modelling and statistical inference. This has led to contradictory and inconsistent conclusions as we shall discuss here.
In a recent work (see chapter 4), we have carried out one of the first studies where mtDNA and NRY data were analysed jointly to model ancient demographic events. Here, we continue along that road and use a simple admixture model (Fig. 1.6) to study the spread of agriculture in Europe, by expanding the modern NRY dataset [Rosser *et al.*, 2000] and by adding modern mtDNA data [Richards *et al.*, 2000]. We also take an Approximate Bayesian Computation (ABC) approach [Beaumont *et al.*, 2002; Blum & François, 2009] using one of the largest aDNA dataset available [Bramanti *et al.*, 2009], to identify the demographic scenarios that could explain both modern and ancient DNA data.
We show for the first time that (i) there are no major contradictions between NRY and mtDNA data, (ii) both exhibit a clear decrease of the Neolithic contribution with geographic distance from the Near-East, (iii) both favour a DDM. But there are also differences between the two markers. We show that (iv) the female effective population size was larger than that of the males, suggesting that the demographic history of males and females was significantly different before and during the Neolithic transition, probably due to differences in the migration patterns and mating systems prior to and after the arrival of agriculture. By combining evidence from both modern and ancient mtDNA we also demonstrate that (v) genetic drift and population structure were extremely important in both HG and farming societies,

explaining why aDNA data can produce many alleles with frequencies that are significantly different from present-day frequencies and (vi) that aDNA also support the DDM. Altogether, we propose a synthetic model of colonization that accounts for both modern and ancient mtDNA and NRY data.

## 3.3 Material and Methods

### 3.3.1 Estimating admixture between Palaeolithic HG and Neolithic farmers using extant genetic data

#### *3.3.1.1 The admixture model*

We applied a Bayesian full-likelihood method, described in Chikhi *et al.* [2001], to make statistical inference on the Neolithic Transition. The original method is implemented in the LEA software [Langella *et al.*, 2001], including a recent parallelized version of it [Giovannini *et al.*, 2009] and has been applied to the Neolithic transition in several regions of the world [Belle *et al.*, 2006; Chikhi *et al.*, 2002; Rasteiro & Chikhi, 2009]. However, it may be worth emphasizing that the idea of using admixture models to study the Neolithic transition is implicit in several previous population genetic studies (e.g. [Barbujani *et al.*, 1995b]). The method used here makes this model very explicit (Fig. 1.6) and assumes that $T$ generations in the past, an 'admixed' population $H$ (representing the European populations), is formed by members of two independent parental populations, $P_1$ (representing the local hunter-gatherers, per instance) and $P_2$ (representing the incoming farmers), whose contributions to $H$ are $p_1$ and $p_2$ ($p_2 = 1 - p_1$), respectively. After the admixture event, the three populations are assumed to evolve independently under pure genetic drift (i.e. mutations after admixture are assumed to be negligible). Therefore, all populations are allowed to have changed in allele frequency since the time of admixture by genetic drift. Changes in allele frequency will depend on both $T$ and on the effective population sizes ($N_1$, $N_2$ and $N_h$). Genetic drift is thus modelled by the three parameters, namely $t_1 = T/N_1$, (drift in the hunter-gatherers (HG) since admixture) $t_2 = T/N_2$ (drift in the Near-Eastern population) and $t_h =$

$T/N_h$ (drift in the admixed population, namely the different European populations analysed). Although very simple, by separating the effects of admixture from drift, the model should be able to capture the essential features of European prehistory as has been shown by simulation [Chikhi *et al.*, 2002; Sousa *et al.*, 2009]. Note also that each analysis of an European population is performed independently with the same parental populations. This means that the method can in principle explain the genetic data in different European populations by varying any of the model parameters. We expect that if the model captures important aspects of the Neolithic transition, the parameters that will vary most are $p_1$ (the admixture parameter) as a function of geographic distance from the Near East and $t_h$, (drift in the admixed population) as a function of both geographical distance and local effective sizes. On the contrary, for data for which an admixture model is unlikely to be meaningful, the same data set could in principle be explained by increasing or decreasing drift in any of the parental populations ($t_1$, $t_2$). This is not what we observe (see below for the validation and in the main text for the use of negative controls).
As noted above, the admixture method is implemented in the LEA software [Langella *et al.*, 2001], which uses a MCMC algorithm to sample the posterior distributions of the model parameters ($p_1$, $t_1$, $t_2$ and $t_h$), using the full information from haplotypes frequencies observed today. For each analysis, LEA was run for 300,000 steps, as it has been shown that it is enough to reach equilibrium for single-locus data [Chikhi *et al.*, 2001, 2002; Giovannini *et al.*, 2009; Sousa *et al.*, 2009].

#### *3.3.1.2 Populations used*

In order to compare the demographic history of both female and male lineages, we selected a large number of modern European and circum-European populations, for which haplogroup frequencies were published for both paternally- and maternally- inherited markers. The Rosser *et al.* [2000] dataset comprises 3616 NRY, for a total of 47 populations. The Richards *et al.* [2000] dataset consists of 4095 individuals typed for their mitochondrial DNA (mtDNA). These data were also compared to the previously analysed NRY data of Semino *et al.* [2000] to determine whether similar trends were observed across the two NRY data. Semino *et*

*al.* [2000] typed more genetic markers (and identified more haplotypes) but for a smaller sample size (n $=$ 1007).

#### *3.3.1.3 Choice of Parental Populations*

Archaeological, linguistic and genetic studies suggest that the Neolithic transition started in the Near East and expanded in several directions, including a Northwest movement towards Europe. To represent the descendants of the Near Eastern Neolithic farmers, most genetic studies (e.g., [Barbujani *et al.*, 1995b; Chikhi *et al.*, 2002; Goldstein & Chikhi, 2002]) have used samples from Turkey, Iraq, Iran, Lebanon, or Syria (i.e. the regions where farming most probably originated). We therefore used the Turkish sample for the Rosser *et al.* [2000] dataset, whereas for the Richards *et al.* [2000] dataset we pooled the Iraq, Syria, Palestine, Druze, Turkey and Kurds samples. To represent the descendants of the Palaeolithic hunter-gathering populations we used the Basque population. We note that under the CDM, all European populations are supposed to be mostly derived from local Palaeolithic ancestors, and could thus be used to that aim. However, based on linguistic and genetic evidence the Basques appear to represent one of the European populations less influenced by the Neolithic transition [Brion *et al.*, 2003; Cavalli-Sforza, 1998; Menozzi *et al.*, 1978; Semino *et al.*, 2000; Wilson *et al.*, 2001]). As an independent test similar to the test performed by Chikhi *et al.* [2002] we also decided also to repeat the admixture analyses by using Sardinia instead of the Basques for the Rosser *et al.* dataset, because this island is considered a genetic isolate [Francalacci *et al.*, 2003; Fraumene *et al.*, 2006], with an independent evolutionary history from the Italian peninsula [Barbujani *et al.*, 1995a]. We also note that since all European population must have had some level of admixture, our approach should provide underestimates of the Near Eastern farmers in Europe.

#### *3.3.1.4 Validation of the admixture analysis with negative controls*

Several European or circum-European populations, for which mtDNA and NRY data are available, are unlikely, due to their geographical location, to have been involved

in the simple expansion and admixture model implicit in the DDM. This was the case of Iceland, Scandinavian countries like Sweden (including the Island of Gotland) and Norway, Baltic countries (Latvia and Lithuania), some Slavic samples (Russia and Belarus) and of the Uralic (Sami, Mari, Estonian and Finnish) and Altaic (Chuvash) language families. These populations were used as negative controls. Indeed, our prediction is that for these populations, the decrease of admixture proportion with increasing geographical distance from the Near East should not hold, or should be much less obvious. We also note that for some populations from the Afroasiatic language family (Algeria and the North Africa sample) the predictions are more difficult to make. We analyse these populations here, to determine whether their admixture level may provide some hint regarding the expansion of the Afro-Asiatic language, but the limited number of samples makes this a conjecture that will need more samples to be tested.

#### *3.3.1.5 Regression Analysis*

A linear regression approach was used to detect, quantify, and assess the significance of any geographical trend in admixture proportions across Europe [Chikhi *et al.*, 2002]. Based on the samples available for the genetic analyses, the geographic distance was calculated from the middle point: i) of Turkey [Rosser *et al.*, 2000] and ii) between Syria and Turkey [Richards *et al.*, 2000]. Given that we do not have access to the exact value of $p_1$ for the samples analysed, but rather to a posterior distribution which presents some level of uncertainty, the regression was performed by repeatedly sampling from the $p_1$ distributions in the following manner. For each of the European samples, one $p_1$ value was randomly sampled from the corresponding posterior distribution. A linear regression was then calculated between this set of values and geographic distance. This process was repeated 1,000 times to obtain the empirical distribution of regression curves. A similar approach was used for $= T/N_h$.

#### *3.3.1.6 $F_{ST}$ analysis*

To further analyse the genetic structure of the populations, and to ascertain the differences between male and female variation patterns we used $F_{ST}$ statistics, computed according to Nei [1977], as it only requires allele frequencies. The pairwise $F_{ST}$ values were calculated for both NRY and mtDNA datasets, using the Near Eastern samples against all the other populations. These values were then plotted against the geographical distance from the same locations used for the regression analyses.

### 3.3.2 aDNA and Coalescent Analysis

#### *3.3.2.1 Populations' datasets*

At the time of writing and analysis, the two largest European aDNA data sets available were those of Haak *et al.* [2005] and of Bramanti *et al.* [2009]. Both present mtDNA data from Central European HG [Bramanti *et al.*, 2009] and from early LBK/AVK (Linear Pottery/Alföld Linear Pottery) farmers [Haak *et al.*, 2005] skeletons, respectively. They were analysed with modern mtDNA data from the same geographical regions following the original authors [Bramanti *et al.*, 2009].

#### *3.3.2.2 Demographic Models: testing for the continuity and discontinuity hypotheses*

The aDNA used in the present study were taken from two studies (see above) which reached opposite conclusions regarding the continuity versus discontinuity hypothesis in Europe. The study of Haak *et al.* [2005] claimed that the change in haplotype frequency between Neolithic and modern samples could not be explain by drift alone, particularly due to the high frequency of the N1a haplotype, which was found at a frequency of 25% in the aDNA samples and is nearly absent in present-day European populations. They thus suggested that the Neolithic farmers were not the ancestors of modern-day Europeans and favoured a continuity hypothesis. Bramanti and colleagues [2009] used a simple panmictic model to ask whether there

was continuity between local Central European HG aDNA samples and modern-day samples from the same geographical region. They also used aDNA samples from Neolithic farmers, and concluded that the continuity hypothesis should be rejected, i.e. that present-day Europeans are not descendants from the local Palaeolithic populations. One serious problem with this study is that assumes totla panmixia and hence cannot actually test for genetic continuity or descontinuity. We show that this model makes unrealistic and self-contradictory assumptions. Their model assumes total panmixia across all Central Europe, across all human populations (i.e. farmers and HG are assumed to be part of the same panmictic population) over the whole period of Europe colonization (45,000 years). Such extreme assumption, as we show, explains why they rarely observed the high $F_{ST}$ values that are computed from real data. We show that by using very simple structured models, the high $F_{ST}$ values observed in real data are actually easily generated.

To do this we performed coalescent simulations under three different sets of models. First, we simulated data under the model of Bramanti and colleagues [2009] to validate our approach and reproduce their results. We named this model Total Panmixia (TP) for the reasons explained above. The TP model assumes that HG and farmers are part of the same panmictic population over Central Europe and were never separated into different populations or communities. The Bramanti model also assumes a single modern female effective population size $N_M$ (12,000,000) and two periods of exponential growth: i) the first starting with an Upper Palaeolithic (UP) population of effective size $N_{UP}$, sampled from an ancestral African female population of constant size 5,000, corresponding to the initial colonization of Central Europe 45,000 years ago and ii) the second following the Neolithic Transition 7,500 years ago, from a population of effective size $N_N$. Both $N_{UP}$ and $N_N$ population sizes were allowed to vary between 10 to 5,000 and 1,000 to 100,000, respectively [Bramanti *et al.*, 2009]. To avoid making the rather strong assumption of panmixia between HG and farmers communities, while keeping the models simple and allowing comparisons with their results, we built two models that are similar but allow for some population structure. In the Split Model (S) we assumed that the Upper Palaeolithic population was structured in two sub-populations of equal size,

45,000 years ago. These sub-populations were assumed to grow independently (no gene flow) until they joined at the beginning of the Neolithic, in Central Europe. For simplicity and to avoid having some Palaeolithic samples in one of the two subpopulations and others in the other subpopulation we assumed that all the Palaeolithic sequences were sampled from the same subpopulation. The main reason for using this model is that it is probably the simplest structured model imaginable under the framework proposed by Bramanti *et al.*. It corresponds, for instance, to a scenario where HG where subdivided into two main populations (one in Central Europe, and the other following a southern route) that joined during the Neolithic, with no genetic contribution from other populations. We also used a more complex splitting model that we named the Split with Differential Growth (SDG) model. The SDG model is similar to the S model but one of the two sub-populations was allowed to have a higher growth rate between 10,000 and 7,500 years ago. It is compatible with a scenario where the 'left' population corresponds to HG, whereas the other one corresponds to Near Eastern farmers arriving and mixing with HG during the Neolithic expansion. This kind of model is an admixture model [Chikhi *et al.*, 2002; Goldstein & Chikhi, 2002]. It is important to note, that for technical reasons, in the SDG model, we constrained one of the subpopulations (deme 1, corresponding to the HG) at the Neolithic to have a size $1/20_{\text{th}}$ of $N_N$. (see Fig. A.1)
Note that all models allowed the same parameters to vary, including the growth rates which were computed on the basis of population size values which in turn were sampled from the priors.

#### *3.3.2.3 Distribution of pairwise $F_{ST}$ values across models and validation of our simulation approach*

We used Bayesian Serial SimCoal software (BayeSSC) [Anderson *et al.*, 2005; Excoffier *et al.*, 2000] to simulate aDNA and modern DNA data, by tracing the ancestry of the female modern samples and incorporating ancient DNA samples of both HG and farmers. We used the same parameter values (and/or priors) for sequence sizes, mutations rates, transition bias, distribution of mutations rate among sites, populations effective sizes and periods of time as in Bramanti *et al.* [2009].

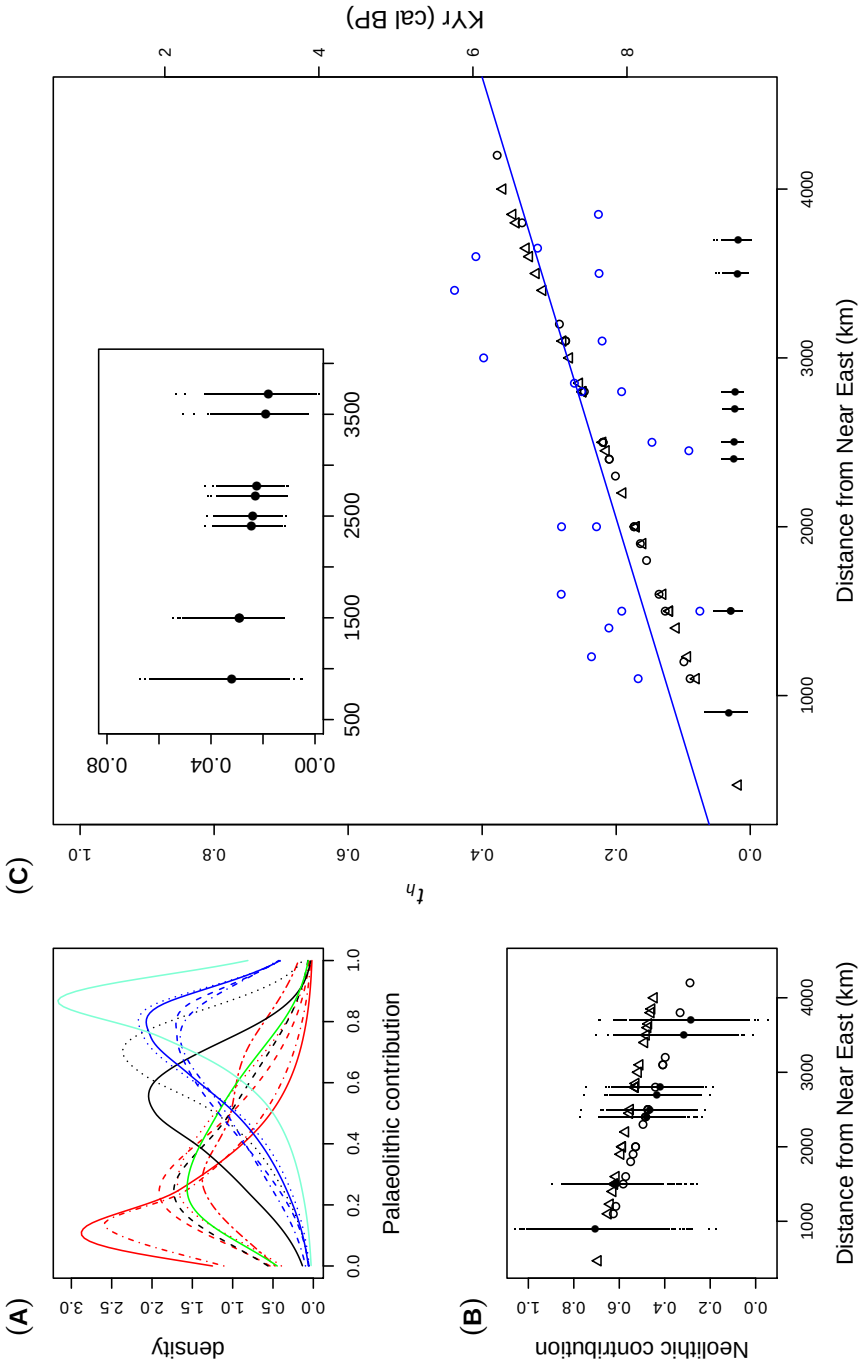
We explored 2,500 parameter combinations using fifty equally spaced values sampled from the priors for both $N_{UP}$ (ranging from 10 to 5,000) and $N_N$ (between 1,000 and 100,000), as in [Bramanti *et al.*, 2009]. For each pairwise combination we performed 500 independent coalescent simulations, hence corresponding to a total of 3,750,000 simulations (1,250,000 simulations for each of the three models). Three sets of sequences were sampled from the coalescent simulations according to the sizes of the observed sequence data (HG, farmers and modern Central Europeans) and their corresponding ages. We then computed the pairwise $F_{ST}$ values in the simulated data and compared them to the values observed in the real data. The proportion of times where the simulated $F_{ST}$ was greater than the observed $F_{ST}$ was recorded, for each combination of $N_{UP}$ and $N_N$ values as in [Bramanti *et al.*, 2009]. We also computed whether the observed $F_{ST}$ values were within the 95% credible interval for each parameter combination. Scripts were written in the R language [Development Core Team, 2009] to create the infiles read by BayeSSC, to analyse the results and to produce the plots in Fig. 3.5. The 2,500 points forming the grid and for which the probabilities were estimated, were used to produce the interpolated plots with the filled.contour R function [Development Core Team, 2009]. The observed pairwise $F_{ST}$ values found by Bramanti and colleagues [2009] and used in this study are: 0.163 for HG *vs.* farmers, 0.0858 for HG *vs.* moderns and 0.058 for farmers *vs.* moderns.

#### *3.3.2.4 Approximate Bayesian Computations (ABC) for model choice and parameter estimation*

In order to determine which of the three demographic models explained best the data and then estimate the demographic parameters of interest we used an ABC approach [Beaumont, 2008; Beaumont *et al.*, 2002]. We performed 1,500,000 simulations for each model (4,500,000 simulations in total) and selected the 1% simulations that best explained the observed data (this was also done using the 0.1% best-fitting simulations and provided the same results). Following Bramanti *et al.* [2009], and to facilitate comparison between studies, we used the three pairwise $F_{ST}$ values used by these authors between the HG, farmers and modern samples

as summary statistics. The simulations were performed with the BayeSCC program, but contrary to the previous section we did not use a grid of values but rather proper a priori distributions. The ABC inference procedure was performed using the abc R package [Csillery *et al.*, 2010]. The postpr function was used to select the best model (estimate the posterior probability of each of the three models). This was done using two approaches (i) the Beaumont *et al.* [2008](2008) multinomial logistic regression (MLR) model, and (ii) the nonlinear conditional heteroscedastic (NCH) model that uses a neural network approach [Blum & François, 2009]. The latter approach uses a non-linear regression correction to minimize departure from non-linearity, that enhances accuracy when compared to the regression algorithm proposed by Beaumont *et al.* [2002; 2010]. For the model that was selected we then we estimated the selected model's parameters of interest ($N_{UP}$ and $N_N$), using the 1% simulations (15,000 values) associated with the shortest Euclidian distances from the observed data. The NCH regression-ABC method, proposed by (Blum and François, 2010), jointly with a logit transformation, was used to estimate the parameters based on the observed and simulated pairwise $F_{ST}$ values.
The model selection approach was validated by calculating the power to recover the true model. For that, we took randomly 1,000 datasets, from the original BayeSSC runs for ABC analysis, for each of the three demographic models. We thus assigned each of these datasets to a model, by using again the function postpr. However, this time we used the pairwise $F_{ST}$ values of the simulated datasets as pseudo-observed summary statistics. Finally, we counted the number of times that the true model was correctly identified (see appendix Table A.1).

**Figure 3.1** ***(facing page)*****: Spatial variation of admixture and drift, across Europe** - In **(A)** are represented the posterior distributions of the Palaeolithic contribution (HG contribution to modern European), for each of the European populations analysed, using mtDNA data. Each curve corresponds to the analysis of a specific hybrid population (Armenia – red, Caucasus – dashed red, Azeri – dotted red, Egypt – dotdash red, Iran – twodash red, Central Mediterranean – black, East Mediterranean – dashed black, West Mediterranean – dotted black, Southeast Europe – green, North and Central Europe – blue, Northeast Europe – dashed blue, Northwest Europe – dotted blue, Alps, dotdash blue and Scandinavia – aquamarine). **(B)** Linear regression of Neolithic contribution, against geographical distance from Near East, using mtDNA data. Mean values for each population are represented by solid circles (mtDNA data) and open triangles and circles (for two different NRY datasets, Rosser *et al.* [2000] and Semino *et al.* [2000], respectively). In **(C)** is represented the linear regression of $t_h$ (drift in the admixed populations) against geographic distance from the Near East for mtDNA data. The close-up shows the mtDNA regression on a different scale for the Y-axis. Mean values for each population are represented both for mtDNA and NRY datasets, with the symbol codes as in **(B)**. Calibrated radiocarbon dates of Neolithic archaeological sites [Pinhasi *et al.*, 2005] (see also table A.2) are also plotted against the distance from the Near East (blue open circles), with the linear regression represented by the blue line.


(A)
density
Palaeolithic contribution
(B)
Neolithic contribution
Distance from Near East (km)
(C)
$t_h$
kYr (cal BP)
Distance from Near East (km)

## 3.4 Results

### 3.4.1 Admixture analyses: The Neolithic contribution decreases with distance from the Near-East, for both NRY and mtDNA data

Figures 3.1A (mtDNA) and A.2 (NRY) show the posterior distributions for $p_1$, the Palaeolithic contribution to the European populations analysed. As expected from simulations [Chikhi *et al.*, 2001; Sousa *et al.*, 2009], the distributions are rather wide and each single population estimate has a large standard error, confirming that population genetic parameters estimated using single locus data are rarely very accurate. Nevertheless, when all populations are considered jointly, a clear geographic pattern is seen in both the new NRY and mtDNA (Fig. 3.1B) datasets. This pattern shows that the proportion of Neolithic genes ($1 - p_1$) decreases from modal values of around 100% in Greece and Cyprus, to 75% in Romania, 30% in France and 20% in Spain (Fig. 3.1B). This confirms previous results theat used another independent NRY data set [Chikhi *et al.*, 2002]. This trend is detected for the first time in mtDNA data, which have repeatedly been claimed to exhibit no SE-NW spatial pattern [Richards *et al.*, 2000, 2002]. Fig. 3.1B shows that the three (two NRY and one mtDNA) datasets produce the same general trend, hence supporting a parallel decrease of female and male lineages from Neolithic farmers in the genome of modern Europeans, as we move away from the Near-East.

### 3.4.2 The Neolithic transition in the Caucasus and European islands: NRY admixture analyses

Another set of new results is found with the NRY samples from the Caucasus (Armenia, Georgia and Ossetia). First, the admixture level of these populations is exactly at the level expected if they had been on a SE-NW expansion axis (i.e. along the general direction of farmers expansion towards Europe during the Neolithic), even though they are geographically located NE of the Fertile Crescent and not NW (Fig. A.3A). Second, when the Caucasus data are analysed independently from the rest of the data, we find a significant geographical trend, as expected if agriculture

has expanded demically from the Near East outwards in several directions, i.e. not just towards Europe (Fig. A.4A), as predicted by Renfrew [1991]. Third, the same analysis performed using populations that are unlikely to have played a major role during the Neolithic transition, due to their geographic location (i.e. negative controls, see SI Material and Methods) exhibit no such trend despite their much larger sample sizes (Fig. A.3B). Fourth, contrary to the negative controls used, several European islands population samples (Cyprus, Sardinia, Ireland and British Isles populations ) appear to also fit within the general decrease in admixture across Europe (Fig. A.4B). Thus, we find clines in in the Caucasus and European Islands, but not in populations from the Eastern/ Northern Europe.

### 3.4.3 Drift in paternal and maternal lineages: NRY and mtDNA data support the DDM but not the same demographic histories

Genetic drift is represented by parameter $t_i$ that represents $t_i$ the ratio of $T$, the time since the admixture event, and $N_i$ the effective size of population $i$ (see Fig.1.6). Thus, genetic drift in the different parental populations is represented by the parameters $t_1$ and $t_2$ for the Palaeolithic and Neolithic populations, respectively. Each of the $t_1$ and $t_2$ posterior distributions is obtained independently by the analysis of one European population (Fig. A.5A-B, A.6A-B). First, we find that the $t_1$ posterior values are always higher than the $t_2$ values suggesting that genetic drift has been more important in the 'Palaeolithic' than in the 'Neolithic' parental population, in agreement with a later population size increase related with the arrival of agriculture. Second, for all the European populations analysed the $t_1$ (and $t_2$) posterior distributions are tightly clustered, rather than spread out, even though each analysis is performed independently. Third, the different $t_1$ posterior values are more diverse (i.e. less clustered) than the $t_2$ distributions, which is expected if the early HG populations were differentiated, due to their smaller effective sizes. Fourth, the $t_1$ and $t_2$ posteriors obtained for the mtDNA datasets support much lower values than the corresponding NRY $t_1$ and $t_2$ posteriors, suggesting a much larger female ($N_f$) than male ($N_m$) population effective size and/or higher female gene flow.

Fifth, Fig. 3.1C shows the results for the parameter $t_h$ which represents drift

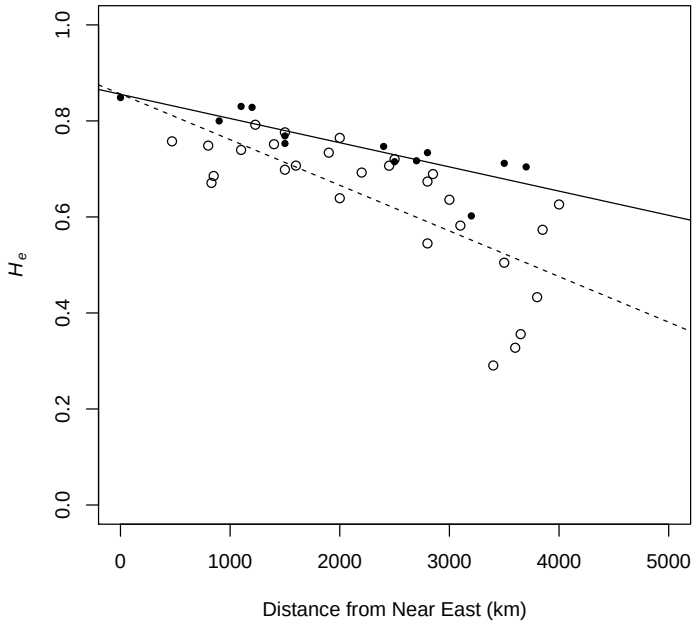



**Figure 3.2: Genetic diversity across Europe** - Expected heterozygozyty $H_e$ values for each European population analysed are regressed against the geographic distance from the Near East, both for NRY (solid circles) and mtDNA (open circles). The linear regressions calculated from these points are represented by the solid (NRY) and dashed (mtDNA) lines.

in the different European populations since the admixture event. We find that for NRY data, $t_h$ is positively correlated with distance from the Near-East and with the earliest date of arrival of agriculture in the different locations based on archaeological artefacts (i.e. drift increases for European populations that had a HG lifestyle for a longer period and admixed later). Sixth, for the mtDNA data, the geographical trend is very different. Similarly low $t_h$ values are observed in the Near-East, but instead of increasing with distance they exhibit (almost) no trend (see close-

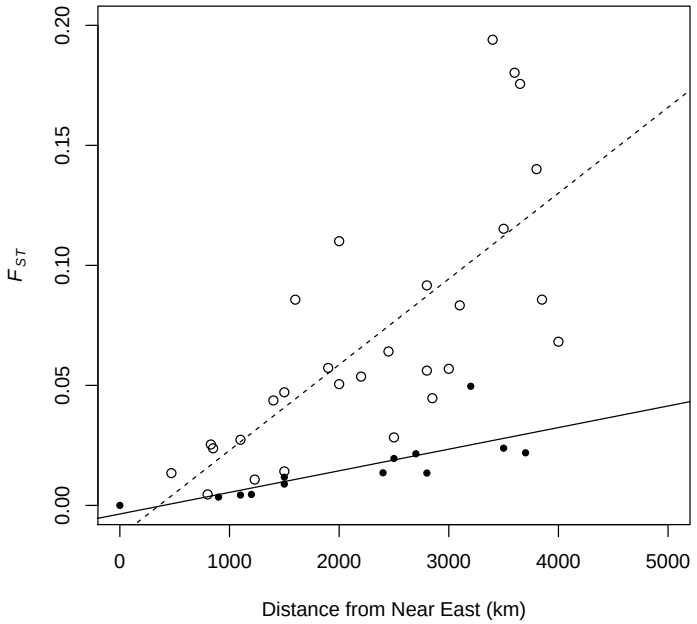



**Figure 3.3: Genetic differentiation across Europe** - Each point represents pairwise $F_{ST}$ values, between European populations and the Near East, regressed against distance from the latter. The symbol and line codes are as in Fig. 3.2.

up in Fig. 3.1C showing a decrease). It thus appears that the mtDNA and NRY $t_h$ results require different explanations for the demographic history of males and females, while favouring both the DDM. Seventh, differences between males and females are also observed when measures of genetic diversity ($H_e$) and differentiation ($F_{ST}$) are regressed against geographic distance from the Near-East. For mtDNA, genetic differentiation between Europeans and Near Easterners increases much less with increasing geographical distance than for NRY data (Fig. 3.2). In agreement with this trend, differences in diversity levels are also less important in mtDNA than in NRY data (Fig.3.3). Both support a higher effective population size of females and/or higher female migration rates.

### 3.4.4 Ancient DNA, coalescent simulations and model identification using ABC

Figure 3.4 represents the three demographic scenarios tested together with their posterior probabilities, using two ABC model choice algorithms on aDNA data [Bramanti *et al.*, 2009]. Whether we use the multinomial logistic regression (MLR) method of Beaumont [2008] or the non-linear heteroscedastic neural network (NCH) approach of Blum and François [2009], the support for the Total Panmixia (TP) model is nil, whereas the best supported model, with a posterior probability $> 0.957$, is the Split with Differential Growth (SDG) model which assumes a differential growth between Neolithic and Palaeolithic farmers. These results suggest that structure is required between HG and farmers to explain the observed data (SDG and S (Split) versus TP) and that differential growth is also required (SDG versus S). Furthermore, the parameters estimated for the SDG suggest that the growth rate in the HG populations, during the Palaeolithic, was very low or null (see table

**Figure 3.4** ***(facing page)*****: Demographic models used in the aDNA analysis and their posterior probabilities** - Three different demographic models were tested using ancient and modern mtDNA data. In the Total Panmixia (TP) model **(A)**, HG and farmers were part of the same panmictic population over Central Europe and were never separated in different populations or communities. This is the model used by Bramanti *et al.* [2009] and assumes a single modern female effective population size $N_M$ and two periods of exponential growth: i) the first starting with an Upper Palaeolithic (UP) population of effective size $N_{UP}$, sampled from an ancestral African female population of constant size, corresponding to the initial colonization of Central Europe 45,000 years ago and ii) the second following the Neolithic Transition 7,500 years ago, from a population of effective size $N_N$. Both $N_{UP}$ and $N_N$ population sizes were allowed to vary using the same priors as in [Bramanti *et al.*, 2009]. In the Split Model (S) **(B)**, the UP population was structured in two sub-populations of equal size, 45,000 years ago. These sub-populations were assumed to grow independently (no gene flow), until they joined together at the beginning of the Neolithic, in Central Europe. The Split with Differential Growth (SDG) model **(C)** is similar to the S model but has a more complex splitting, in which one of the two sub-populations was allowed to have a higher growth rate between 10,000 and 7,500 years ago. In **(D)** are represented the posterior probabilities under each model, calculated using the ABC framework, for two different types of post-rejection adjustments: multinomial logistic regression (MLR: white bars) and non-linear heteroscedastic neural network (NCH: grey bars).

(A) Total Panmixia (TP)
(B) Split (S)
(C) Split with Differential Growth (SDG)
$N_{UP}$
$N_N$
$N_M$
T
Present
(D)
Posterior probability
0.0
0.2
0.4
0.6
0.8
1.0
MLR
NCH
0.000
0.000
0.036
0.043
0.964
0.957
TP
S
SDG

3.1).

**Table 3.1: Demographic parameters estimated under the Split with Differential Growth (SDG) model.** Weighted ($\omega$) median, 5% and 95% percentiles values are represented for $N_N$ and $N_{UP}$. Deme 1 and 2 correspond to the demes without and with differential growth, respectively (see Fig. A.1).

| | $\omega$ **Median** | $\omega$ **5% Perc.** | $\omega$ **95% Perc.** | **Prior** |
|---|---|---|---|---|
| $N_N$ | | | | |
| Total | 18 374.80 | 3 529.00 | 77 274.60 | U: 1 000 – 100 000 |
| Deme 1 | 967.10 | 185.74 | 4 067.09 | |
| Deme 2 | 17 407.70 | 3 343.26 | 73 207.51 | |
| $N_{UP}$ | | | | |
| Total | 2 248.12 | 297.64 | 4 717.56 | U: 10 – 5 000 |
| Deme 1 | 1 124.06 | 148.82 | 2 358.78 | |
| Deme 2 | 1 124.06 | 148.82 | 2 358.78 | |

**Table 3.2: Probability of simulated $F_{ST}$ values being higher than observed ones ($P_{S>O}$), in the aDNA analysis.** Maximum values of $P_{S>O}$, for each of the models and pairwise comparisons analysed in this study.

| **Models** | $P_{S>O}$ | | |
|---|---|---|---|
| | HG *v.s.* Farmers | HG *v.s.* Modern | Farmers *v.s.* Modern |
| TP (this study) | 0.018 | 0.032 | 0.152 |
| TP [Bramanti *et al.*, 2009] | 0.022 | 0.028 | – |
| S | 0.132 | 0.278 | 0.192 |
| SDG | 0.990 | 1.000 | 0.612 |

The same kind of results, but using another approach, is showed in Fig. 3.5. This figure represents the estimated probability of obtaining $F_{ST}$ values that are equal or higher than those observed in the real data ($P_{S>O}$), for the three scenarios. The data simulated under the TP model (Fig. 3.5A-C) show results identical to those obtained by Bramanti and colleagues [2009], hence validating our simulation approach and the exagerated simplicity of the model used by these authors. For this model, the parameter space explaining the observed data is extremely limited. However, as soon as structure is incorporated in the models (S and SDG), the

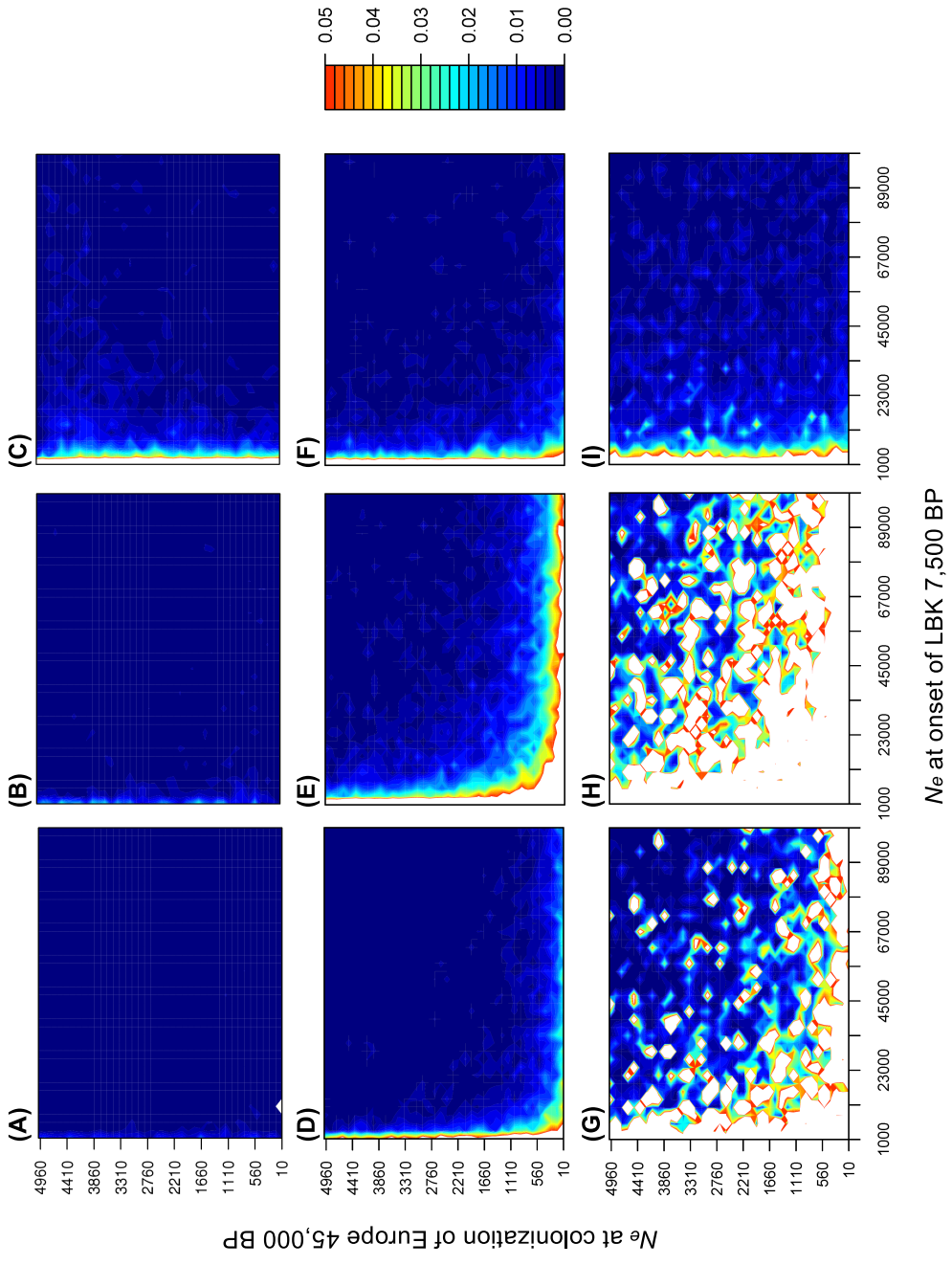
number of parameter combinations ($N_{UP}$ and $N_N$) for which large $F_{ST}$ values are observed becomes very large hence allowing for many realistic scenarios to explain the observed data. This is true for the S model (Fig. 3.5D-F) and even more when we introduce differential growth in the model (Fig. 3.5G-I). For instance, the probability of $P_{S>O}$ values in the SDG model panels can be as high as 0.99 for the HG *vs.* farmer comparisons or as high as one for the HG *vs.* modern European comparison, showing that simple structured models produce high $F_{ST}$ values for reasonable parameter values. Conversely, the simulations for the TP model have maximum $P_{S>O}$ values of 0.018 for the first comparison and 0.032 for the latter, in agreement with the values found by Bramanti and colleagues [2009] (see table 3.2).

## 3.5 Discussion

### 3.5.1 Both contemporary NRY and mtDNA data support DDM, but tell different demographic histories

Our analysis, using contemporary data, suggests that there is a parallel decrease in the NRY and mtDNA Neolithic contributions to the European populations with increasing distance from the Near-East. This is not compatible with a model of cultural diffusion and requires demic movement of both male and female farmers, from the Near-East, as agriculture spread into Europe, in agreement with archaeo-

**Figure 3.5 *(facing page)*: Probability of obtaining genetic differentiation values close to the observed in the real data** - The panels in each row correspond to data simulated under **(A, B, C)** the TP model, **(D, E, F)** the S model and **(G, H, I)** the SDG model (see Fig. 3.4, for models definitions). Each column corresponds to a specific pairwise $F_{ST}$ comparison, namely between HG and early farmers **(A, D, G)**, HG and modern Europeans **(B, E, H)**, and early farmers and modern Europeans **(C, F, I)**. The x- and y-axis represent the values used for the female effective size $N_N$ (at the onset of the Central European Neolithic 7,500 years ago) and $N_{UP}$ (45,000 years ago), respectively. The colour key represents the probability of obtaining a $F_{ST}$ value equal or greater than that observed.The white shaded area corresponds to parameter combinations for which this probability is greater than 0.05.


(A)
(B)
(C)
(D)
(E)
(F)
(G)
(H)
(I)
0.05
0.04
0.03
0.02
0.01
0.00
4960
4410
3860
3310
2760
2210
1660
1110
560
10
1000
23000
45000
67000
89000
Ne at colonization of Europe 45,000 BP
Ne at onset of LBK 7,500 BP

logical data [Bocquet-Appel *et al.*, 2009; Gkiasta *et al.*, 2003; Pinhasi *et al.*, 2005]. This parallel decrease also suggests that both males and females admixed with the local Palaeolithic populations that inhabited Europe at the time, resulting in a progressive dilution of the Near-East genes. We also found that the demic diffusion process was centrifugal, with samples from the Caucasus fitting in the general trend, as was already suggested by Renfrew [1991] and others [Balanovsky *et al.*, 2011] and in agreement with linguistic data too [Gray & Atkinson, 2003]. Moreover, the European islands appear also to fit within this trend. This suggests that the sea did not represent major barrier to the Neolithic expansion and that the peopling of these islands was not subjected to major drift effects or radically different admixture histories compared to neighbouring continental populations [Bocquet-Appel *et al.*, 2009].

It therefore appears that, when we use one coherent statistical framework, both datasets from male and female markers (mtDNA [Richards *et al.*, 2000] and NRY [Rosser *et al.*, 2000]), support the DDM. These results are at odds with the original conclusions drawn by Richards *et al.* [2000] (i.e. using only mtDNA), who advocated that mtDNA data favoured the CDM. However, they are in agreement with the clines described by Rosser *et al.* [2000] (i.e. only with NRY data). It is worth noting that the methods used by the two studies are not comparable. Richards *et al.* [Richards *et al.*, 2000] used the age of mtDNA mutations and haplogroups to date major demographic events. This kind of approach has been criticised as it can lead to misinterpretation of the data [Barbujani & Chikhi, 2006; Barbujani *et al.*, 1998; Goldstein & Chikhi, 2002]. Rosser *et al.* [2000] used spatial autocorrelation methods instead, to identify statistically significant clines. This method has been similarly criticised, as a cline in itself does not indicate the time at which it was established. Model-based approaches, like those applied here, explicitly state the assumptions used to make inference and are probably the most suitable to infer demographic parameters [Chikhi & Beaumont, 2005; Chikhi *et al.*, 2002; Currat & Excoffier, 2005], such as the Neolithic contribution to European populations.

The fact that extant NRY and mtDNA both support the DDM does not imply that other details of the male and female demography were identical, particularly in re-

lation with the amount of drift experienced by each sex [Wilkins, 2006]. Indeed, our results point to a higher $N_f$ over $N_m$, in agreement with the larger coalescence times for mtDNA [Tang *et al.*, 2002; Wilder *et al.*, 2004]. But before addressing this issue and proposing a model accounting for these results we turn to the aDNA results.

### 3.5.2 aDNA supports Demic Diffusion

The first aDNA study using model-based approaches, on samples identified as Linear Pottery Culture (LBK), argued in favour of CDM [Haak *et al.*, 2005]. Later, the same LBK data was compared to samples from Palaeolithic/Mesolithic archaeological sites and modern data from the same region, by Bramanti *et al.* [2009]. They interpreted the genetic differentiation observed in the real data as being too high to 'be explained by population continuity alone' [2009], hence arguing for a Neolithic immigration in Central Europe. Their study thus disagreed with that undertaken previously by Haak *et al.* [2005]. These studies [Bramanti *et al.*, 2009; Haak *et al.*, 2005] had in common that all DNA samples, ancient and modern alike, were assumed to belong to the same panmictic population (see Fig. 3.4A). While this may seem surprising, the model assumed in these two studies is the one that we call Total Panmixia. This model surprisingly assumes that there was no population structure and that HG and farmers were allowed to mate freely, making the distinction between HG and farmers unclear.

What our new aDNA simulation framework suggests is that it is actually possible to explain the large genetic differentiation between samples if we explicitly model both population structure and different population growth rates between Neolithic and Palaeolithic populations before they admixed. In a recent work, Haak and colleagues [2010] also allowed for some population structure, namely between populations of Central Europe and the Near-East. Their results suggested an affinity between the first LBK farmers and modern Near-Easterners, but they still could not explain the high population differentiation encountered between the LBK farmers and present-day Central European populations. On the contrary, our SDG model, could explain the high $F_{ST}$ values encountered between HG and farmers and be-

tween farmers (or HG) and modern-day Central Europeans. We believe that the main difference with the Haak *et al.* study [2010] is that they did not allow variable population growth rates in their simulations. However, by varying the growth rates between HG and farmers, as between the onset of farming and the following period, we could explain these high $F_{ST}$ values.
Differential growth between farmers and HG is supported by anthropological and archaeological data [Galeta & Bruzek, 2009; Shennan, 2009]. Indeed, at the onset of the Neolithic expansion in the Near-East and in the front of the wave of expansion, it has been shown that a very high growth rate is expected from the colonizing populations until their size reaches the new carrying capacity ceilings [Shennan, 2009]. Interestingly, our estimates suggest that the female growth rate remained quasi-constant during the Palaeolithic, and that there was an expansion with the advent of farming, which is also in agreement with archaeological data [Bocquet-Appel *et al.*, 2005; Gignoux *et al.*, 2011]. Such an increase in $N_f$ could also be explained by an increase in gene flow following the arrival of farming, for instance if it was accompanied by a change in post-marital residence patterns in females.

### 3.5.3 Towards an integrated model of Neolithic transition

Altogether, the work presented here allows us to draw a coherent integrated model for the Neolithic transition in Europe which accounts for both the congruent admixture results between mtDNA and NRY data, their difference in terms of diversity and differentiation (drift), and the constraints imposed by the aDNA data. On that basis, we propose (i) an establishment of farming communities in Europe by a demic diffusion process, with an origin in the Near-East, in agreement with archaeological [Bocquet-Appel *et al.*, 2009; Galeta & Bruzek, 2009; Gkiasta *et al.*, 2003; Pinhasi *et al.*, 2005; Price *et al.*, 2001] and anthropological studies [Bentley *et al.*, 2003; Bocquet-Appel, 2002; Pinhasi & von Cramon-Taubadel, 2009], along with a process of admixture with the local HG [Bentley *et al.*, 2003]; (ii) a spread in different directions from the Near-East, with the Caucasus and European Islands being part of this gradual expansion. Furthermore, we propose that (iii) both male and fe-

male farmers were involved in this demic movement, and that (iv) the demographic histories of the two sexes were probably different during and perhaps before the Neolithic transition. In particular, we propose that the difference in the amount of drift experienced by males and females can be explained by a change in the patterns of gene flow and by a shift in human mating systems, from polygyny to monogamy during to the Neolithic transition. Below we go through the rationale and data that corroborate this scenario.

As noted above, one of our main results is that $N_f > N_m$ and/or that migration rates were higher in females compared with males (Fig. 3.1C). Anthropological, linguistic and archaeological evidence suggest that the transition from hunting-gathering to farming or herding communities usually leads to an increase in patrilocality (i.e. when the marital residence is the groom's birthplace) due to the fact that males tend to control and inherit wealth (i.e the land or the herds), hence leading to higher female migration rates [Baker & Jacobsen, 2006; Bentley *et al.*, 2002, 2008; Cavalli-Sforza & Minch, 1997; Fortunato & Jordan, 2010; Haak *et al.*, 2008; Langergraber *et al.*, 2007]. Given that forager communities do not accumulate wealth, migration patterns are more likely to be symmetrical, and this is indeed what has been observed. In other words, sedentism that accompanied the Neolithic transition [Bellwod & Oxenham, 2008] is expected to have led to a decrease in male gene flow, whereas female gene flow would either have remained constant or would have increased to compensate the decrease in male gene flow. This would explain two of our results, namely the higher mtDNA diversity, the higher NRY differentiation, and the higher difficulty found by several authors to identify clines in mtDNA data, compared to NRY. Interestingly, this would also be in agreement with the larger coalescent times described for mtDNA compared to NRY [Tang *et al.*, 2002; Wilder *et al.*, 2004] and would partly explain the results and interpretation of Richard *et al.* [2000].

Another cultural change that is thought to have taken place in Europe during the Neolithic transition is a shift from polygyny to monogamy [Fortunato, 2011; Lagerlöf, 2010]. In fact, several Neolithic burials [Bentley *et al.*, 2008; Haak *et al.*, 2008] show evidences of nuclear families, which may reflect a monogamous marriage

system. A shift from polygyny to monogamy would have the effect of decreasing male variance in reproductive success, since more males would now be able to mate, and consequently would increase the effective population size of males. This could result in a signal of population growth in NRY data that would be more recent compared to that observed in mtDNA and is exactly what Dupanloup and colleagues [2003] have argued and found. Our results are in good agreement with theirs. Indeed, we found that $t_h$ increased in males but not in females as we moved away from the Near East (Fig. 3.1C), with $t_h$ being the ratio of $T$, the time since the admixture event, and $N_h$, the effective size of the admixed population. Given that $T$ necessarily decreases as we move away from the Near East, an increase of this ratio suggests that the decrease of $T$ was compensated by a rapid increase in $N_h$. In other words, the admixture process between HG and farmers led to a very rapid increase in the effective population size of male whereas this increase was more limited in females. Indeed, a shift from polygyny to monogamy would have less influence on $N_f$, which would anyway be higher than that of males, due to their lower variance in reproductive success. Altogether, a model in which human societies began to adopt farming as a means of subsistence, with the correlated patrilocality and monogamy as a mating system, would be in agreement with all the results presented here, including the aDNA (for instance, it was rather impressive to find that the most probable scenarios, independently inferred no significant growth in Palaeolithic females), and allow us to put in a single picture, results from several genetic and anthropological studies.

While we claim that a more coherent picture emerges from our results, we cannot claim that other scenarios could not also explain the results. Many layers of complexities could be added. For instance, female hypergamy (i.e. the fact that lower social status women are more likely to mate with males from a higher status than the opposite) has been described in several human migration and colonization events [Quintana-Murci *et al.*, 2008; Salzano, 2004; Thomas *et al.*, 2006], and it is believed that it probably happened during the Neolithic transition in Europe [Bentley *et al.*, 2003], with HG females marrying into farmer communities [Bentley *et al.*, 2009]. Qualitatively, female hypergamy would increase female mobility and lead to

low levels of mtDNA genetic differentiation between populations. Thus, one should expect lower mtDNA gradients and (almost) no geographic trend in drift, which is exactly what we see. The exclusion of HG males would lead to an increase of NRY genetic differentiation, explaining the clear geographic trend found in genetic drift. However, we must add that this scenario, which may indeed have taken place, would not as easily fit with the admixture patterns that we find and which are similar in males and females. Thus, at this stage, we would be cautious before arguing for or against female hypergamy. We should also insist on the fact that the patterns identified here correspond to global patterns, and are not in contradiction with regional studies arguing against the demic diffusion. Several processes are likely to have taken place during the millennia corresponding to the arrival of farming communities in Europe. Similarly, it is increasingly clear that different routes (coastal or continental) were followed by different groups of humans. Still, the genetic data point to a major input from Near-eastern populations. This cannot be explained by cultural diffusion at a European scale, and as we have argued repeatedly, using the age of haplogroups or haplotypes to reconstruct human prehistory still awaits formal validation, despite the large literature that uses it [Barbujani & Chikhi, 2006; Barbujani *et al.*, 1998; Chikhi, 2009].

## 3.6 Conclusion

Our study represents the first attempt to integrate contemporary mtDNA and NRY data, together with aDNA. This has allowed us to draw a coherent picture of the Neolithic Transition in Europe, which not only provides an explanation for the patterns of genetic diversity found today and in our past, but also for the apparent contradiction between phylogeographic and model-based studies. The aDNA modelling approach described here could be applied to other aDNA datasets and we are applying it to unpublished data from an Iberian Neolithic population (see apendix B). The results from these independent data appear to validate the suggestion that structured models with varying growth rates explain better the genetic distances observed between ancient and modern DNA than simpler models. The Neolithic

transition in Europe is one of the most studied periods of human prehistory and the source of much debate. It is our hope that the work presented here may help provide a consistent framework to address certain aspects of this 'long-standing' controversy.

**Acknowledgements**
The authors are grateful to C. van Schaik, A. Coutinho, G. Gomes, V. Sousa, J. Salmona, J. Alves, C. Gamba and S. Davis for useful comments on earlier versions of this manuscript. The simulations were partly carried out on the HULK simulation server (European Commission grant MEXT-CT-2004-14338 to G. Gomes) and on the HERMES High Performance Computing Centre (FCT grant H200741/re-equip/2005 to P. Fernandes). R.R. was supported by FCT grant (ref. SFRH/BD/30821/2006). L.C. was partly funded by the CNRS and the FCT grant PTDC/BIA-BDE/71299/2008. Travels between Toulouse and Lisbon were made possible thanks to A Coutinho, E. Danchin, C. Thébaud and B Crouau-Roy. A. Barelli is also thanked for her support.

## 3.7 References

AMMERMAN, A.J. & CAVALLI-SFORZA, L.L. (1984). *The Neolithic transition and the genetics of populations in Europe*. Princeton University Press, Princeton.

ANDERSON, C.N.K., RAMAKRISHNAN, U., CHAN, Y.L. & HADLY, E.A. (2005). Serial simCOAL: a population genetics model for data from multiple populations and points in time. *Bioinformatics*, **21**, 1733–4.

BAKER, M. & JACOBSEN, J. (2006). A human capital-based theory of postmarital residence rules. *J Law Econ Organ*, **23**, 208–241.

BALANOVSKY, O., DIBIROVA, K., DYBO, A., MUDRAK, O., FROLOVA, S., POCHESHKHOVA, E., HABER, M., PLATT, D., SCHURR, T., HAAK, W., KUZNETSOVA, M., RADZHABOV, M., BALAGANSKAYA, O., ROMANOV, A., ZAKHAROVA, T., SORIA HERNANZ, D.F., ZALLOUA, P., KOSHEL, S., RUHLEN, M., RENFREW, C., WELLS, R.S., TYLER-SMITH, C. & AND, E.B. (2011). Parallel evolution of genes and languages in the Caucasus region. *Mol Biol Evol*.

BALARESQUE, P., BOWDEN, G., ADAMS, S., LEUNG, H., KING, T., ROSSER, Z., GOODWIN, J., MOISAN, J., RICHARD, C., MILLWARD, A. *et al.* (2010). A predominantly Neolithic origin for European paternal lineages. *PLoS biology*, **8**, e1000285.

BALTER, M. (2009). Archaeology: ancient DNA says Europe's first farmers came from afar. *Science*, **325**, 1189.

BARBUJANI, G. & CHIKHI, L. (2006). Population genetics: DNAs from the European Neolithic. *Heredity*, **97**, 84–85.

BARBUJANI, G., BERTORELLE, G., CAPITANI, G. & SCOZZARI, R. (1995a). Geographical structuring in the mtDNA of Italians. *Proc Natl Acad Sci U S A*, **92**, 9171–9175.

BARBUJANI, G., SOKAL, R.R. & ODEN, N.L. (1995b). Indo-European origins: a computer-simulation test of five hypotheses. *Am J Phys Anthropol*, **96**, 109–32.

BARBUJANI, G., BERTORELLE, G. & CHIKHI, L. (1998). Evidence for Paleolithic and Neolithic gene flow in Europe. *Am J Hum Genet*, **62**, 488–492.

BEAUMONT, M. (2008). *Joint determination of topology, divergence time, and immigration in population trees*, 135–154. McDonald Institute for Archaeological Research, Cambridge.

BEAUMONT, M.A., ZHANG, W. & BALDING, D.J. (2002). Approximate Bayesian Computation in population genetics. *Genetics*, **162**, 2025–35.

BEAUMONT, M.A., NIELSEN, R., ROBERT, C., HEY, J., GAGGIOTTI, O., KNOWLES, L., ESTOUP, A., PANCHAL, M., CORANDER, J., HICKERSON, M., SISSON, S.A., FAGUNDES, N., CHIKHI, L., BEERLI, P., VITALIS, R., CORNUET, J.M., HUELSENBECK, J., FOLL, M., YANG, Z., ROUSSET, F., BALDING, D. & EXCOFFIER, L. (2010). In defence of model-based inference in phylogeography. *Mol Ecol*, **19**, 436–446.

BELLE, E.M.S., LANDRY, P.A. & BARBUJANI, G. (2006). Origins and evolution of the Europeans' genome: evidence from multiple microsatellite loci. *Proc R Soc B*, **273**, 1595–1602.

BELLWOD, P. & OXENHAM, M. (2008). *The expansions of farming societies and the role of the Neolithic Demographic Transition*, 13–34. Springer.

BELLWOOD, P. (2004). *First Farmers: the origins of agricultural societies*. Blackwell Publishing, Oxford.

BENTLEY, R.A., PRICE, T.D., LÜNING, J., GRONENBORN, D., WAHL, J. & FULLAGAR, P.D. (2002). Human migration in early Neolithic Europe. *Curr Anthropol*, **43**, 799–804.

BENTLEY, R.A., CHIKHI, L. & PRICE, T.D. (2003). The Neolithic transition in Europe: comparing broad scale genetic and local scale isotopic evidence. *Antiquity*, **77**, 63–66.

BENTLEY, R.A., WAHP, J., PRICE, T.D. & ATKINSON, T.C. (2008). Isotopic signatures and hereditary traits: snapshot of a Neolithic community in Germany. *Antiquity*, **82**, 290–304.

BENTLEY, R.A., LAYTON, R.H. & TEHRANI, J. (2009). Kinship, marriage, and the genetics of past human dispersals. *Hum Biol*, **81**, 159–79.

BLUM, M.G.B. & FRANÇOIS, O. (2009). Non-linear regression models for Approximate

Bayesian Computation. *Stat Comput*, **20**, 63–73.

BOCQUET-APPEL, J.P. (2002). Paleoanthropological traces of a Neolithic demographic transition. *Curr Anthropol*, **43**, 637–650.

BOCQUET-APPEL, J.P., DEMARS, P.Y., NOIRET, L. & DOBROWSKY, D. (2005). Estimates of Upper Palaeolithic meta-population size in Europe from archaeological data. *J Archaeol Sci*, **32**, 1656–1668.

BOCQUET-APPEL, J.P., NAJI, S., LINDEN, M.V. & KOZLOWSKI, J.K. (2009). Detection of diffusion and contact zones of early farming in Europe from the space-time distribution of 14C dates. *J Archaeol Sci*, **36**, 807–820.

BRAMANTI, B., THOMAS, M.G., HAAK, W., UNTERLAENDER, M., JORES, P., TAMBETS, K., ANTANAITIS-JACOBS, I., HAIDLE, M.N., JANKAUSKAS, R., KIND, C.J., LUETH, F., TERBERGER, T., HILLER, J., MATSUMURA, S., FORSTER, P. & BURGER, J. (2009). Genetic discontinuity between local hunter-gatherers and central Europe's first farmers. *Science*, **326**, 137–140.

BRION, M., SALAS, A., GONZÁLEZ-NEIRA, A., LAREU, M.V. & CARRACEDO, A. (2003). Insights into Iberian population origins through the construction of highly informative Y-chromosome haplotypes using biallelic markers, STRs, and the MSY1 minisatellite. *Am J Phys Anthropol*, **122**, 147–161.

CAVALLI-SFORZA, L.L. (1998). The Basque population and ancient migrations in Europe. *Munibe (Antropología-Arqueología)*, **6 (Suppl)**, 129–137.

CAVALLI-SFORZA, L.L. & MINCH, E. (1997). Paleolithic and Neolithic lineages in the European mitochondrial gene pool. *Am J Hum Genet*, **61**, 247–254.

CHIKHI, L. (2009). Update to Chikhi et al.'s "Clinal variation in the nuclear DNA of europeans" (1998): genetic data and storytelling–from archaeogenetics to astrologenetics? *Hum Biol*, **81**, 639–643.

CHIKHI, L. & BEAUMONT, M.A. (2005). *Modelling human genetic history*. John Wiley, New York.

CHIKHI, L., DESTRO-BISOL, G., BERTORELLE, G., PASCALI, V. & BARBUJANI, G. (1998). Clines of nuclear DNA markers suggest a largely Neolithic ancestry of the European gene pool. *Proc Natl Acad Sci U S A*, **95**, 9053–9058.

CHIKHI, L., BRUFORD, M.W. & BEAUMONT, M.A. (2001). Estimation of admixture proportions: a likelihood-based approach using Markov chain Monte Carlo. *Genetics*, **158**, 1347–1362.

CHIKHI, L., NICHOLS, R.A., BARBUJANI, G. & BEAUMONT, M.A. (2002). Y genetic data support the Neolithic demic diffusion model. *Proc Natl Acad Sci U S A*, **99**, 11008–11013.

CSILLERY, K., FRANCOIS, O. & BLUM, M.G.B. (2010). abc: estimation and model selection with Approximate Bayesian Computation (ABC).

CURRAT, M. & EXCOFFIER, L. (2005). The effect of the Neolithic expansion on European molecular diversity. *Proc R Soc B*, **272**, 679–688.

DEVELOPMENT CORE TEAM, R. (2009). R: A language and environment for statistical computing.

DUPANLOUP, I., PEREIRA, L., BERTORELLE, G., CALAFELL, F., PRATA, M.J., AMORIM, A. & BARBUJANI, G. (2003). A recent shift from polygyny to monogamy in humans is suggested by the analysis of worldwide Y-chromosome diversity. *J Mol Evol*, **57**, 85–97.

DUPANLOUP, I., BERTORELLE, G., CHIKHI, L. & BARBUJANI, G. (2004). Estimating the impact of prehistoric admixture on the genome of Europeans. *Mol Biol Evol*, **21**, 1361–1372.

EXCOFFIER, L., NOVEMBRE, J. & SCHNEIDER, S. (2000). SimCoal: a general coalescent program for the simulation of molecular data in interconnected populations with arbitrary demography. *J Hered*, **91**, 506–9.

FAGUNDES, N.J.R., RAY, N., BEAUMONT, M., NEUENSCHWANDER, S., SALZANO, F.M., BONATTO, S.L. & EXCOFFIER, L. (2007). Statistical evaluation of alternative models of human evolution. *Proc Natl Acad Sci U S A*, **104**, 17614–9.

FORTUNATO, L. (2011). Reconstructing the history of marriage strategies in Indo-European-speaking societies: monogamy and polygyny. *Hum Biol*, **83**, 87–105.

FORTUNATO, L. & JORDAN, F. (2010). Your place or mine? a phylogenetic comparative analysis of marital residence in Indo-European and Austronesian societies. *Philos Trans R Soc B*, **365**, 3913–22.

FRANCALACCI, P., MORELLI, L., UNDERHILL, P.A., LILLIE, A.S., PASSARINO, G., USELI, A., MADEDDU, R., PAOLI, G., TOFANELLI, S., CALÒ, C.M., GHIANI, M.E., VARESI, L., MEMMI, M., VONA, G., LIN, A.A., OEFNER, P. & CAVALLI-SFORZA, L.L. (2003). Peopling of three mediterranean islands (corsica, sardinia, and sicily) inferred by y-chromosome biallelic variability. *Am J Phys Anthropol*, **121**, 270–279.

FRAUMENE, C., BELLE, E.M.S., CASTRÌ, L., SANNA, S., MANCOSU, G., COSSO, M., MARRAS, F., BARBUJANI, G., PIRASTU, M. & ANGIUS, A. (2006). High resolution analysis and phylogenetic network construction using complete mtDNA sequences in sardinian genetic isolates. *Mol Biol Evol*, **23**, 2101–2111.

GALETA, P. & BRUZEK, J. (2009). Demographic model of the neolithic transition in central Europe. *Documenta Praehistorica*, **36 ( Neolithic Studies 16)**, 139–150.

GIGNOUX, C.R., HENN, B.M. & MOUNTAIN, J.L. (2011). Rapid, global demographic ex-

pansions after the origins of agriculture. *Proc Natl Acad Sci U S A*, **108**, 6044–9.

GIOVANNINI, A., ZANGHIRATI, G., BEAUMONT, M.A., CHIKHI, L. & BARBUJANI, G. (2009). A novel parallel approach to the likelihood-based estimation of admixture in population genetics. *Bioinformatics*, **25**, 1440–1441.

GKIASTA, M., RUSSELL, T., SHENNAN, S. & STEELE, J. (2003). Neolithic transition in Europe: the radiocarbon revisited. *Antiquity*, **77**, 45–62.

GOLDSTEIN, D.B. & CHIKHI, L. (2002). Human migrations and population structure: what we know and why it matters. *Annu Rev Genomics Hum Genet*, **3**, 129–152.

GRAY, R.D. & ATKINSON, Q.D. (2003). Language-tree divergence times support the Anatolian theory of Indo-European origin. *Nature*, **426**, 435–9.

HAAK, W., FORSTER, P., BRAMANTI, B., MATSUMURA, S., BRANDT, G., TÄNZER, M., VILLEMS, R., RENFREW, C., GRONENBORN, D., ALT, K.W. & BURGER, J. (2005). Ancient DNA from the first european farmers in 7500-year-old neolithic sites. *Science*, **310**, 1016–1018.

HAAK, W., BRANDT, G., DE JONG, H.N., MEYER, C., GANSLMEIER, R., HEYD, V., HAWKESWORTH, C., PIKE, A.W.G., MELLER, H. & ALT, K.W. (2008). Ancient DNA, strontium isotopes, and osteological analyses shed light on social and kinship organization of the later stone age. *Proc Natl Acad Sci U S A*, **105**, 18226–31.

HAAK, W., BALANOVSKY, O., SANCHEZ, J.J., KOSHEL, S., ZAPOROZHCHENKO, V., ADLER, C.J., DER SARKISSIAN, C.S.I., BRANDT, G., SCHWARZ, C., NICKLISCH, N., DRESELY, V., FRITSCH, B., BALANOVSKA, E., VILLEMS, R., MELLER, H., ALT, K.W. & AND, A.C. (2010). Ancient DNA from european early neolithic farmers reveals their Near Eastern affinities. *PLoS Biol*, **8**, e1000536.

LAGERLÖF, N.P. (2010). Pacifying monogamy. *J Econ Growth*, **15**, 235–262.

LANGELLA, O., CHIKHI, L. & BEAUMONT, M. (2001). LEA (likelihood-based estimation of admixture) : a program to simultaneously estimate admixture and the time since admixture. *Mol Ecol Notes*, **1**, 357–358.

LANGERGRABER, K.E., SIEDEL, H., MITANI, J.C., WRANGHAM, R.W., REYNOLDS, V., HUNT, K. & VIGILANT, L. (2007). The genetic signature of sex-biased migration in patrilocal chimpanzees and humans. *PLoS One*, **2**, e973.

MALMSTRÖM, H., GILBERT, M.T.P., THOMAS, M.G., BRANDSTRÖM, M., STORA, J., MOLNAR, P., ANDERSEN, P.K., BENDIXEN, C., HOLMLUND, G., GÖTHERSTRÖM, A. & WILLERSLEV, E. (2009). Ancient DNA reveals lack of continuity between neolithic hunter-gatherers and contemporary scandinavians. *Curr Biol*, **19**, 1758–62.

MENOZZI, P., PIAZZA, A. & CAVALLI-SFORZA, L. (1978). Synthetic maps of human gene

frequencies in Europeans. *Science*, **201**, 786–792.

MITHEN, S. (2007). Did farming arise from a misapplication of social intelligence? *Philos Trans R Soc Lond B Biol Sci*, **362**, 705–718.

NEI, M. (1977). F-statistics and analysis of gene diversity in subdivided populations. *Ann Hum Genet*, **41**, 225–233.

PINHASI, R. & VON CRAMON-TAUBADEL, N. (2009). Craniometric data supports demic diffusion model for the spread of agriculture into Europe. *PLoS One*, **4**, e6747.

PINHASI, R., FORT, J. & AMMERMAN, A.J. (2005). Tracing the origin and spread of agriculture in Europe. *PLoS Biol*, **3**, e410.

PRICE, T.D., BENTLEY, R.A., LÜNING, J., GRONENBORN, D. & WAHL, J. (2001). Prehistoric human migration in the Linearbandkeramik of Central Europe. *Antiquity*, **75**, 593–603.

QUINTANA-MURCI, L., QUACH, H., HARMANT, C., LUCA, F., MASSONNET, B., PATIN, E., SICA, L., MOUGUIAMA-DAOUDA, P., COMAS, D., TZUR, S., BALANOVSKY, O., KIDD, K.K., KIDD, J.R., VAN DER VEEN, L., HOMBERT, J.M., GESSAIN, A., VERDU, P., FROMENT, A., BAHUCHET, S., HEYER, E., DAUSSET, J., SALAS, A. & BEHAR, D.M. (2008). Maternal traces of deep common ancestry and asymmetric gene flow between Pygmy hunter-gatherers and Bantu-speaking farmers. *Proc Natl Acad Sci U S A*, **105**, 1596–601.

RASTEIRO, R. & CHIKHI, L. (2009). Revisiting the peopling of Japan: an admixture perspective. *J Hum Genet*, **54**, 349–354.

RENFREW, C. (1991). Before Babel: Speculations on the Origins of Linguistic Diversity. *Camb Archaeol J*, **1**, 3–23.

RICHARDS, M. (2003). The neolithic invasion of Europe. *Annu Rev Anthropol*, **32**, 135–162.

RICHARDS, M., MACAULAY, V., HICKEY, E., VEGA, E., SYKES, B., GUIDA, V., RENGO, C., SELLITTO, D., CRUCIANI, F., KIVISILD, T., VILLEMS, R., THOMAS, M., RYCHKOV, S., RYCHKOV, O., RYCHKOV, Y., GÖLGE, M., DIMITROV, D., HILL, E., BRADLEY, D., ROMANO, V., CALÌ, F., VONA, G., DEMAINE, A., PAPIHA, S., TRIANTAPHYLLIDIS, C., STEFANESCU, G., HATINA, J., BELLEDI, M., RIENZO, A.D., NOVELLETTO, A., OPPENHEIM, A., NØRBY, S., AL-ZAHERI, N., SANTACHIARA-BENERECETTI, S., SCOZARI, R., TORRONI, A. & BANDELT, H.J. (2000). Tracing european founder lineages in the Near Eastern mtDNA pool. *Am J Hum Genet*, **67**, 1251–1276.

RICHARDS, M., MACAULAY, V., TORRONI, A. & BANDELT, H.J. (2002). In search of geographical patterns in European mitochondrial DNA. *Am J Hum Genet*, **71**, 1168–1174.

ROSSER, Z.H., ZERJAL, T., HURLES, M.E., ADOJAAN, M., ALAVANTIC, D., AMORIM, A.,

AMOS, W., ARMENTEROS, M., ARROYO, E., BARBUJANI, G., BECKMAN, G., BECKMAN, L., BERTRANPETIT, J., BOSCH, E., BRADLEY, D.G., BREDE, G., COOPER, G., CÔRTE-REAL, H.B., DE KNIJFF, P., DECORTE, R., DUBROVA, Y.E., EVGRAFOV, O., GILISSEN, A., GLISIC, S., GÖLGE, M., HILL, E.W., JEZIOROWSKA, A., KALAYDJIEVA, L., KAYSER, M., KIVISILD, T., KRAVCHENKO, S.A., KRUMINA, A., KUCINSKAS, V., LAVINHA, J., LIVSHITS, L.A., MALASPINA, P., MARIA, S., MCELREAVEY, K., MEITINGER, T.A., MIKELSAAR, A.V., MITCHELL, R.J., NAFA, K., NICHOLSON, J., NØRBY, S., PANDYA, A., PARIK, J., PATSALIS, P.C., PEREIRA, L., PETERLIN, B., PIELBERG, G., PRATA, M.J., PREVIDERÉ, C., ROEWER, L., ROOTSI, S., RUBINSZTEIN, D.C., SAILLARD, J., SANTOS, F.R., STEFANESCU, G., SYKES, B.C., TOLUN, A., VILLEMS, R., TYLER-SMITH, C. & JOBLING, M.A. (2000). Y-chromosomal diversity in Europe is clinal and influenced primarily by geography, rather than by language. *Am J Hum Genet*, **67**, 1526–1543.

SALZANO, F.M. (2004). Interethnic variability and admixture in latin America–social implications. *Rev Biol Trop*, **52**, 405–15.

SEMINO, O., PASSARINO, G., OEFNER, P.J., LIN, A.A., ARBUZOVA, S., BECKMAN, L.E., BENEDICTIS, G.D., FRANCALACCI, P., KOUVATSI, A., LIMBORSKA, S., MARCIKIAE, M., MIKA, A., MIKA, B., PRIMORAC, D., SANTACHIARA-BENERECETTI, A.S., CAVALLI-SFORZA, L.L. & UNDERHILL, P.A. (2000). The genetic legacy of Paleolithic Homo sapiens sapiens in extant Europeans: a Y chromosome perspective. *Science*, **290**, 1155–1159.

SHENNAN, S. (2009). Evolutionary demography and the population history of the European early Neolithic. *Hum Biol*, **81**, 339–55.

SOUSA, V.C., FRITZ, M., BEAUMONT, M.A. & CHIKHI, L. (2009). Approximate bayesian computation without summary statistics: the case of admixture. *Genetics*, **181**, 1507–1519.

TANG, H., SIEGMUND, D.O., SHEN, P., OEFNER, P.J. & FELDMAN, M.W. (2002). Frequentist estimation of coalescence times from nucleotide sequence data using a tree-based partition. *Genetics*, **161**, 447–59.

THOMAS, M.G., STUMPF, M.P.H. & HÄRKE, H. (2006). Evidence for an apartheid-like social structure in early anglo-saxon England. *Proc R Soc B*, **273**, 2651–7.

WILDER, J., MOBASTER, Z. & HAMMER, M. (2004). Genetic evidence for unequal effective population sizes of human females and males. *Mol Biol Evol*, **21**, 2047–2057.

WILKINS, J.F. (2006). Unraveling male and female histories from human genetic data. *Curr Opin Genet Dev*, **16**, 611–7.

WILSON, J.F., WEISS, D.A., RICHARDS, M., THOMAS, M.G., BRADMAN, N. & GOLD-

STEIN, D.B. (2001). Genetic evidence for different male and female roles during cultural transitions in the British Isles. *Proc Natl Acad Sci U S A*, **98**, 5078–83.

ZVELEBIL, M. & ZVELEBIL, K. (1998). Agricultural transition and Indo-European dispersals. *Antiquity*, **62**, 574–583.

# 4. Investigating sex-biased migration during the Neolithic transition in Europe, using an explicit spatial simulation framework

Rita Rasteiro[1], Pierre-Antoine Bouttier[1,†], Vítor C. Sousa[1,‡] and Lounés Chikhi[1,2,3]

[1]Instituto Gulbenkian de Ciência, Rua da Quinta Grande, 6, 2780-156 Oeiras, Portugal; [2]CNRS, Laboratoire Évolution et Diversité Biologique (EDB), Bât. 4R3 b2, 118 Route de Narbonne, 31062 Toulouse cédex 9, France; [3]Université de Toulouse, UPS, EDB, Bât. 4R3 b2, 118 Route de Narbonne, 31062 Toulouse cédex 9, France; [†]New address: Université de Grenoble and CNRS, Laboratoire Jean Kutzmann, France; [‡]New address: Department of Genetics, Rutgers University, NJ, USA

**Development of the simulation framework:** R Rasteiro, P-A Bouttier, VC Sousa and L Chikhi

**Analysis:** R Rasteiro

**Manuscript:** R Rasteiro and L Chikhi

textbfCitation: Rasteiro R, Bouttier P-A, Sousa VC and Chikhi L (2012) Investigating sex-biased migration during the Neolithic transition in Europe, using an explicit spatial simulation framework. *Proc R Soc B* (advanced online)

## 4.1 Summary

Cultural practices can deeply influence genetic diversity patterns. The Neolithic transitions that took place at different times and locations around the world led to major cultural and demographic changes that influenced and therefore left their marks on human genetic diversity patterns. Several studies on the European Neolithic transition suggest that mtDNA and Y-chromosome data can exhibit different patterns, which could be due to different demographic histories for females and males. Archaeological and anthropological data suggest that the transition from hunter-gatherers to farmers' societies is probably associated with changes in social organization, particularly in postmarital residence rules (i.e. patrilocality, matrilocality or bilocality). The movements of humans and genes associated with these rules can be seen as sex-biased short-range migrations. We developed a new individual-based simulation approach to explore the genetic consequences of 45 different scenarios, where we varied the patterns of postmarital residence and admixture between hunter-gatherers and farmers. We recorded mtDNA and Y-chromosome data and analysed their diversity patterns within and between populations, through time and space. We also collected published mtDNA and Y-chromosome data from European and Near-Eastern populations in order to identify the scenarios that would best explain them. We show that (i) different postmarital residence systems can lead to different patterns of genetic diversity and differentiation, (ii) asymmetries between mtDNA and Y-chromosome can be due to different behaviours between males and females, but also to different mutations rates (iii) patrilocality in farmers explains the present patterns of genetic diversity better than matrilocality or bilocality. Moreover, we found that (iv) the genetic diversity of farmers change depending on the hunter-gatherers postmarital residence rules even though they are assumed to disappear more than 5000 years ago in our simulations.

**Keywords**: postmarital residence, migration, spatial expansion, Neolithic, Palaeolithic, admixture

## 4.2 Introduction

The Neolithic transition was one of the greatest cultural transitions in human prehistory [Bellwood, 2004; Davis, 2005; Mithen, 2007]. The demographic and cultural changes that it triggered unquestionably changed how human genes, cultures and languages are distributed around the world today [Ammerman & Cavalli-Sforza, 1984; Bellwood, 2004; Davis, 2005; Mithen, 2007]. Although Europe is probably the most studied area, there are still major disagreements among archaeologists [Bellwood, 2004; Diamond & Bellwood, 2003; Gkiasta *et al.*, 2003; Pinhasi *et al.*, 2005] and among geneticists [Barbujani *et al.*, 1995, 1998; Belle *et al.*, 2006; Chikhi *et al.*, 2002; Richards *et al.*, 2000, 2002] on how the transition into farming-based societies happened in this region, and on how archaeological and genetic data should be interpreted [Chikhi, 2009]. As a first approximation, the Neolithic transition has mainly been modelled by considering one or the other of the following alternative scenarios: the Cultural Diffusion model (CDM) [Zvelebil & Zvelebil, 1998] and the Demic Diffusion model (DDM) [Ammerman & Cavalli-Sforza, 1984; Feldman & Cavalli-Sforza, 1976; Itan *et al.*, 2009]. The CDM proposes that agriculture and related technologies arrived in Europe without a significant movement of farmers. It predicts that there should be no or very little genetic contribution in Europe from the Near-Eastern populations. In the DDM the spread of Neolithic innovations was a consequence of the movement of people that either eliminated or integrated the less densely populated hunter-gatherer (HG) societies [Ammerman & Cavalli-Sforza, 1984]. A movement of genes is thus predicted, even though its genetic consequences are much more complex than is usually acknowledged (e.g. [Chikhi *et al.*, 2002; Currat & Excoffier, 2005]). In the last fifteen years, the CDM has gained momentum, mainly from the support of mitochondrial DNA (mtDNA) analyses [Richards *et al.*, 2000, 2002], despite some criticisms that suggest that the mitochondrial data actually support the DDM (e.g. [Barbujani *et al.*, 1998; Goldstein & Chikhi, 2002]). NRY (non-recombining region of the Y-chromosome) data were also interpreted in favour of the CDM by some authors [Semino *et al.*, 2000], but other studies have generated opposite conclusions [Balaresque *et al.*, 2010; Belle

*et al.*, 2006] results of both the mtDNA and NRY are thus controversial. However, if we assume that mtDNA and NRY data could indeed be interpreted in different ways, one favouring CDM and the other the DDM respectively, this would open the possibility that the demographic histories of females and males were different (e.g. [Bentley *et al.*, 2003; Cavalli-Sforza & Minch, 1997]). For instance, differences in migration patterns after marriage could lead to major differences in terms of genetic diversity within and between populations when comparing mtDNA and NRY data. This is why it is very important to analyse jointly these two markers, rather than independently as is too often done to identify possible causes for the differences obtained beyond stochasticity (see chapter 3).

Archaeological and anthropological data suggest that the transition from a hunter-gatherer to a farmer society is correlated with drastic changes in lifestyle [Wilkins & Marlowe, 2006] and probably also with changes in postmarital residence systems. Moreover, the majority of today's human populations (ca. 74%) are patrilocal (i.e. the woman moves to her husband's birthplace after marriage) [Baker & Jacobsen, 2006; Langergraber *et al.*, 2007], but HG societies appear to be more variable, with bilocality (both males and females can move after marriage, with no clear bias towards one of the sexes) and matrilocality (higher male migration rates) practices being more frequent than in other societies (i.e. farmers and pastoralists) [Marlowe, 2004]. This observation has led to the suggestion that patrilocality started to increase after the emergence of agriculture [Fortunato, 2011; Marlowe, 2004; Wilkins, 2006; Wilkins & Marlowe, 2006].

However, there has been no formal test assessing whether there was such a shift during the Neolithic transition in Europe, using genetic data. Our aim is to study the impact of different postmarital systems on genetic diversity and to investigate if genetic data can give us any indication on whether such an increase in patrilocality indeed occurred after the Neolithic transition. Here, we use realistic spatial forward simulations of individuals and record their NRY and mtDNA data to explore a wide spectrum of scenarios where HG and Farmer populations are allowed to be either patrilocal, bilocal or matrilocal. These postmarital rules are modelled by varying the male and female migration rates.

## 4.3 Material and Methods

### 4.3.1 General Framework

To address questions related with the changes in postmarital rules during the European Neolithic Transition, we developed a new forward spatial simulation approach that incorporates both geographical and demographic data, as well as several types of genetic markers. The general principle is very similar to that followed by the SPLATCHE and SPLATCHE2 software [Currat *et al.*, 2004; Ray *et al.*, 2010]. Like in those software, space is divided into "layers", which are themselves subdivided into demes, as in a two-dimensional stepping-stone model. Our framework allows to simulate different "layers" (such as HG and Farmers), inhabiting the same geographical space as in Currat and Excoffier [2004]. Each deme can exchange migrants, at a certain rate ($m$), with up to four neighbours depending on its geographical location relative to the edges. Each deme is characterized by a carrying capacity ($K$) and a friction ($F$) values (Fig. 4.1) which can be different between layers. Density is logistically regulated within each deme (either in the HG or Farmers layers), with intrinsic $K$ and growth rate ($r$). Mating between layers (HG and Farmers) is modelled with an admixture parameter ($\gamma$). See appendices C.1.1 and C.1.2, for details on these parameters.

However, contrary to SPLATCHE and SPLATCHE2, our approach is not based on the coalescent. It uses a forward individual- rather than backward/coalescent gene-based simulation framework, where the demographic and genetic simulations are carried simultaneously. While computationally slower, it also has several advantages. We can: (i) model complex situations that occur in human societies (e.g variation in male and female migration rates) more easily, (ii) follow multilocus genotypes within individuals and (iii) simulate all the individuals of a deme. This last point is particularly important to study the Neolithic Transition, as one of the assumptions of the coalescent is that the effective population size is large compared to the sample size. This is unlikely to have been the case in founder HG and Farmers demes, particularly if there was high variance in reproductive success (i.e. multiple coalescent events, not allowed by standard coalescent theory, but that are incorporated in

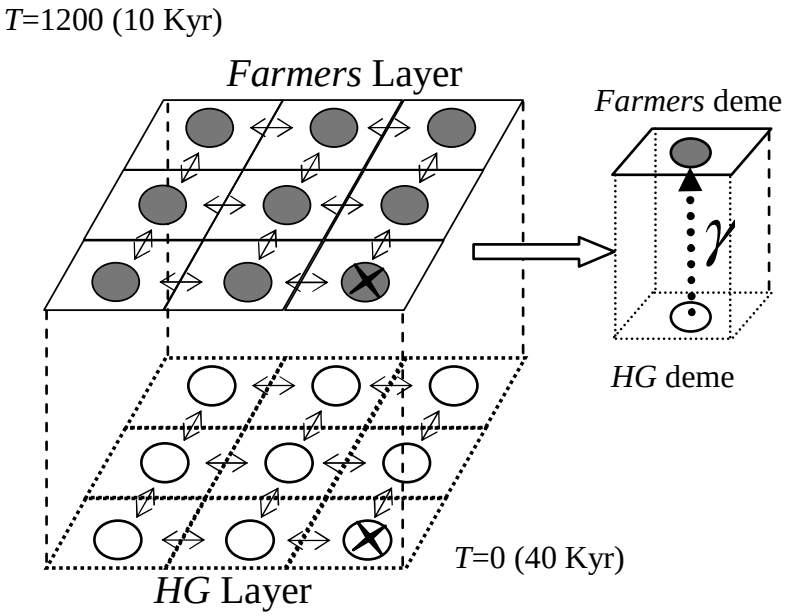



**Figure 4.1: Model of spatial expansion** - Two different layers (Farmers and HG) occupying the same geographical space. Demes are numbered using rows and columns, with deme 0_0 being the upper-left corner deme. The cross, in the bottom-right corner deme (deme 9_9 in the 10×10 lattice or deme 29_29 in the 30×30 lattice), indicates where the expansion starts at time *T*. Admixture ($\gamma$) represents gene flow between layers. In our simulations, admixture was unidirectional from the HG to the Farmers layer.

SPLATCHE2, which uses a generation by generation algorithm).

The fact that our approach aims at simulating in a realistic manner the movement of individuals, rather than that of genes leads to several other differences: (i) foundation events must involve at least one male and one female; (ii) the Maynard-Smith and Slatkin Maynard-Smith & Slatkin [1973] logistic growth formula is used and corrected (formula 4.1) to account for the fact that growth is limited by the number of reproductive females,

$$N_{t+1} = 2N_{f,t}\frac{1+r}{1+r\frac{2N_{f,t}}{K}} \tag{4.1}$$

where $N_{f,t}$ is the number of females at generation *t*; (iii) growth is not deterministic, as the number of individuals in generation *t+1* is drawn from a Poisson distribution, with mean $N_{t+1}$, as given by equation (4.1); and (iv) the number of migrants in the different directions is also stochastically drawn from binomial distributions; (v) sex-biased migration can be simulated using a sex ratio migration parameter given by $mSR = m_f/(m_f + m_m)$, where $m_f$ and $m_m$ are the female and male migration rates, respectively. This parameter is applied after the number of migrants in each direction is calculated. Details related with the growth formula, migration (including *mSR* parameter) and algorithm are in appendices C.1.3, C.1.4 and C.2, respectively.

### 4.3.2 Neolithic transition model

To study the properties of spatial expansions during the Neolithic transition we simulated NRY and mtDNA data assuming a regular lattice. We assumed that (i) there were two different layers, each corresponding to the HG and Farmer layers, (ii) the first wave of expansion by HG started 40 kyr ago (1600 generations ago, assuming a generation time of 25 years [Currat & Excoffier, 2005]), corresponding to $T$ = 0), (ii) the second wave started 10 kyr ago ($T$ = 1200 generations) to represent the spread of the Farmers [Currat & Excoffier, 2005].

Due to the computational cost of the simulations, our scenarios (see below) were tested with regular lattices of 100 demes (i.e. 10 by 10) per layer. The most likely scenarios were then also tested in 30 by 30 lattices (900 demes per layer). For all simulations, both the HG and Farmer expansions started at the bottom-right corner deme (Fig. 4.1). While, a Lotka-Volterra [Lotka, 1932; Volterra, 1931] competition model could be incorporated in our logistic growth formula to eliminate the HG populations, we decided to model the HG extinction by increasing the friction to 1 and reducing $K$ in the HG layer at a time related to the size of the lattice used in the simulations. Once again, this was due to the computational cost of the simulations.

Thus, the HG populations were led to extinction at $T$ = 1300 (7.5 kyr ago) in the 10×10 lattice and $T$ = 1400 (5 kyr ago) in the 30×30 lattice simulations. These values were chosen to reflect the fact that it takes more time for the Farmers to occupy the available space in the 30×30 lattice simulations. As a consequence, cohabitation between HG and Farmers could be variable in space for a particular simulation and between the 10×10 and 30×30 sets of simulations.

### 4.3.3 Variable parameters: sex-biased migration and admixture

We were interested in determining the genetic consequences of sex-related migration patterns, in both HG and Farmer societies. All combinations of bilocal, patrilocal or matrilocal societies were simulated corresponding to a total of nine scenarios (table C.1), using the *mSR* parameter. When $mSR = 0.5$, males and females have the same probability to migrate (bilocality). For $mSR > 0.5$, males migrate at a lower rate (patrilocality), whereas the opposite is true when $mSR < 0.5$ (matrilocality). The following values of *mSR* (0.25, 0.5, and 0.75) were used to test matrilocality, bilocality and patrilocality, respectively. Thus, all individuals of a deme will conform to the postmarital residence system of the layer.

In all simulations, we assumed unidirectional admixture, from the HG to the Farmers layer. We used the same framework as in [Currat & Excoffier, 2005], in agreement with anthropological data suggesting that asymmetrical gene flow occurs when a dominant group invades a new region, like it is supposed to have happened during the Bantu expansion [Quintana-Murci *et al.*, 2008] or in the colonization of Brazil by Europeans [Salzano, 2004]. Five values of $\gamma$ (0, 0.25, 0.5, 0.75 and 1) were used for each of the nine scenarios above, for a total of 45 different simulation sets carried out in the 10×10 lattices. For each set, 500 independent replicates were run. The results of the above simulations strongly suggested that the most probable scenarios were those with Farmers patrilocality. We repeated these scenarios using a 30×30 lattice (i.e. on a wider geographical region), using four values for $\gamma$ (0, 0.25, 0.5 and 0.75), to determine whether significantly different results would be observed with a larger lattice corresponding to a larger area. No major differences

were observed in the genetic diversity within demes, in agreement with Hamilton *et al.* [Hamilton *et al.*, 2005], who also compared lattices with different sizes in their spatial simulations (30×30 and 50×50).

### 4.3.4 Fixed Parameters

To reduce the number of simulations required we fixed the values of $K$, $r$ and $m$ to those from Currat and Excoffier [2005], who calibrated and tested them to simulate the effect of the Neolithic expansion in Europe. We performed some additional tests using different values and found that the values in [Currat & Excoffier, 2005] usually produced $H_e$ and $F_{ST}$ values that were close to the observed data (not shown). The carrying capacity of hunter-gatherers ($H_e$) was set to 40, corresponding to a density of 0.064 individuals per $Km^2$ (see [Currat & Excoffier, 2005]). $K_F$ was set to be 20 times larger than $K_{HG}$ ($K_F = 800$). Both values correspond to 625 $Km^2$ demes. The growth rates used were $r_{HG} = 0.4$ and $r_F = 0.8$ and the migration rate was $m = 0.25$, as in [Currat & Excoffier, 2005]. All the simulations were done under a random mating framework, using a male to female ratio of 1:1. The NRY and mtDNA allele frequencies in the starting deme, both for HG and Farmers, were sampled from the same allelic distributions, using present-day Near-Eastern populations for the mtDNA [Richards *et al.*, 2000] and NRY [Rosser *et al.*, 2000] data. This was done to limit the number of simulations required to reproduce similar levels of genetic diversity in simulated and real data.

### 4.3.5 Summary statistics

Since we were interested in migration patterns of males and females, the comparison of the different scenarios to real data was evaluated by measures of genetic diversity and differentiation. Thus, we computed the mean expected heterozygosity ($H_e$) and mean $F_{ST}$ [Nei, 1977] for NRY and mtDNA simulated data, across generations and only for demes along the diagonal. In the 10×10 lattices, we sampled the ten diagonal demes, with deme 9_9 (bottom-right corner) being the starting deme and deme 0_0 (upper-left corner) the last colonized (Fig. 4.1). In the pairwise $F_{ST}$

analyses, we compared all the demes in the diagonal against the starting deme (9_9). This allowed us to study the trajectory of these statistics through time and space (in the expansion axis), for the two types of markers jointly.
To compare our simulations results with real data, we computed a regression for $H_e$ and $F_{ST}$ values, using published NRY [Rosser *et al.*, 2000] and mtDNA [Richards *et al.*, 2000] data from present-day European and Near-Eastern populations. We used these datasets because they are two large-scale studies that address the Neolithic transition in Europe, despite being around ten years old, represent some of the best data available. Indeed, more recent studies tend to focus on specific haplogroups which can be different across studies, and thus cannot be used here. For the real data, the pairwise $F_{ST}$ values were obtained comparing European against Near-Eastern populations.

## 4.4 Results

As expected, our simulations show that both $H_e$ and $F_{ST}$ values differ for Y-linked and mtDNA markers when migration patterns differ in males and females. However, our results also demonstrate complex patterns that would have been difficult to predict without simulations. We will concentrate on the patterns observed for the Farmers, since they correspond to the modern populations.

### 4.4.1 General results across all scenarios

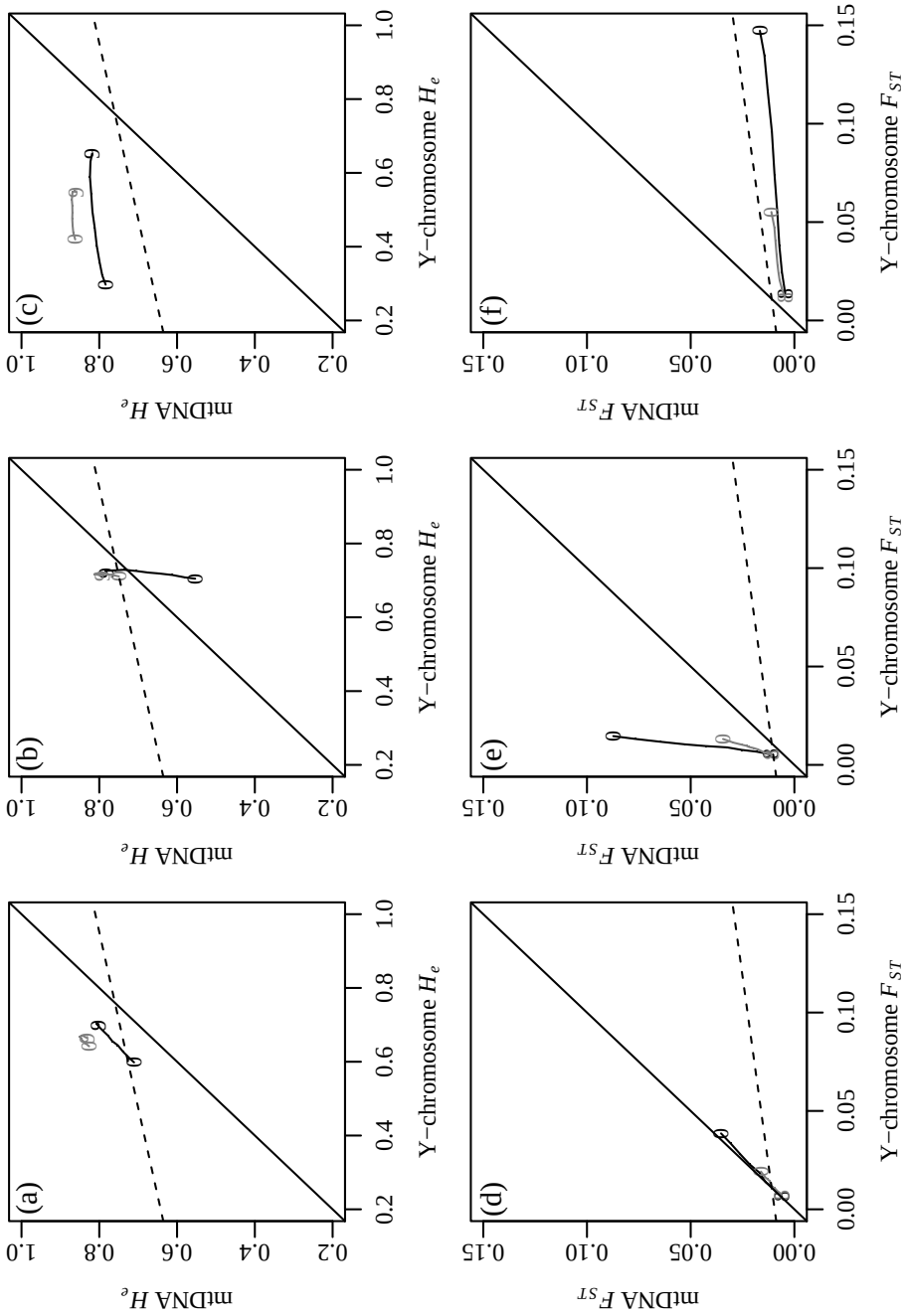
Looking at all samples obtained at a particular generation, we found that as time goes from generation 1300 to 1600 the set of $H_e$ values becomes more concentrated in one region (Fig. 4.2a-c; see also Fig. C.1). In other words, we found fewer differences on levels of genetic diversity across modern populations (generation 1600) compared to ancient populations (generation 1300).
At the genetic differentiation level, the $F_{ST}$ values against the starting deme (9_9) increase with distance from it, as expected. This increase can be significantly greater for the NRY compared to mtDNA data (as in Fig. 4.2f), or the opposite (as in Fig. 4.2e) depending on the scenarios (see below), but the $F_{ST}$ values increase

with distance from the starting deme. As generations go, $F_{ST}$ values between the starting and last deme decrease with time (Fig. 4.2d-f), in agreement with the fact that $H_e$ values are increasingly similar among samples. Thus, in these simulations modern populations are genetically less differentiated than ancient ones.

### 4.4.2 No admixture scenarios

In the scenarios without admixture (Fig. 4.2), the $H_e$ values decrease along the axis of the expansion, with the highest values being observed in the starting deme (9_9) and the lowest in the last colonized deme (0_0). This is observed for all generations sampled. Moreover, these points typically move, as a group and across generations, from higher to lower NRY diversity and from lower to higher mtDNA diversity. In other words, present-day populations have more mtDNA diversity and slightly less NRY diversity than ancient populations, whichever the postmarital residence pattern. Note that this is true when the set of samples from the diagonal are analysed as a group but not necessarily for each sample individually, due to the fact that the points are also more compact, as we noted above. For instance, the starting deme (9_9) loses NRY diversity (Fig. 4.2) whereas the last colonized deme actually sees its NRY diversity increase. Another very striking result was that the three scenarios (bi-, matri- and patrilocality) exhibit clearly differentiated patterns

**Figure 4.2 *(facing page)*: Genetic diversity and differentiation in modern populations, under no admixture** - Panels (a) to (c) represent average $H_e$ values whereas panels (d-f) represent average $F_{ST}$ values. Only the Farmer's populations were sampled since they represent modern populations. The different columns correspond to scenarios where Farmers were bilocal (a and d), matrilocal (b and e), and patrilocal (c and f), respectively. $H_e$ and $F_{ST}$ values were computed for the demes located in the diagonal of the $10\times10$ lattice. A line was drawn going through all demes between the plotted numbers (9 and 0) that represent the coordinates of deme 9_9 (the first deme to be colonized: bottom-right corner) and 0_0 (the last: upper-left corner). Each colour represents a time step (black and grey are generations 1300 and 1600, respectively), for which the summary statistics were calculated ($T = 1600$ is the present-day generation). The solid line represents cases where NRY and mtDNA values are equal. The dashed line is the regression obtained with the real observed data.


(a)
(b)
(c)
(d)
(e)
(f)
mtDNA $H_e$
Y−chromosome $H_e$
mtDNA $F_{ST}$
Y−chromosome $F_{ST}$
0.2
0.4
0.6
0.8
1.0
0.00
0.05
0.10
0.15

(Fig. 4.2). This can be seen in the way the points are arranged in “parallel lines” through time.

In bilocality scenarios, with no admixture, the points are arranged in a direction parallel to the dashed line corresponding to equal values for the x and y axes (i.e. for mtDNA and NRY data). Interestingly, we observed that, despite the bilocality $H_e$ values were higher for mtDNA than for NRY data (Fig. 4.2a), whereas $F_{ST}$ values were quite similar (Fig. 4.2d). The difference in $H_e$ values is probably due to differences in the mutation rates, higher in mtDNA (see appendix C.3). Indeed, when we repeated these simulations by assuming the same mutation rate for the two markers, we found symmetrical results (not shown).

In the matrilocal scenarios, all demes had similar NRY $H_e$, hence generating values forming “lines” parallel to the y-axis (Fig. 4.2b). As expected, the mtDNA $F_{ST}$ values were higher than the NRY $F_{ST}$ values (Fig. 4.2e), that were themselves very similar between demes (i.e. the gene flow between demes was high), generating “lines” near-parallel to the y-axis. On the contrary, in scenarios with Farmer patrilocality (and still no admixture), a similar behaviour is seen but inverted for the two markers (i.e. higher NRY $F_{ST}$), and with almost no variation along the y-axis (Fig. 4.2f). Similarly, the $H_e$ values also show this behaviour (Fig. 4.2c). A particularly interesting result was that this trend was parallel to the regression obtained from the real data from modern populations (solid line in Fig. 4.2). This was true for both $F_{ST}$ and $H_e$ values and was not observed in the other scenarios (matrilocality and bilocality).

### 4.4.3 Influence of HG postmarital behaviour on the Farmers genetic diversity

In the scenarios with admixture between HG and Farmers, some significant changes are found on the level of genetic diversity (Fig. 4.3; see also Fig. C.1 and C.2): First, compared to the no admixture scenarios, the sets of points are shifted towards lower NRY diversity when $\gamma$ increases, whereas mtDNA diversity does not change very much or shows a slight increase. Thus, in our simulations, admixture leads to

a decrease in NRY diversity in all cases compared to the no admixture scenarios. Second, scenarios with patrilocality in HG populations generated fewer changes relative to the no admixture scenarios in NRY diversity compared to bilocality and matrilocality. This is true whether the Farmers were patrilocal, matrilocal or bilocal and can be seen in Fig. 4.3 where the points with a P (HG patrilocality) are closer to the points of the no admixture scenarios (Fig. 4.3a, 4.3d and 4.3g) compared to the points with a B (HG bilocality) and even more with an M (HG matrilocality). In other words, in situations where HG could mate with Farmers, the postmarital behaviour of HG populations clearly leads to differences in the distribution of Farmers genetic diversity, in modern populations that have the same postmarital residence system. Third, when Farmers are patrilocal, a higher admixture rate (Fig. 4.3i) would tend to blur this effect and make $H_e$ pattern almost indistinguishable, whichever postmarital behaviour the HG may have had. However, the simulations that seem to better fit the trend of the observed data are the ones from patrilocality in Farmers, whatever the HG's postmarital behaviour is and whatever the admixture rate is.

**Figure 4.3** ***(facing page)*****: Genetic diversity in present-day Farmers, under admixture** - $H_e$ values that were computed for the demes located in the diagonal of the $10\times10$ lattice, between the starting deme (9_9) and the last to be colonized (0_0) represented by 9 and 0, respectively. To make the panels easier to read a line was drawn going through all demes between these two points, but the other demes identifications are not represented. Each column corresponds to one value of the admixture parameter $\gamma$ (0, 0.5 and 1) and each row corresponds to one postmarital residence system for the Farmers layer (bilocality, matrilocality and patrilocality). Within each panel the three possible scenarios for the postmarital residence system of the HG are represented. Each colour and letter represent a different residence pattern in the HG layer (colours blue, green and black and letters B, M and P correspond to scenarios where HG are bilocal, matrilocal and patrilocal, respectively). Cases where NRY and mtDNA have the same $H_e$ values would fall on the solid line. The dashed line is the regression obtained for the real (i.e. observed) data.

γ = 0
γ = 0.5
γ = 1
Bilocality
Matrilocality
Patrilocality
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
mtDNA $H_e$
Y−chromosome $H_e$
0.5
0.7
0.9
0.2
0.4
0.6
0.8
1.0
M
B
P

### 4.4.4 Influence of HG postmarital behaviour on the Farmers genetic differentiation

Interestingly, in a 10×10 lattice, the modern samples $F_{ST}$ values seem less affected by the postmarital residence system of the HG, than the corresponding $H_e$ values (Fig. C.3). In particular, the analyses of only the last generation data show that the $F_{ST}$ values were nearly identical across all HG scenarios with and without admixture. Conversely, in the generations that follow the admixture events there were clear differences between the no admixture and admixture scenarios (Fig. C.4). However, in the scenarios analysed using a larger lattice (30×30) was possible to separate postmarital residence system of the HG, on the basis of $F_{ST}$ values (Fig. C.5).

## 4.5 Discussion

Altogether, our simulations allowed us to study the effect of i) variable migration rates in males and females within the HG and Farmers layers and ii) variable admixture between layers, on the patterns of genetic diversity and differentiation in present-day populations.

### 4.5.1 Main results: (i) first farmers were patrilocal and (ii) different postmarital residence systems have a different impact on human genetic patterns

Patrilocality was the most probable scenario among Farmers. It was particularly obvious in the no admixture scenarios, but was also found in the scenarios with admixture. This result agrees with ancient DNA (aDNA) and Strontium isotope analyses that suggested patrilocality in Linear [Bentley *et al.*, 2002] and Corded [Haak *et al.*, 2008] Ware Culture burials from Germany. Cultural phylogenetics studies also suggest that patrilocality started to increase after the advent of agriculture [Fortunato, 2011; Fortunato & Jordan, 2010].
Changes in postmarital residence systems were also found to lead to different ge-

netic patterns in present-day populations. In particular, the Farmers' $H_e$ values changed significantly depending on whether the HG were patrilocal, bilocal or matrilocal. Thus, it appears that even though HG populations disappear as far back as 5000 years before the present in our simulations, they influence present-day patterns in modern-day populations.

### 4.5.2 Behaviour of summary statistics

Pairwise $F_{ST}$ statistics were much less influenced than $H_e$ values by the postmarital residence pattern of the HG populations. While $F_{ST}$ values were different across scenarios after the start of admixture, this signal disappeared in the modern samples. However, when a larger lattice ($30\times30$) is analysed (corresponding to a larger geographic area) it was possible to distinguish between the postmarital residence systems of the HG populations. This is compatible with the notion that the degree of genetic differentiation between two demes depends of their geographical distance, on the migration rate between local demes and on the time since the populations started expanding. If migration rates are large and/or necessary time has passed, then it may be necessary to use large lattices to avoid this homogenizing effect in $F_{ST}$ values. This $F_{ST}$ statistics' dependence on geographical distances implies that inferences based on local/regional sampling is valid only for the most recent history, while sampling from more distant places may be able to recover older patterns, a point that has been stressed by Wilkins and Marlowe [2006].
Furthermore, the access to the genetic composition of ancient HG populations may be not only useful but necessary to provide us with significant information on this issue. In other words, aDNA may be required to allow us to determine the postmarital behaviour of HG populations in Europe before and after the Neolithic transition. Currently, the number of aDNA studies about the Neolithic transition are slowly increasing [Bramanti *et al.*, 2009; Haak *et al.*, 2005, 2010; Malmström *et al.*, 2009] but are unfortunately limited to the mtDNA. Our results suggest that obtaining NRY DNA from the same samples would be particularly important, as was done in a recent study [Lacan *et al.*, 2011].

To identify the most probable scenario we focused on the trend observed in the statistics of both simulated and real data. Our approach was thus to some extent qualitative. To obtain a better fit, one would also need to consider the spread of populations in both the simulated and real data (not just the regression slope). In theory, simulating different scenarios should allow us to better tune migration rates, and identify the original level of diversity in both HG and Farmers populations, that are compatible with the observed modern-day data.

### 4.5.3 Mutation rates can generate asymmetries between mtDNA and NRY data

Although mtDNA and NRY data are often presented as symmetrical counter parts of the female and male demography, respectively, this is not necessarily that simple. The difference in mutation rates can generate an asymmetry between mtDNA and NRY $H_e$ values in bilocal scenarios with no sex-related variance in reproductive success (Fig. 4.2a and 4.3a). This is something to keep in mind when analysing differences observed in real mtDNA and NRY data because such differences are often interpreted in terms of differences in male and female behaviours [Seielstad *et al.*, 1998; Wilder *et al.*, 2004; Wilkins, 2006].

### 4.5.4 Admixture decreases Farmers NRY genetic diversity

We found this surprising at first, as it is usually assumed that regions where populations admix will exhibit higher levels of genetic diversity. However, the underlying assumption is that admixing populations have similar $N_e$. Several studies have shown that during spatial expansions the expanding population is diluted [Chikhi *et al.*, 2002; Edmonds *et al.*, 2004; Klopfstein *et al.*, 2006]. We thus believe that as admixture took place between populations with different sizes (i.e. HG having much smaller populations than the Farmers), the incoming population will dilute the Farmers genetic diversity and led to the decrease in NRY genetic diversity. This can be seen in the simulations where the decrease is observed even when both HG and Farmers had the same pattern of postmarital rules. However, this is not

necessarily a general result as mtDNA genetic diversity did not always decrease. Again, the difference in mutation rates between mtDNA and NRY markers may interact in a complex way with demographic parameters leading to asymmetries in present-day data.

### 4.5.5 Comparison with other sex-biased migration studies

Until now, the inference of patterns of sex-biased migration have relied mainly on the comparison and estimation of dispersal from pairwise NRY and mtDNA $F_{ST}$ values [47][48] and by cultural phylogenetics [Fortunato, 2011; Fortunato & Jordan, 2010; Jordan *et al.*, 2009] in modern populations.

Hamilton *et al.* [2005] also used spatial framework, using NRY and mtDNA data, to study matrilocal and patrilocal groups from northern Thailand. In their study the authors applied a modified version of SPLATCHE and were not interested in detecting shifts in postmarital residence patterns, which were assumed to be invariant in their simulations. Instead, their aim was to compare male and female migration rates in known patrilocal and matrilocal societies that would explain present-day levels of genetic differentiation and diversity. $H_e$re our aim was to understand how postmarital residence interacts with admixture between different societies, to generate differences in maternally or paternally-inherited markers analysed jointly.

Model-based approaches have many advantages as they allow us to identify parameters that have a significant impact on the data. However they also rely on strong assumptions. In our study, it was necessary to make assumptions on the level and patterns of gene flow, carrying capacities and genetic make-up of the founder populations, which suggests that some of the conclusions presented here should not be taken at face value. Hitherto, we believe that the general trends identified are to some extent robust. For instance, our simulations were performed on a $10\times10$ lattice. But when we repeated the patrilocal scenarios on a $30\times30$ lattice we found essentially the same results, the main difference being that the power to identify scenarios was increased in the $30\times30$ lattice.

The simulated framework introduced here owes much to the work of Currat *et al.*

[2005], but is sufficiently different to represent an interesting alternative to identify the critical assumptions that are robust and those that are not, and the type of data required to separate scenarios. Altogether, our simulations helped identify important parameters and scenarios, together with data that would be needed to study the Neolithic transition in Europe (NRY aDNA), but much work is still necessary.

## 4.6 Conclusion

There are still very few studies that have dealt with the kind of complex scenarios that involve the characterization of the expansion of two demographically different populations across the same geographical area when migration patterns and admixture levels vary, and those that exist do not deal with sex-biased migration [Currat & Excoffier, 2005][Currat & Excoffier, 2004]. Our work provides some of the first insights into the consequences of complex demographic changes that probably took place during the European Neolithic, on present-day human genetic patterns.

**Acknowledgements**

Part of this work was performed using HPC resources from CALMIP, Toulouse (Grant 2010-P1038). We are grateful to B. Parreira for the stimulating comments and discussions. We would also like to thank C. Gamba and I. Alves and anonymous reviewers for reading and constructively commenting a previous version of the manuscript. RR and VCS were supported by FCT grants (ref. SFRH/BD/30821/2006 and SFRH/BD/22224/2005, respectively). PAB was supported by funding from the IGC. LC was funded by the FCT projects PTDC/BIA-BDE/71299/2006 and PTDC/BIA-BEC/100176/2008 and grant no. CD-AOOI-07-003 from the Institut Français de la Biodiversité, Programme Biodiversité des îles de l'Océan Indien. This work was also partly funded by the "Laboratoire d'Excellence (LABEX)entitled TULIP (ANR-10-LABX-41)".

## 4.7 References

AMMERMAN, A.J. & CAVALLI-SFORZA, L.L. (1984). *The Neolithic transition and the genetics of populations in Europe*. Princeton University Press, Princeton.

BAKER, M. & JACOBSEN, J. (2006). A human capital-based theory of postmarital residence rules. *J Law Econ Organ*, **23**, 208–241.

BALARESQUE, P., BOWDEN, G., ADAMS, S., LEUNG, H., KING, T., ROSSER, Z., GOODWIN, J., MOISAN, J., RICHARD, C., MILLWARD, A. *et al.* (2010). A predominantly Neolithic origin for European paternal lineages. *PLoS biology*, **8**, e1000285.

BARBUJANI, G., BERTORELLE, G., CAPITANI, G. & SCOZZARI, R. (1995). Geographical structuring in the mtDNA of Italians. *Proc Natl Acad Sci U S A*, **92**, 9171–9175.

BARBUJANI, G., BERTORELLE, G. & CHIKHI, L. (1998). Evidence for Paleolithic and Neolithic gene flow in Europe. *Am J Hum Genet*, **62**, 488–492.

BELLE, E.M.S., LANDRY, P.A. & BARBUJANI, G. (2006). Origins and evolution of the Europeans' genome: evidence from multiple microsatellite loci. *Proc R Soc B*, **273**, 1595–1602.

BELLWOOD, P. (2004). *First Farmers: the origins of agricultural societies*. Blackwell Publishing, Oxford.

BENTLEY, R.A., PRICE, T.D., LÜNING, J., GRONENBORN, D., WAHL, J. & FULLAGAR, P.D. (2002). Human migration in early Neolithic Europe. *Curr Anthropol*, **43**, 799–804.

BENTLEY, R.A., CHIKHI, L. & PRICE, T.D. (2003). The Neolithic transition in Europe: comparing broad scale genetic and local scale isotopic evidence. *Antiquity*, **77**, 63–66.

BRAMANTI, B., THOMAS, M.G., HAAK, W., UNTERLAENDER, M., JORES, P., TAMBETS, K., ANTANAITIS-JACOBS, I., HAIDLE, M.N., JANKAUSKAS, R., KIND, C.J., LUETH, F., TERBERGER, T., HILLER, J., MATSUMURA, S., FORSTER, P. & BURGER, J. (2009). Genetic discontinuity between local hunter-gatherers and central Europe's first farmers. *Science*, **326**, 137–140.

CAVALLI-SFORZA, L.L. & MINCH, E. (1997). Paleolithic and Neolithic lineages in the European mitochondrial gene pool. *Am J Hum Genet*, **61**, 247–254.

CHIKHI, L. (2009). Update to Chikhi et al.'s "Clinal variation in the nuclear DNA of europeans" (1998): genetic data and storytelling–from archaeogenetics to astrologenetics? *Hum Biol*, **81**, 639–643.

CHIKHI, L., NICHOLS, R.A., BARBUJANI, G. & BEAUMONT, M.A. (2002). Y genetic data support the Neolithic demic diffusion model. *Proc Natl Acad Sci U S A*, **99**, 11008–11013.

CURRAT, M. & EXCOFFIER, L. (2004). Modern humans did not admix with Neanderthals during their range expansion into Europe. *PLoS Biol*, **2**, e421.

CURRAT, M. & EXCOFFIER, L. (2005). The effect of the Neolithic expansion on European molecular diversity. *Proc R Soc B*, **272**, 679–688.

CURRAT, M., RAY, N. & EXCOFIER, L. (2004). SPLATCHE: a program to simulate genetic diversity taking into account environmental heterogeneity. *Mol Ecol Notes*, **4**, 139–142.

DAVIS, S.J.M. (2005). Why domesticate food animals? Some zoo-archaeological evidence

from the Levant. *J Archaeol Sci*, **32**, 1408–1416.

DIAMOND, J. & BELLWOOD, P. (2003). Farmers and their languages: the first expansions. *Science*, **300**, 597–603.

EDMONDS, C.A., LILLIE, A.S. & CAVALLI-SFORZA, L.L. (2004). Mutations arising in the wave front of an expanding population. *Proc Natl Acad Sci*, **101**, 975–9.

FELDMAN, M.W. & CAVALLI-SFORZA, L.L. (1976). Cultural and biological evolutionary processes, selection for a trait under complex transmission. *Theor Popul Biol*, **9**, 238–259.

FORTUNATO, L. (2011). Reconstructing the history of residence strategies in Indo-European-speaking societies: neo-, uxori-, and virilocality. *Hum Biol*, **83**, 107–28.

FORTUNATO, L. & JORDAN, F. (2010). Your place or mine? a phylogenetic comparative analysis of marital residence in Indo-European and Austronesian societies. *Philos Trans R Soc B*, **365**, 3913–22.

GKIASTA, M., RUSSELL, T., SHENNAN, S. & STEELE, J. (2003). Neolithic transition in Europe: the radiocarbon revisited. *Antiquity*, **77**, 45–62.

GOLDSTEIN, D.B. & CHIKHI, L. (2002). Human migrations and population structure: what we know and why it matters. *Annu Rev Genomics Hum Genet*, **3**, 129–152.

HAAK, W., FORSTER, P., BRAMANTI, B., MATSUMURA, S., BRANDT, G., TÄNZER, M., VILLEMS, R., RENFREW, C., GRONENBORN, D., ALT, K.W. & BURGER, J. (2005). Ancient DNA from the first european farmers in 7500-year-old neolithic sites. *Science*, **310**, 1016–1018.

HAAK, W., BRANDT, G., DE JONG, H.N., MEYER, C., GANSLMEIER, R., HEYD, V., HAWKESWORTH, C., PIKE, A.W.G., MELLER, H. & ALT, K.W. (2008). Ancient DNA, strontium isotopes, and osteological analyses shed light on social and kinship organization of the later stone age. *Proc Natl Acad Sci U S A*, **105**, 18226–31.

HAAK, W., BALANOVSKY, O., SANCHEZ, J.J., KOSHEL, S., ZAPOROZHCHENKO, V., ADLER, C.J., DER SARKISSIAN, C.S.I., BRANDT, G., SCHWARZ, C., NICKLISCH, N., DRESELY, V., FRITSCH, B., BALANOVSKA, E., VILLEMS, R., MELLER, H., ALT, K.W. & AND, A.C. (2010). Ancient DNA from european early neolithic farmers reveals their Near Eastern affinities. *PLoS Biol*, **8**, e1000536.

HAMILTON, G., STONEKING, M. & EXCOFFIER, L. (2005). Molecular analysis reveals tighter social regulation of immigration in patrilocal populations than in matrilocal populations. *Proc Natl Acad Sci U S A*, **102**, 7476–80.

ITAN, Y., POWELL, A., BEAUMONT, M.A., BURGER, J. & THOMAS, M.G. (2009). The origins of lactase persistence in Europe. *PLoS Comput Biol*, **5**, e1000491.

JORDAN, F.M., GRAY, R.D., GREENHILL, S.J. & MACE, R. (2009). Matrilocal residence is

ancestral in Austronesian societies. *Proc R Soc B*, **276**, 1957–64.

KLOPFSTEIN, S., CURRAT, M. & EXCOFFIER, L. (2006). The fate of mutations surfing on the wave of a range expansion. *Mol Biol Evol*, **23**, 482–490.

LACAN, M., KEYSER, C., RICAUT, F.X., BRUCATO, N., DURANTHON, F., GUILAINE, J., CRUBÉZY, E. & LUDES, B. (2011). Ancient DNA reveals male diffusion through the Neolithic Mediterranean route. *PNAS*, **early edition**.

LANGERGRABER, K.E., SIEDEL, H., MITANI, J.C., WRANGHAM, R.W., REYNOLDS, V., HUNT, K. & VIGILANT, L. (2007). The genetic signature of sex-biased migration in patrilocal chimpanzees and humans. *PLoS One*, **2**, e973.

LOTKA, A.J. (1932). The growth of mixed populations : two species competing for a common food supply. *Journal of Washington Academy of Sciences*, **22**, 461–469.

MALMSTRÖM, H., GILBERT, M.T.P., THOMAS, M.G., BRANDSTRÖM, M., STORA, J., MOLNAR, P., ANDERSEN, P.K., BENDIXEN, C., HOLMLUND, G., GÖTHERSTRÖM, A. & WILLERSLEV, E. (2009). Ancient DNA reveals lack of continuity between neolithic hunter-gatherers and contemporary scandinavians. *Curr Biol*, **19**, 1758–62.

MARLOWE, F. (2004). Marital residence among foragers. *Curr Anthropol*, **45**, 277–283.

MAYNARD-SMITH, J. & SLATKIN, M. (1973). The stability of predator-prey systems. *Ecology*, **54**, 384–391.

MITHEN, S. (2007). Did farming arise from a misapplication of social intelligence? *Philos Trans R Soc Lond B Biol Sci*, **362**, 705–718.

NEI, M. (1977). F-statistics and analysis of gene diversity in subdivided populations. *Ann Hum Genet*, **41**, 225–233.

PINHASI, R., FORT, J. & AMMERMAN, A.J. (2005). Tracing the origin and spread of agriculture in Europe. *PLoS Biol*, **3**, e410.

QUINTANA-MURCI, L., QUACH, H., HARMANT, C., LUCA, F., MASSONNET, B., PATIN, E., SICA, L., MOUGUIAMA-DAOUDA, P., COMAS, D., TZUR, S., BALANOVSKY, O., KIDD, K.K., KIDD, J.R., VAN DER VEEN, L., HOMBERT, J.M., GESSAIN, A., VERDU, P., FROMENT, A., BAHUCHET, S., HEYER, E., DAUSSET, J., SALAS, A. & BEHAR, D.M. (2008). Maternal traces of deep common ancestry and asymmetric gene flow between Pygmy hunter-gatherers and Bantu-speaking farmers. *Proc Natl Acad Sci U S A*, **105**, 1596–601.

RAY, N., CURRAT, M., FOLL, M. & EXCOFFIER, L. (2010). SPLATCHE2: a spatially explicit simulation framework for complex demography, genetic admixture and recombination. *Bioinformatics*, **26**, 2993–2994.

RICHARDS, M., MACAULAY, V., HICKEY, E., VEGA, E., SYKES, B., GUIDA, V., RENGO,

C., SELLITTO, D., CRUCIANI, F., KIVISILD, T., VILLEMS, R., THOMAS, M., RYCHKOV, S., RYCHKOV, O., RYCHKOV, Y., GÖLGE, M., DIMITROV, D., HILL, E., BRADLEY, D., ROMANO, V., CALÌ, F., VONA, G., DEMAINE, A., PAPIHA, S., TRIANTAPHYLLIDIS, C., STEFANESCU, G., HATINA, J., BELLEDI, M., RIENZO, A.D., NOVELLETTO, A., OPPENHEIM, A., NØRBY, S., AL-ZAHERI, N., SANTACHIARA-BENERECETTI, S., SCOZARI, R., TORRONI, A. & BANDELT, H.J. (2000). Tracing european founder lineages in the Near Eastern mtDNA pool. *Am J Hum Genet*, **67**, 1251–1276.

RICHARDS, M., MACAULAY, V., TORRONI, A. & BANDELT, H.J. (2002). In search of geographical patterns in European mitochondrial DNA. *Am J Hum Genet*, **71**, 1168–1174.

ROSSER, Z.H., ZERJAL, T., HURLES, M.E., ADOJAAN, M., ALAVANTIC, D., AMORIM, A., AMOS, W., ARMENTEROS, M., ARROYO, E., BARBUJANI, G., BECKMAN, G., BECKMAN, L., BERTRANPETIT, J., BOSCH, E., BRADLEY, D.G., BREDE, G., COOPER, G., CÔRTE-REAL, H.B., DE KNIJFF, P., DECORTE, R., DUBROVA, Y.E., EVGRAFOV, O., GILISSEN, A., GLISIC, S., GÖLGE, M., HILL, E.W., JEZIOROWSKA, A., KALAYDJIEVA, L., KAYSER, M., KIVISILD, T., KRAVCHENKO, S.A., KRUMINA, A., KUCINSKAS, V., LAVINHA, J., LIVSHITS, L.A., MALASPINA, P., MARIA, S., MCELREAVEY, K., MEITINGER, T.A., MIKELSAAR, A.V., MITCHELL, R.J., NAFA, K., NICHOLSON, J., NØRBY, S., PANDYA, A., PARIK, J., PATSALIS, P.C., PEREIRA, L., PETERLIN, B., PIELBERG, G., PRATA, M.J., PREVIDERÉ, C., ROEWER, L., ROOTSI, S., RUBINSZTEIN, D.C., SAILLARD, J., SANTOS, F.R., STEFANESCU, G., SYKES, B.C., TOLUN, A., VILLEMS, R., TYLER-SMITH, C. & JOBLING, M.A. (2000). Y-chromosomal diversity in Europe is clinal and influenced primarily by geography, rather than by language. *Am J Hum Genet*, **67**, 1526–1543.

SALZANO, F.M. (2004). Interethnic variability and admixture in latin America–social implications. *Rev Biol Trop*, **52**, 405–15.

SEIELSTAD, M.T., MINCH, E. & CAVALLI-SFORZA, L.L. (1998). Genetic evidence for a higher female migration rate in humans. *Nat Genet*, **20**, 278–80.

SEMINO, O., PASSARINO, G., OEFNER, P.J., LIN, A.A., ARBUZOVA, S., BECKMAN, L.E., BENEDICTIS, G.D., FRANCALACCI, P., KOUVATSI, A., LIMBORSKA, S., MARCIKIAE, M., MIKA, A., MIKA, B., PRIMORAC, D., SANTACHIARA-BENERECETTI, A.S., CAVALLI-SFORZA, L.L. & UNDERHILL, P.A. (2000). The genetic legacy of Paleolithic Homo sapiens sapiens in extant Europeans: a Y chromosome perspective. *Science*, **290**, 1155–1159.

VOLTERRA, V. (1931). *Variations and fluctuations of the numbers of individuals in animal species living together*, 409–448. McGraw-Hill, New York.

WILDER, J.A., KINGAN, S.B., MOBASHER, Z., PILKINGTON, M.M. & HAMMER, M.F.

(2004). Global patterns of human mitochondrial DNA and Y-chromosome structure are not influenced by higher migration rates of females versus males. *Nat Genet*, **36**, 1122–5.

WILKINS, J.F. (2006). Unraveling male and female histories from human genetic data. *Curr Opin Genet Dev*, **16**, 611–7.

WILKINS, J.F. & MARLOWE, F.W. (2006). Sex-biased migration in humans: what should we expect from genetic data? *Bioessays*, **28**, 290–300.

ZVELEBIL, M. & ZVELEBIL, K. (1998). Agricultural transition and Indo-European dispersals. *Antiquity*, **62**, 574–583.

# 5. SINS: forward simulation of individuals through time and space

Rita Rasteiro[1], Pierre-Antoine Bouttier[1,†], Damien Monier[1], Vítor C. Sousa[1,‡] and Lounès Chikhi[1,2,3]

[1]Instituto Gulbenkian de Ciência, Rua da Quinta Grande, 6, 2780-156 Oeiras, Portugal; [2]CNRS, Laboratoire Évolution et Diversité Biologique (EDB), Bât. 4R3 b2, 118 Route de Narbonne, 31062 Toulouse cédex 9, France; [3]Université de Toulouse, UPS, EDB, Bât. 4R3 b2, 118 Route de Narbonne, 31062 Toulouse cédex 9, France; [†]New address: Université de Grenoble and CNRS, Laboratoire Jean Kutzmann, France; [‡]New address: Department of Genetics, Rutgers University, NJ, USA.

**Development of the simulation framework:** R Rasteiro, P-A Bouttier, D Monier, VC Sousa and L Chikhi

**Manuscript:** R. Rasteiro and L. Chikhi

## 5.1 Summary

SINS is a computer program that simulates genetic data under complex demographic scenarios using a spatial framework. Space is divided into layers, which are themselves subdivided into demes that harbour male and female individuals. Each deme is characterized by carrying capacity ($K$) and friction ($F$) values which define the maximum population size and the difficulty to move into that deme, re-

spectively. SINS allows the user to simulate (i) variable $K$ and $F$ maps across time and space, (ii) expansions from multiple sources, (iii) contractions and habitat fragmentation, (iv) admixture and competition between populations from two or more layers corresponding to the same geographical space, (v) variance in reproductive success in males and females and (vi) sex-biased migration. The program uses an individual-based approach to simulate forward in time several types of molecular markers (sequences, SNPs and microsatellites) and genetic objects (X and Y chromosomes, autosomes and mitochondrial DNA). The flexibility of SINS should allow its application to many species and evolutionary questions. A companion program, SINS-Stat, samples SINS genetic outputs and performs several population genetic statistics.

**Availability:** SINS and SINS-Stat are freely available at [TBA], together with a user guide (appendix D) and examples.

## 5.2 Introduction

SINS (for Simulating INdividuals in Space) is a program that simulates the demography of populations and their resulting genetic diversity in a spatial setting. It is an individual-based tool that incorporates both geographical and demographic data, allowing to generate several types of genetic markers. SINS owes much of its conception to SPLATCHE [Currat *et al.*, 2004], recently been upgraded to SPLATCHE2 [Ray *et al.*, 2010]. SINS and SPLATCHE share significant features, but are complementary due to some differences that we detail below.

## 5.3 Methods

### 5.3.1 Demography

SINS uses a 2D bouncing edges stepping-stone model framework, where demes (populations) are connected by gene flow (with migration rate $m$) and arranged in a grid (layer). Several layers can occupy the same geographical space, and individuals from different layers can interact either by competition, admixture or both.

Each deme is characterized by carrying capacity (*K*) and friction (*F*) values, which can be different among demes and layers and can change with time. Migration is constrained by the *F* value of neighbouring demes. Expansion, contraction and fragmentation of populations are simulated by defining appropriate *K* and *F* maps. Expansions can start from multiple sources and at different times. Mating between individuals from different layers can be asymmetrical and is modelled by an admixture parameter ($\gamma$). These features are, apart from minor differences, shared with SPLATCHE (one layer) and SPLATCHE2 (a maximum of two layers). We now outline several features that are specific to SINS:

1. Population size is logistically regulated within each deme, with intrinsic growth rate ($r_i$ for layer *i*), but the specificity of SINS is that (i) foundation events must involve at least one male and one female, (ii) the Maynard-Smith and Slatkin [1973] logistic growth formula is used and corrected (equation 5.1) to account for the fact that growth is limited by the number of reproductive females. This avoids unrealistic situations where a female may have a biologically unrealistic number of offspring. The Lotka-Volterra model is used to incorporate competition into the logistic growth equation (5.1), through a parameter $\alpha_{ij}$ that varies between 0 and 1 and represents the pressure exerted by populations from layer *j* over populations from layer *i*. Thus, the population size in a deme from layer *i* at time *t+1* is calculated as:

$$N_{i,t+1} = 2N_{f,i,t}\frac{1+r_i}{1+\sum_{j=1}^{nlayer} r_i\alpha_{ij}\frac{2N_{f,i,t}}{K_i}} \tag{5.1}$$

   where $N_{f,i,t}$ is the number of reproductive females in the same deme from layer *i*, at time *t* and *nlayer* is the number of layers. Another difference is that (iii) growth is not deterministic, with the actual values of population size drawn from a Poisson distribution with mean $N_{i,t+1}$, as given by equation (5.1).
2. Random mating is assumed by default, but it is possible to simulate the variance in the reproductive success of individuals, by giving as input the percentage of males and females that reproduce.

3. Sex-biased migration is simulated through parameter *mSR* (equation 5.2) that allows the user to vary the ratio of female ($m_f$) to male ($m_m$) migration rates.

$$mSR = \frac{m_f}{m_f + m_m} \tag{5.2}$$

4. Most processes are stochastic such that the total number of migrants, the direction of migration events and the deme population sizes are drawn from statistical distributions.

### 5.3.2 Genetics

SINS simulates individuals whose sex is defined by their sexual chromosomes (XX for the female and XY for the males). Multilocus genotypes can be simulated for several types of markers (SNPs, microsatellites and sequences). The program assumes that the loci are either independent or totally linked. It is thus possible to simulate autosomes, sexual chromosomes and mitochondrial DNA (mtDNA), making it possible to follow the parallel history of any set of chromosomes and compare, for instance, Y-chromosome and mtDNA data from the same populations.
The genetic make-up of founding populations can be taken from pre-specified allele frequencies (from observed or simulated data). When several layers are simulated, it is also possible to found a new population by sampling the corresponding deme from another layer.

### 5.3.3 Outputs and Summary Statistics

SINS produces demographic and genetic outputs. The demographic output is a single text file, where the number of individuals is recorded for each deme, layer and generation time. The genetic outputs are divided by chromosome/locus. For each locus, one file is created with the genotypes of all individuals, on all demes and layers, for the time steps chosen by the user. Each individual is identified by its layer, deme, sex and time step. Moreover, the parents of each individual are also recorded, together with their original birth deme.

A companion program (SINS-Stat) is also available to sample individuals from specified demes and layers and to compute single locus population genetic statistics.

## 5.4 Implementation

Both SINS and SINS-Stat are written in Java and require JRE 1.6 to be installed. Depending on the size of the simulated world and the number of generations, large amounts of RAM may be required for SINS to run. Since it is a non-graphical console program, it can be easily used in computer clusters, hence decreasing the execution time (see user guide to details).

## 5.5 Discussion

Recent years have seen the development of coalescent-based programs to simulate complex demographic histories. The advantage of such programs is that they are extremely efficient because only the sampled genealogies are simulated. In contrast, with forward simulations the whole population has to be simulated. While this makes forward methods computationally less efficient, they are beginning starting to be used again once more [Balloux, 2001; Guillaume & Rougemont, 2006; Neuenschwander *et al.*, 2008; Peng & Kimmel, 2005]. Forward simulations are intuitively easier to grasp and code, particularly in the case of complex scenarios that involve spatial expansions or selection. The two types of approaches should thus be complementary rather than opposed, depending on the questions one asks. For instance, in a recent study simulating the spatial expansion of North African population across Gibraltar, Currat *et al.*, [2010] used modified versions of SPLATCHE incorporating both coalescent and forward simulations for markers under selection. Similarly, SINS should be seen as a program that is complementary to SPLATCHE. It incorporates features that are more realistic, but will probably be difficult to use for species with very large population sizes. However, the development and growth of computing power should make it possible for SINS to be useful for statistical inference under increasingly complex scenarios. These would include features such as sex-biased migration and variance in the reproductive success together with ex-

pansions from Pleistocene *refugia* or contractions due to environmental fluctuations or habitat loss and fragmentation. To our knowledge, no other forward simulation program is capable of simulating individuals from populations with different demographic histories, inhabiting the same geographic space (i.e. layers).

**Acknowledgements**
We are grateful to I. Alves and C. Chaouiya for useful comments on an earlier version of the manuscript. This work was funded by Fundação para a Ciência e Tecnologia [SFRH/BD/30821/2006 to R.R., SFRH/BD/22224/2005 to V.S., PTDC/BIA-BDE/71299/2006 and PTDC/BIA-BEC/100176/2008 to L.C.), Instituto Gulbenkian de Ciência [P.A.B.,D.M.] and Institut Français de la Biodiversité, Programme Biodiversité des Îles de l'Océan Indien [CD-AOOI-07-003 to L.C.].

## 5.6 References

BALLOUX, F. (2001). Easypop (version 1.7): a computer program for population genetics simulations. *J Hered*, **92**, 301–302.

CURRAT, M., RAY, N. & EXCOFIER, L. (2004). SPLATCHE: a program to simulate genetic diversity taking into account environmental heterogeneity. *Mol Ecol Notes*, **4**, 139–142.

CURRAT, M., POLONI, E.S. & SANCHEZ-MAZAS, A. (2010). Human genetic differentiation across the Strait of Gibraltar. *BMC Evol Biol*, **10**, 237.

GUILLAUME, F. & ROUGEMONT, J. (2006). Nemo: an evolutionary and population genetics programming framework. *Bioinformatics*, **22**, 2556–2557.

MAYNARD-SMITH, J. & SLATKIN, M. (1973). The stability of predator-prey systems. *Ecology*, **54**, 384–391.

NEUENSCHWANDER, S., HOSPITAL, F., GUILLAUME, F. & GOUDET, J. (2008). quantiNemo: an individual-based program to simulate quantitative traits with explicit genetic architecture in a dynamic metapopulation. *Bioinformatics*, **24**, 1552–1553.

PENG, B. & KIMMEL, M. (2005). simuPOP: a forward-time population genetics simulation environment. *Bioinformatics*, **21**, 3686–3687.

RAY, N., CURRAT, M., FOLL, M. & EXCOFFIER, L. (2010). SPLATCHE2: a spatially explicit simulation framework for complex demography, genetic admixture and recombination. *Bioinformatics*, **26**, 2993–2994.

# 6. General Discussion

The increasing availability of genetic data from current human populations has allowed geneticists and archaeologists to reconstruct their demographic history. Several past demographic events led to major cultural, social and demographic changes that most likely influenced and therefore left their marks on human genetic diversity patterns, being the Neolithic transition considered to be one of the most important ones [Mithen, 2007]. Such changes have been suggested either by archaeological and anthropological data, but there have been very few studies to address these issues from a genetic point of view. In this thesis, we decided to use model-based approaches to study the consequences of the cultural, social and demographic shifts that followed the Neolithic transition, on the patterns of genetic diversity.

## 6.1 Neolithic transition in Japan and Europe

First, in chapter 2, we decided to test this approach on the colonization of Japan, by hunter-gatherers (HG) Jomon and rice cultivators Yayoi. While geographically Japan is a relatively small region, the genetic contribution of these two populations on modern Japanese was yet the focus of much dispute, between archaeologists, anthropologists and geneticists. When we published this work, there were no data available from mtDNA and the Pan-Asian HUGO SNP consortium database [Ngamphiw *et al.*, 2011] was not yet released. Nevertheless, using published Y-chromosome data, our results clearly point to a demic diffusion process, similar to the process that was suggested for Europe. However, we could not pinpoint the origin of the Yayoi farmers in mainland Asia. More studies using more *loci* are needed to answer this question.

In chapter 3, the same admixture approach is applied to the European Neolithic. Chikhi *et al.* [2002] had already applied it to Europe, using Y-chromosome data, but we decided to integrate in the same study mtDNA and Y chromosome data. Indeed, one of the major limitations in published studies is the fact that they either use mtDNA or Y-chromosome data. Moreover, different statistical methodologies were used on the different markers. This has led to the claim that the different markers favour opposite models for the Neolithic transition. For the first time, we used the same methodological approaches for both contemporary mtDNA and Y-chromosome data. We found that both Y-chromosome and mtDNA data clearly favour a demic diffusion process, but that they also identify key differences in the female and male demographic histories, most likely related with sex-related differences in effective size and migration rates. These differences are probably related to the various shifts in cultural practices and lifestyles that followed the Neolithic Transition, such as sedentism [Bellwood, 2004], the shift from polygyny to monogamy [Dupanloup *et al.*, 2003; Fortunato, 2011a] or the increase of patrilocal residence systems [Fortunato, 2011b; Wilkins & Marlowe, 2006].

In the same study, we also analyzed ancient and modern data from Central Europe, using an ABC approach. We found that the patterns of genetic diversity, encountered in current and ancient populations, demonstrate that both modern and ancient mtDNA support the demic diffusion model. Our results also show that we need to incorporate ancient population structure and differential growth between Neolithic and Palaeolithic populations to explain the patterns of genetic diversity found today and in our past. We also applied this model approach to the Iberian Neolithic (see appendix B) and found the same kind of results.
Altogether, the study of chapter 3 represents the first attempt to integrate under the same framework contemporary mtDNA and NRY data, together with aDNA, and provides an explanation for the patterns of genetic diversity that we see today.

## 6.2 Spatial expansion and the European Neolithic

The results of chapter 3 made us believe that other kind of models should be used to study the consequence of space, structure and also of culture on the patterns of genetic diversity. Indeed, in human population genetic studies, is being increasingly recognised the influence of social structure and cultural practices in the today's patterns of human genetic diversity and demography [Hamilton *et al.*, 2005; Wilkins, 2006; Wilkins & Marlowe, 2006]. For our study, we were particularly interested in models that that incorporate space, heterogeneous environments and sex-biased processes and that allow to simulate different population groups inhabiting the same geographic space.

At the beginning of this thesis, SPLATCHE [Currat *et al.*, 2004] was the only available computer program that simulated genetic data under complex demographic scenarios, using a spatial framework. However, the available version just allowed simulating one population group. Moreover, SPLATCHE is a coalescence-based program and although it is extremely efficient, because only the sampled genealogies are simulated, there are certain constraints on the complexity of scenarios possible to simulate. Thus, we started to develop SINS (chapter 5), which also simulates the demography of populations and their resulting genetic diversity in a spatial expansion setting. We wanted to apply it to questions related not only with Human Evolution, but also with Conservation Genetics (other of the scopes of our research group). SINS owes much of its conception to SPLATCHE, but it is a forward individual-based tool and the whole population has to be simulated. While this makes SINS computationally less efficient, it also allows simulating certain population demographic scenarios, which were not otherwise possible with SPLATCHE, such as sex-biased migration. We believe that both SPLATCHE and SINS approaches should be seen as complementary, rather than opposed, depending on the questions one asks.

In chapter 4, we applied SINS to study several aspects of the Neolithic transition

from hunter-gathering to farming societies in Europe. We were particularly interested in the consequences of various post-marital residence systems (i.e. patrilocality, matrilocality and bilocality) and admixture between hunter-gatherers (HG) and farmers on patterns of genetic diversity, during the spatial expansions that led to the colonization of Europe by humans. We compared the genetic diversity of Y-chromosome and mtDNA data, in order to infer the male and female demographic histories, respectively. Our results suggest that (i) different post-marital residence systems can lead to different patterns of genetic diversity and differentiation, (ii) patrilocality (when the women move to their husband's birthplace) explains the present patterns of genetic diversity better than matrilocality (the men follow their wives) or bilocality (both sexes can move with equal probability).

The study of chapter 4 is not a full inference study, as it would require simulating the HG and farmers initial genetic diversity from some statistical distribution. Instead, due to the limited speed of the simulations, we decided to use the allelic frequencies observed today as a starting distribution. However, the actual allelic frequencies in specific simulations were not fixed and could vary between simulations. Nevertheless, we believe that much work is still needed to understand how spatial expansions influence present-day allele frequencies and how this could be robust to ancient allele frequencies.

In this thesis, we did not use full potentialities of SINS, such as heterogeneous environments fluctuations along time and space. In chaper 4, we only have tested scenarios in a homogeneous environment, with rectangular lattices. Indeed, we did not consider the effect of present and past geography and land topology on the distribution of individuals in space. We also did not consider past climatic changes, such as glaciations, and the resulting population contractions and expansions. In fact, the increase of human populations in Europe after the Last Maximum Glaciation is believed to have had a great impact on human genetic diversity [Barbujani & Chikhi, 2000; Semino *et al.*, 2000]. In principle, we could increase the complexity of the scenarios and add more realism to the models, by using geographic infor-

mation systems to create the carrying capacity and friction maps used in the SINS simulations.

Another interesting study, would be to construct samples similar to those found in European Neolithic transition aDNA studies [Bramanti *et al.*, 2009; Haak *et al.*, 2005, 2010; Lacan *et al.*, 2011; Malmström *et al.*, 2009] (appendix B), look at the distribution of $F_{ST}$ values and compare them to the observed data. This kind of study could be coupled with the above scenarios as real data are typically obtained from individuals living sometimes hundreds of *km* from each other and spread over centuries or millennia.

## 6.3 Perspectives

#### *6.3.0.1 SINS' new features*

Other features are implemented or are soon to be implemented in a modified version of the SINS framework and would allow simulating even more complex scenarios. These new features include different mating systems, long-distance migration and sex-biased admixture but, they are not yet validated. In fact, the general framework of SINS was validated for publication (see appendix C.4), under the simplest situation: we simulated the genetic evolution of a population by creating a scenario with conditions similar to a Wright-Fisher model, and for which we know what to expect due to Population Genetics theory.

Currently in SINS, we assume that individuals mate randomly. However, populations are rarely panmictic. In humans, several mating systems have been described (monogamy or polygamy), that may significantly influence the genetic patterns of a population, namely the female and male effective population sizes [Wilkins, 2006]. Anthropological evidence says that humans are moderate polygynous [Lagerlöf, 2010], but in Europe monogamy is suggested to be old and associated with the introduction of agriculture [Fortunato, 2011a]. In fact, it has been reported as a

response to class cleavages, in order to appease non-elite men against rebellion [Lagerlöf, 2010]. This is consistent with Y-chromosome data pointing to a decline in variance in male reproductive success after the advent of farming [Dupanloup *et al.*, 2003] and also with archaeological evidence of monogamy in Neolithic burials [Bentley *et al.*, 2008; Haak *et al.*, 2008]. The implementation of different mating systems and, at the same time, the introduction of variance in the reproductive success is one of the new features of SINS. It would be interesting to apply it to the European Neolithic and to simulate the shift from polygyny to monogamy in several scenarios of European colonization by HG and farmers.

The implementation of long-distance migration events is another feature that is crucial to understand several phenomena of postglacial recolonization, which have been described in many species [Hewitt, 2000]. Exploring these alternative demographic scenarios using simulations could be informative too.

The new sex-biased admixture parameter allows to study the influence of limited admixture by one sex, on patterns of genetic diversity. This could apply to female hypergamy, phenomenon that is described in several human migration and colonization events [Carvajal-Carmona *et al.*, 2000; Quintana-Murci *et al.*, 2008; Salzano, 2004; Thomas *et al.*, 2006], and it is also believed to have happened during the Neolithic transition in Europe [Bentley *et al.*, 2003], with HG females marrying into farmer communities [Bentley *et al.*, 2009].

#### *6.3.0.2 SINS' in an ABC framework*

In order to increase the complexity of models it is necessary to have a good knowledge of the parameters used to avoid incertitude. Recent years have seen the development of new inferential methods in population genetics, named Approximate Bayesian Computation (ABC) [Beaumont, 2008; Beaumont *et al.*, 2002]. They require genetic data to be simulated within a particular model or sets of models. After the data have been simulated, they are compared to real or observed data. If the

simulated data are very different from the real data, they are simply rejected. However, when they are arbitrarily close, the parameters that were used to obtain the simulated data are saved and used for inference. The rationale is that parameters that generate data close to the real ones are more likely than those producing data that are very different. It is possible to incorporate an ABC framework in SINS, either for model parameter estimation or selection of the best demographic scenario. This was recently done, but still much is needed to test it using simulated data sets, for which the parameter values are known and applying it to real datasets.

The incorporation of and ABC framework and the development and growth of computing power, should make it possible for SINS to be useful for statistical inference under increasingly complex scenarios. In fact, a parallel approach is being developed to use it in computing cluster and decrease the computing time of the simulations.

## 6.4 Conclusion

Overall, the work presented in this thesis points to a scenario where the demic diffusion model had an important role in the dissemination of agriculture, and thus genes, in certain parts of the world (Japan and Europe). Furthermore, although there are some discrepancies between males and females, both sexes support demic diffusion during the European Neolithic. These differences are probably due to sex-biased processes, such as sex-biased migration, that leave their mark in contemporary patterns of genetic diversity.

Here we studied the influence of sex-biased migrations systems, but almost nothing is known about the influence of mating systems in human populations' genetic diversity. Still much is needed to be done to understand the influence of space, structure and culture in the genetic make-up of human populations. Indeed, we believe that our spatial forward framework could be used to explore (i) the consequences of various spatial processes and (ii) the influence of social structure and different cultural practices, on today's patterns of human genetic diversity and de-

mography.

We thus believe that this thesis represents a significant advance in our understanding of one of the most important economic, demographic and cultural transitions in human Prehistory: the Neolithic transition.

## 6.5 References


BARBUJANI, G. & CHIKHI, L. (2000). *Genetic population structure of Europeans inferred from nuclear and mitochondrial DNA polimorphisms*, 119–130. McDonald Institute for Archaeological Research, Cambridge.

BEAUMONT, M. (2008). *Joint determination of topology, divergence time, and immigration in population trees*, 135–154. McDonald Institute for Archaeological Research, Cambridge.

BEAUMONT, M.A., ZHANG, W. & BALDING, D.J. (2002). Approximate Bayesian Computation in population genetics. *Genetics*, **162**, 2025–35.

BELLWOOD, P. (2004). *First Farmers: the origins of agricultural societies*. Blackwell Publishing, Oxford.

BENTLEY, R.A., CHIKHI, L. & PRICE, T.D. (2003). The Neolithic transition in Europe: comparing broad scale genetic and local scale isotopic evidence. *Antiquity*, **77**, 63–66.

BENTLEY, R.A., WAHP, J., PRICE, T.D. & ATKINSON, T.C. (2008). Isotopic signatures and hereditary traits: snapshot of a Neolithic community in Germany. *Antiquity*, **82**, 290–304.

BENTLEY, R.A., LAYTON, R.H. & TEHRANI, J. (2009). Kinship, marriage, and the genetics of past human dispersals. *Hum Biol*, **81**, 159–79.

BRAMANTI, B., THOMAS, M.G., HAAK, W., UNTERLAENDER, M., JORES, P., TAMBETS, K., ANTANAITIS-JACOBS, I., HAIDLE, M.N., JANKAUSKAS, R., KIND, C.J., LUETH, F., TERBERGER, T., HILLER, J., MATSUMURA, S., FORSTER, P. & BURGER, J. (2009). Genetic discontinuity between local hunter-gatherers and central Europe's first farmers. *Science*, **326**, 137–140.

CARVAJAL-CARMONA, L.G., SOTO, I.D., PINEDA, N., ORTÍZ-BARRIENTOS, D., DUQUE, C., OSPINA-DUQUE, J., MONTOYA, M.M.P., ALVAREZ, V.M., BEDOYA, G. & RUIZ-LINARES, A. (2000). Strong Amerind/White sex bias and a possible sephardic contribution among the founders of a population in Northwest Colombia. *Am J Hum Genet*, **67**, 1287–1295.

CHIKHI, L., NICHOLS, R.A., BARBUJANI, G. & BEAUMONT, M.A. (2002). Y genetic data support the Neolithic demic diffusion model. *Proc Natl Acad Sci U S A*, **99**, 11008–11013.

CURRAT, M., RAY, N. & EXCOFIER, L. (2004). SPLATCHE: a program to simulate genetic diversity taking into account environmental heterogeneity. *Mol Ecol Notes*, **4**, 139–142.

DUPANLOUP, I., PEREIRA, L., BERTORELLE, G., CALAFELL, F., PRATA, M.J., AMORIM, A. & BARBUJANI, G. (2003). A recent shift from polygyny to monogamy in humans is suggested by the analysis of worldwide Y-chromosome diversity. *J Mol Evol*, **57**, 85–97.

FORTUNATO, L. (2011a). Reconstructing the history of marriage strategies in Indo-European-speaking societies: monogamy and polygyny. *Hum Biol*, **83**, 87–105.

FORTUNATO, L. (2011b). Reconstructing the history of residence strategies in Indo-European-speaking societies: neo-, uxori-, and virilocality. *Hum Biol*, **83**, 107–28.

HAAK, W., FORSTER, P., BRAMANTI, B., MATSUMURA, S., BRANDT, G., TÄNZER, M., VILLEMS, R., RENFREW, C., GRONENBORN, D., ALT, K.W. & BURGER, J. (2005). Ancient DNA from the first european farmers in 7500-year-old neolithic sites. *Science*, **310**, 1016–1018.

HAAK, W., BRANDT, G., DE JONG, H.N., MEYER, C., GANSLMEIER, R., HEYD, V., HAWKESWORTH, C., PIKE, A.W.G., MELLER, H. & ALT, K.W. (2008). Ancient DNA, strontium isotopes, and osteological analyses shed light on social and kinship organization of the later stone age. *Proc Natl Acad Sci U S A*, **105**, 18226–31.

HAAK, W., BALANOVSKY, O., SANCHEZ, J.J., KOSHEL, S., ZAPOROZHCHENKO, V., ADLER, C.J., DER SARKISSIAN, C.S.I., BRANDT, G., SCHWARZ, C., NICKLISCH, N., DRESELY, V., FRITSCH, B., BALANOVSKA, E., VILLEMS, R., MELLER, H., ALT, K.W. & AND, A.C. (2010). Ancient DNA from european early neolithic farmers reveals their Near Eastern affinities. *PLoS Biol*, **8**, e1000536.

HAMILTON, G., STONEKING, M. & EXCOFFIER, L. (2005). Molecular analysis reveals tighter social regulation of immigration in patrilocal populations than in matrilocal populations. *Proc Natl Acad Sci U S A*, **102**, 7476–80.

HEWITT, K. (2000). The genetic legacy of the Quartenary ice ages. *Nature*, **405**, 907–913.

LACAN, M., KEYSER, C., RICAUT, F.X., BRUCATO, N., DURANTHON, F., GUILAINE, J., CRUBÉZY, E. & LUDES, B. (2011). Ancient DNA reveals male diffusion through the Neolithic Mediterranean route. *PNAS*, **early edition**.

LAGERLÖF, N.P. (2010). Pacifying monogamy. *J Econ Growth*, **15**, 235–262.

MALMSTRÖM, H., GILBERT, M.T.P., THOMAS, M.G., BRANDSTRÖM, M., STORA, J., MOLNAR, P., ANDERSEN, P.K., BENDIXEN, C., HOLMLUND, G., GÖTHERSTRÖM, A. & WILLERSLEV, E. (2009). Ancient DNA reveals lack of continuity between neolithic hunter-gatherers and contemporary scandinavians. *Curr Biol*, **19**, 1758–62.

MITHEN, S. (2007). Did farming arise from a misapplication of social intelligence? *Philos*

*Trans R Soc Lond B Biol Sci*, **362**, 705–718.

NGAMPHIW, C., ASSAWAMAKIN, A., XU, S., SHAW, P.J., YANG, J.O., GHANG, H., BHAK, J., LIU, E., TONGSIMA, S., & THE HUGO PAN-ASIAN SNP CONSORTIUM (2011). PanSNPdb: The Pan-Asian SNP Genotyping Database. *PLoS ONE*, **6**, e21451.

QUINTANA-MURCI, L., QUACH, H., HARMANT, C., LUCA, F., MASSONNET, B., PATIN, E., SICA, L., MOUGUIAMA-DAOUDA, P., COMAS, D., TZUR, S., BALANOVSKY, O., KIDD, K.K., KIDD, J.R., VAN DER VEEN, L., HOMBERT, J.M., GESSAIN, A., VERDU, P., FROMENT, A., BAHUCHET, S., HEYER, E., DAUSSET, J., SALAS, A. & BEHAR, D.M. (2008). Maternal traces of deep common ancestry and asymmetric gene flow between Pygmy hunter-gatherers and Bantu-speaking farmers. *Proc Natl Acad Sci U S A*, **105**, 1596–601.

SALZANO, F.M. (2004). Interethnic variability and admixture in latin America–social implications. *Rev Biol Trop*, **52**, 405–15.

SEMINO, O., PASSARINO, G., OEFNER, P.J., LIN, A.A., ARBUZOVA, S., BECKMAN, L.E., BENEDICTIS, G.D., FRANCALACCI, P., KOUVATSI, A., LIMBORSKA, S., MARCIKIAE, M., MIKA, A., MIKA, B., PRIMORAC, D., SANTACHIARA-BENERECETTI, A.S., CAVALLI-SFORZA, L.L. & UNDERHILL, P.A. (2000). The genetic legacy of Paleolithic Homo sapiens sapiens in extant Europeans: a Y chromosome perspective. *Science*, **290**, 1155–1159.

THOMAS, M.G., STUMPF, M.P.H. & HÄRKE, H. (2006). Evidence for an apartheid-like social structure in early anglo-saxon England. *Proc R Soc B*, **273**, 2651–7.

WILKINS, J.F. (2006). Unraveling male and female histories from human genetic data. *Curr Opin Genet Dev*, **16**, 611–7.

WILKINS, J.F. & MARLOWE, F.W. (2006). Sex-biased migration in humans: what should we expect from genetic data? *Bioessays*, **28**, 290–300.

# A. Appendix: Admixture in Europe

## A.1 Supplementary Tables

**Table A.1: Validation of the ABC model selection procedure.** Each line corresponds to the percentage of times that a model was assigned to each of the models, by a higher posterior probability.

| | % Attribution | | |
|---|---|---|---|
| | **TP** | **S** | **SDG** |
| **TP** | 74.7 | 21.3 | 4.0 |
| **S** | 44.7 | 38.1 | 17.2 |
| **SDG** | 2.6 | 9.6 | 87.8 |

**Note:** When data are simulated under the S model our results show that a significant proportion of the data sets are identified as being generated under another model (and as many as 44.7% are assigned to the TP model). This is less the case for the data generated under the TP model (but still they represent as much as 25% altogether) and even less under the SDG model. Thus despite non negligible error rates, these simulations suggest that there is a bias favouring the TP model, and much less the S and SDG models. One reason for this is that the ABC algorithm used here followed the procedure of Bramanti and colleagues [Bramanti *et al.*, 2009], and was only based on three statistics, which were available. However, the results also show that the SDG model is the model which is most easily identified with nearly 88% of positive results. Given that the results obtained from the real data provide no support for the TPM, and less than 5% for the S model, we are confident that the inference of the model is unlikely to be incorrect hence demonstrating the importance of differential growth. This explains why Haak *et al.* [2010] were unable to explain the observed $F_{ST}$ values with their split model.

**Table A.2: Calibrated radiocarbon dates of Neolithic archaeological sites.** Location and type of Neolithic culture (EN – Early Neolithic, LBK – Linear Pottery Culture) are also represented in this table [Pinhasi *et al.*, 2005].

| Location | Archaeological site | Culture | Dates (Yrs cal BP) |
|---|---|---|---|
| Georgia | Arkb1 | Pottery Neolithic | 7 937 |
| Cyprus | Cypro-EPPNB | Kissonega-Mylouthkia | 10 494 |
| Greece | Knossos | EN | 8 946 |
| Bulgaria | Polyanista-Platoto1 | EN | 8 145 |
| Czech Republic | Bylany | LBK | 7 604 |
| Slovakia | Sturovo | LBK | 7 146 |
| Romania | Trestiana | Starcevo,Cri | 7 539 |
| Yugoslavia | Apatin | Starcevo | 7 932 |
| Hungary | Endrod | Köros | 7 765 |
| Poland | Strezelce | LBK | 7 150 |
| Italy | Praia di Mare | EN | 8 324 |
| Germany | Klein Denkte | LBK | 8 803 |
| Netherlands | Geleen | LBK | 7 317 |
| Denmark | Christiansholm Mose | Neolithic | 6 139 |
| France | Pontcharaud | Epicardial | 7 930 |
| Belgium | Omal | LBK | 7 412 |
| Scotland | Boghead Mound | Neolithic | 6 839 |
| Cornwall | Carn Bea | Neolithic | 5 761 |
| East Anglia | Strawberry Hill | Neolithic | 7 677 |
| Ireland | Carrowmore | Neolithic | 6 038 |
| Spain | Cueva del Nasciemento | EN | 7 637 |
| Portugal | Pena D'Água | Cardial | 7 629 |
| Northern Sweden | Skoteholm | Neolithic | 6 297 |
| Lithuania | Daktariske | Neolithic | 6 317 |

## A.2 Supplementary Figures

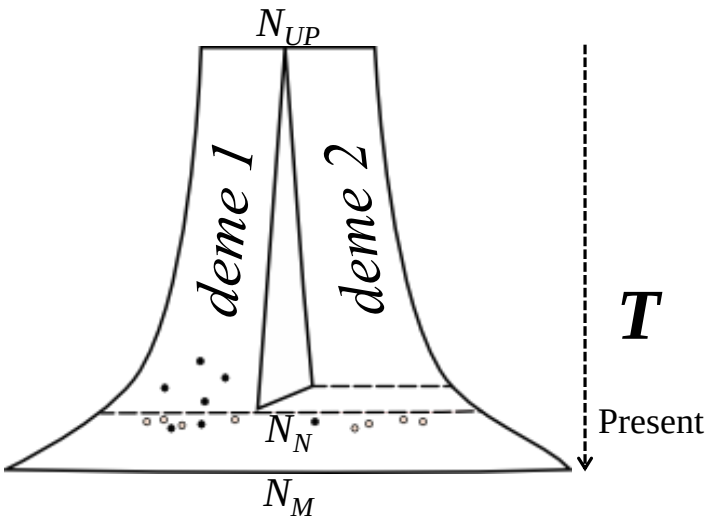



**Figure A.1: Split with differential growth model (SDG)** - Name of the demes are written in the figure

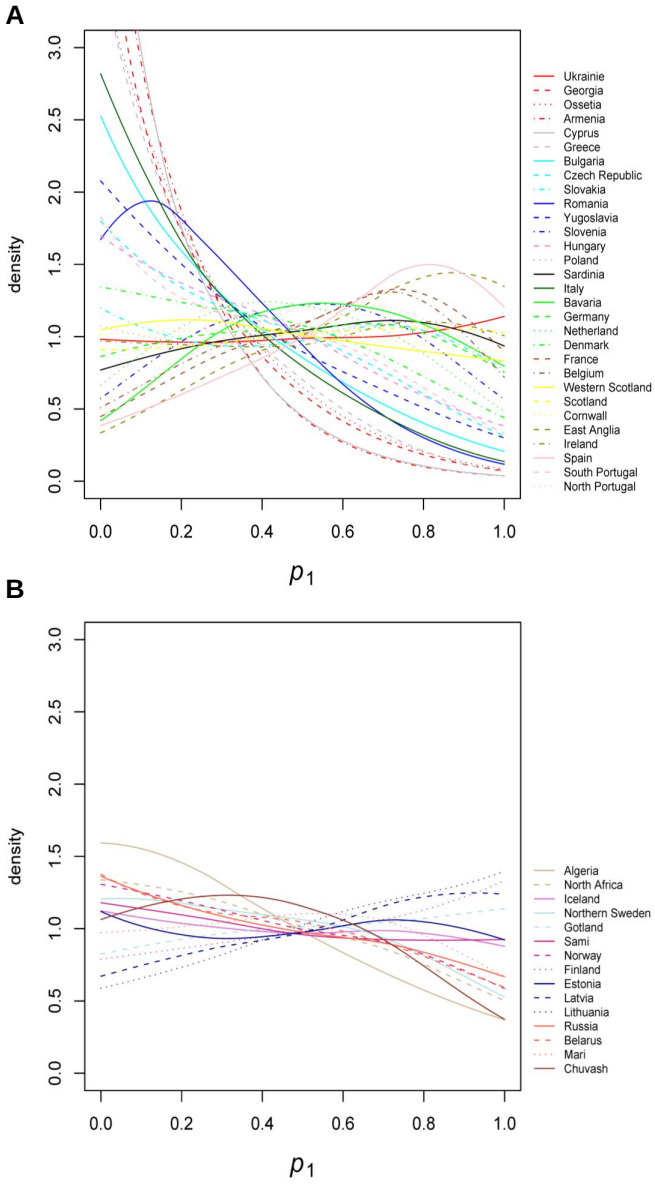



**Figure A.2: Palaeolithic contribution to modern European ($p_1$) posterior distributions** - Each curve corresponds to the analysis of a specific hybrid (European) population using NRY data [Rosser *et al.*, 2000]. In **(A)** are represented all the populations used in this study and in **(B)** are the populations used as negative control (see section 3.3.1.4).

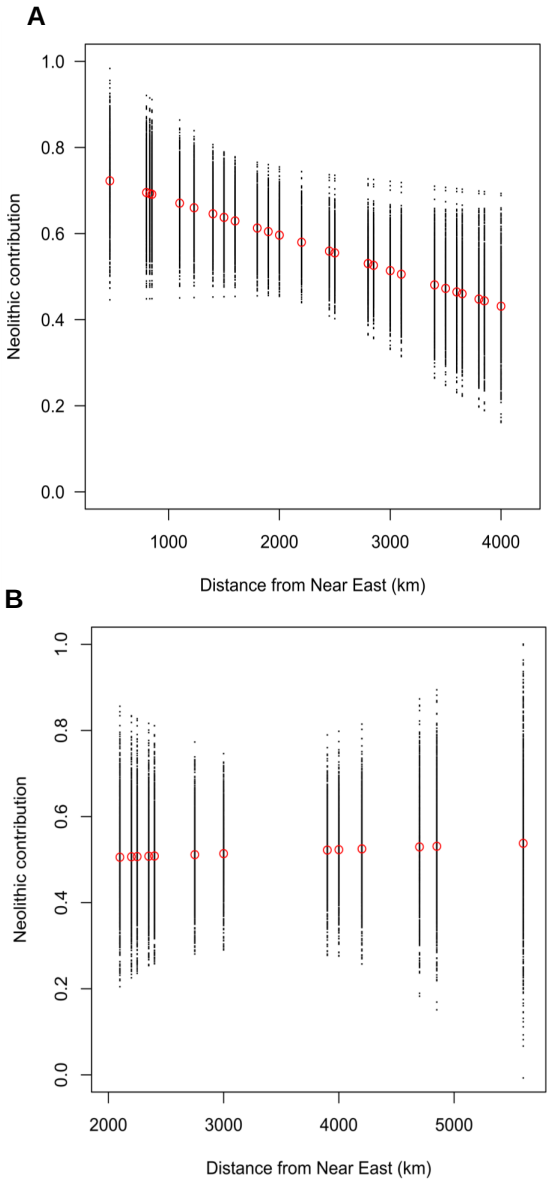



**Figure A.3: Linear regression of Neolithic contribution ($1 - p_1$), against geographical distance from the Near East, using NRY data** - In **(A)** are represented all the populations used in this study and in **(B)** are the populations used as negative control (see chapter 3, section 3.3.1.4). Mean values for each population are represented by red circles.

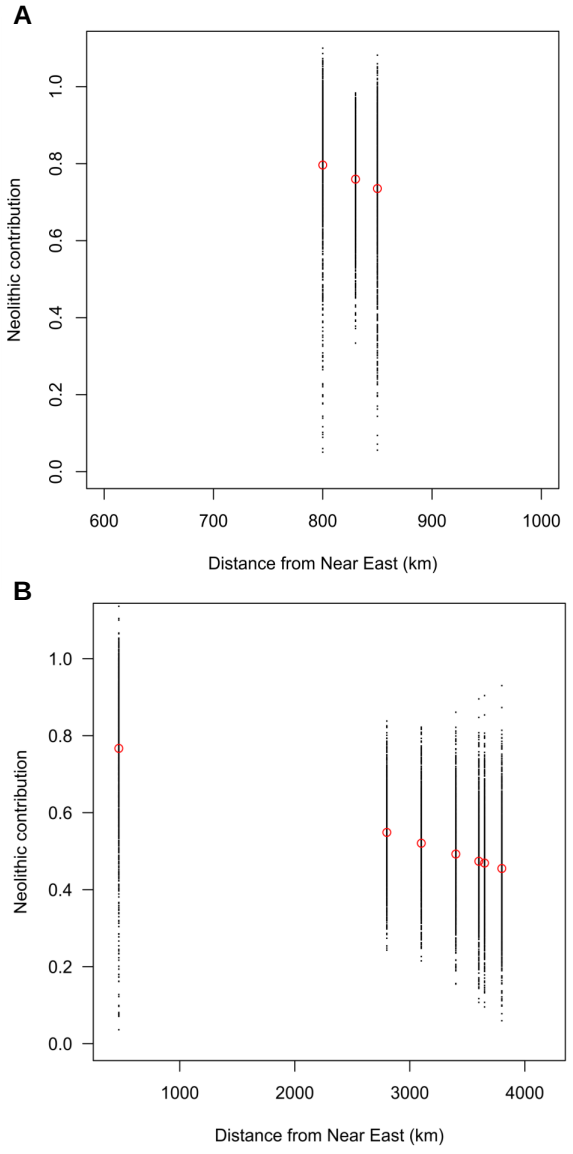



**Figure A.4: Caucasus and European Islands: linear regression of Neolithic contribution ($1 - p_1$), against geographical distance from the Near East, using NRY data** - In **(A)** are represented the Caucasus populations (note the different scale on the x-axis) and in **(B)** are the European Islands population samples (Cyprus, Sardinian, UK and Ireland) used in this study. Mean values for each population are represented by red circles.

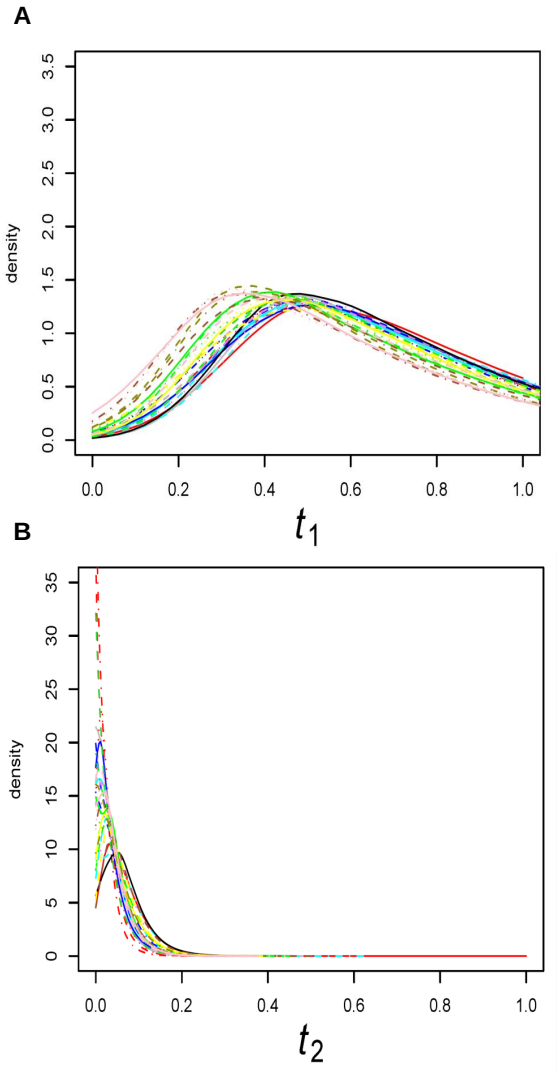



**Figure A.5: Distributions of the $t_i$'s for all populations, using NRY** - **(A)** Posterior distributions of $t_1$. The different curves represent the amount of genetic drift, since the admixture event, between the present sample of Basques and the ancestral populations of *HG* that interbred with the incoming farmers. **(B)** Posterior distributions of $t_2$. As in (A), but for the drift between the Near East and the first farmer populations. The colour codes are as in Fig. A.2A.

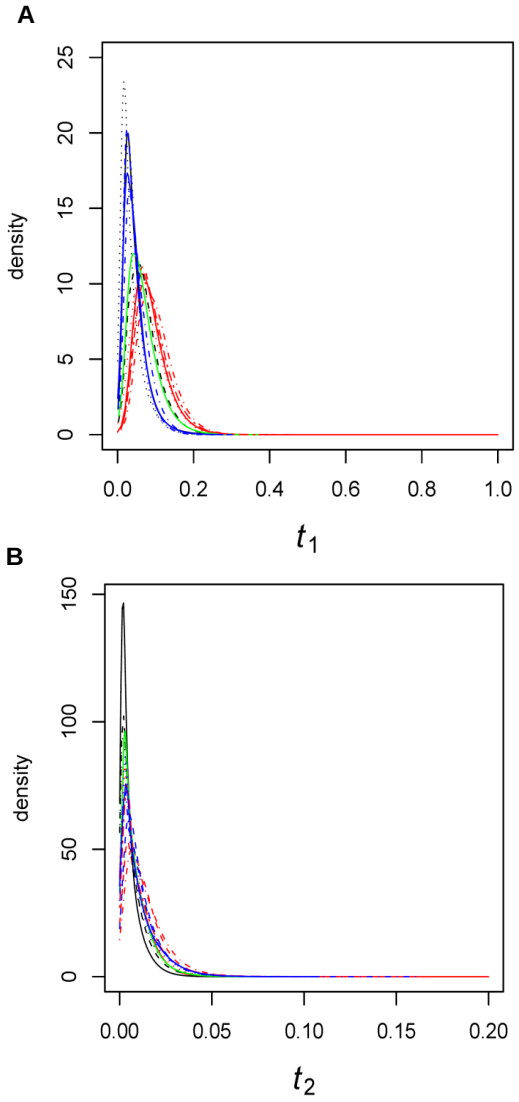



**Figure A.6: Distributions of the $t_i$'s for all populations, using mtDNA** - **(A)** Posterior distributions of $t_1$. **(B)** Posterior distributions of $t_2$ (see Fig. A.5 for more detailed explanation. Note that the panel B has a different scale on the x-axis compared to panel A and Fig. A.5

# B. Appendix: Neolithic transition in the Iberian Peninsula

**Citation:** C Gamba, E Fernandez, M Tirado, M F Deguillon, M H Pemonge, P Utrilla, M Edo, M Molist, Rita Rasteiro, Lounès Chikhi and E Arroyo-Pardo (2012) Ancient DNA from an Early Neolithic Iberian population supports a pioneer colonization by first farmers. *Mol Ecol* 21: 25-56

---

In this paper, we collaborated on the statistical analyses of the aDNA data. Particularly, it were used the same demographic models and R scripts developed for the analyses presented in chapter 3,

# Ancient DNA from an Early Neolithic Iberian population supports a pioneer colonization by first farmers

C. GAMBA,* E. FERNÁNDEZ,*† M. TIRADO,* M. F. DEGUILLOUX,‡ M. H. PEMONGE,‡ P. UTRILLA,§ M. EDO,¶ M. MOLIST,** R. RASTEIRO,†† L. CHIKHI†††§§ and E. ARROYO-PARDO*

*Laboratorio de Genética Forense y Genética de Poblaciones, Facultad de Medicina, Pabellón 7, 4ª Planta, Universidad Complutense de Madrid, Avenida Complutense s/n, 28040 Madrid, Spain, †Instituto de Arqueologia e Paleociências, Universidade do Algarve, 8005-139 Faro, Portugal, ‡UMR 5199 PACEA, Laboratoire d'Anthropologie Des Populations Passées et Présentes, Université Bordeaux 1, 33405 Talence cedex, France, §Departamento de Ciencias de la Antigüedad, Universidad de Zaragoza, 50009 Zaragoza, Spain, ¶Departament de Prehistòria, Història Antiga i Arqueologia, Universitat de Barcelona, 08032 Barcelona, Spain, **Departamento de Prehistoria, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain, ††Instituto Gulbenkian de Ciência, P-2780-156 Oeiras, Portugal, ‡‡CNRS, Université Paul Sabatier, ENFA; UMR 5174 EDB (Laboratoire Evolution & Diversité Biologique); 118 route de Narbonne, F-31062 Toulouse, France, §§Université de Toulouse; UMR 5174 EDB, F-31062 Toulouse, France*

**Abstract**

**The Neolithic transition has been widely debated particularly regarding the extent to which this revolution implied a demographic expansion from the Near East. We attempted to shed some light on this process in northeastern Iberia by combining ancient DNA (aDNA) data from Early Neolithic settlers and published DNA data from Middle Neolithic and modern samples from the same region. We successfully extracted and amplified mitochondrial DNA from 13 human specimens, found at three archaeological sites dated back to the Cardial culture in the Early Neolithic (Can Sadurní and Chaves) and to the Late Early Neolithic (Sant Pau del Camp). We found that haplogroups with a low frequency in modern populations—N* and X1—are found at higher frequencies in our Early Neolithic population (~31%). Genetic differentiation between Early and Middle Neolithic populations was significant ($F_{ST}$ ~0.13, $P < 10^{-5}$), suggesting that genetic drift played an important role at this time. To improve our understanding of the Neolithic demographic processes, we used a Bayesian coalescence-based simulation approach to identify the most likely of three demographic scenarios that might explain the genetic data. The three scenarios were chosen to reflect archaeological knowledge and previous genetic studies using similar inferential approaches. We found that models that ignore population structure, as previously used in aDNA studies, are unlikely to explain the data. Our results are compatible with a pioneer colonization of northeastern Iberia at the Early Neolithic characterized by the arrival of small genetically distinctive groups, showing cultural and genetic connections with the Near East.**

*Keywords*: ancient DNA, Iberian Peninsula, mitochondrial DNA, Neolithic

*Received 8 July 2011; revision received 5 October 2011; accepted 11 October 2011*

## Introduction

The Neolithic transition that transformed Europe arose in the Near East more than 10 000 BP and spread towards Western and Central Europe during an expansion that lasted several millennia (Price 2000). It represents a major if not the most important economic revolution to take place in Western and Central Europe. Foraging was replaced by agriculture and animal farming, eliciting a simultaneous demographic response characterized by a sharp increase in birth rate (Bocquet-Appel & Bar-Yosef

Correspondence: Cristina Gamba, Fax: +34 913941576;
E-mail: cristinagamba@med.ucm.es

2008). The last 50 years have witnessed a major scientific controversy over how this spread took place, basically focused on its demographic or cultural nature, usually defined by two extreme models of *cultural diffusion* (CDM) and *demic diffusion* (DDM) (Childe 1964; Zvelebil 2001). Intermediate models, such as *Infiltration*, *Leapfrog colonization*, *Folk migration*, *Frontier mobility*, *Elite dominance* or *Contact*, have been also suggested (Zvelebil 2001). In the Iberian Peninsula, archaeological data suggest a dual model (coastal DDM and inland CDM) (Bernabeu 1997; Fernández & Gómez 2009) and radiocarbon dates support a rapid spread of Neolithic culture in a framework of a maritime pioneer colonization (Zilhão 2001), which suppose the arrival of small Neolithic groups to coastal areas.

To quantify the relative genetic contribution of Near Eastern Neolithic populations to the European gene pool, modern genetic variability has been explored with contradictory results depending on the type of markers studied and/or the approaches involved in data analyses (Richards 2003; Dupanloup *et al.* 2004; Barbujani & Chikhi 2006; Soares *et al.* 2010). Whereas phylogeographic studies using Y chromosome and mitochondrial DNA suggested limited contributions (around 20–30%) and concluded in favour of the CDM (Richards *et al.* 2000; Semino *et al.* 2000), simulation analyses performed with some of these data provided a much wider range of possible contributions, perhaps as much as 80%, depending on the populations analysed (Chikhi *et al.* 2002), and favoured a DDM.

Ancient DNA (aDNA) analyses have made a step forward in the last few years. Although this approach has some limitations, such as a restricted number of samples, it provides valuable first-hand information about human ancestry. The Neolithic transition in Central Europe has been explored using aDNA and up to 42 Neolithic skeletons associated with the Linear Pottery Culture [LBK, *Linearbandkeramik*, 5500–4900 cal. before the common era (BCE)] were successfully typed (Haak *et al.* 2005, 2010). Moreover, the Mesolithic background in the same region was also studied (Bramanti *et al.* 2009). The LBK Neolithic population showed high frequency of haplogroup N1a (~25%), a haplogroup that is very rare in the modern-day European population (0.2%). This result was interpreted as suggesting a Palaeolithic ancestry for modern Europeans (Haak *et al.* 2005). This haplogroup was then proposed to be a genetic signature of the Neolithic spread in Central Europe, which was supported by its absence in the Mesolithic population of the surrounding areas (Bramanti *et al.* 2009). Haplogroup N1a was later detected in one Neolithic individual from a French Megalithic burial, suggesting that these interpretations might be incorrect (Deguilloux *et al.* 2011). The dispersion of this lineage through pioneer Neolithic groups to western France was proposed by these authors. Recently, Haak *et al.* (2010) analysed all LBK mitochondrial lineages available at the time and showed that they had affinities with modern populations from the Near East. They concluded that it favoured the DDM with an important genetic contribution during the Neolithic spread towards Europe, hence contradicting the previous study of Haak *et al.* (2005).

While many 'phylogeographical' studies have tended to use network-based 'methods' to 'reconstruct' the demographic history of human populations, there is now an increasing recognition that evolutionary factors (e.g. mutation rate and genetic drift) are intrinsically noisy, that haplogroups cannot be identified to populations (Barbujani *et al.* 1998) or cultures and that model-based approaches are necessary to make progress in the statistical analyses of complex population scenarios (Beaumont *et al.* 2010), such as the Neolithic contribution to the European gene pool.

The colonization of Europe is thought to have taken place following two main routes, namely the Central European and the Mediterranean route of Neolithic spread. In contrast to the former, the Mediterranean route is linked to the Cardial pottery complex instead of the LBK and has been poorly studied. In this framework, Iberia represents an interesting case study, as it is located at the westernmost edge of the Neolithic expansion. The only Iberian Neolithic aDNA study in that region was carried out on Middle Neolithic (MN) samples (3500–3000 cal. BCE) from northeastern Iberia (Sampietro *et al.* 2007). Haplogroup frequencies were very similar to those of present-day populations and were interpreted as favouring a genetic continuity between Neolithic and modern-day Iberian populations. In the light of these divergent results between the Iberian Peninsula and Central Europe (because only the Haak *et al.* 2005 study had then been published), the authors assumed that two different mechanisms of Neolithic diffusion were involved: *cultural diffusion* in Central Europe and *demic diffusion* in the Mediterranean (Sampietro *et al.* 2007). However, some bias could have been introduced considering the MN data (3500–3000 cal. BCE) as representative of the first Neolithic settlers of the region (around 5500 BCE) owing to the age gap between them.

Here, we address this issue by presenting the first Early Neolithic (EN) (Cardial and post-Cardial pottery cultures) aDNA data from northeastern Iberian specimens. Data from these first Neolithic settlers were compared with later inhabitants of the same region in a diachronic context using different simulation scenarios.

Our results show that the simple panmictic models assumed by previous aDNA studies (with the exception

of Haak *et al.* 2010; R. Rasteiro and L. Chikhi, submitted) are rejected. We show that the genetic data support structured models that are compatible with a pioneer colonization by the first Neolithic groups in northeastern Iberia.

## Materials and methods

### *Sample information*

Forty-nine samples from a minimum of 22 individuals found in three Neolithic sites of northeastern Iberia were studied: Can Sadurní ($N = 7$, Barcelona province, 5475–5305 cal. BCE, Blasco *et al.* 2005), Chaves ($N = 3$, Huesca province, 5329–4999 cal. BCE, Utrilla *et al.* 2008) and Sant Pau del Camp ($N = 12$, Barcelona province, 4250–3700 cal. BCE, Molist *et al.* 2008) (see sites location in Fig. 1 and sample description in Table S1, Supporting information). Can Sadurní and Chaves samples are dated back to the EN and are associated with the Cardial culture. Sant Pau del Camp samples are slightly more recent and are dated back to Epicardial or Late EN. Can Sadurní archaeological site is a cave located at the Garraf mountains, 450 m above sea level and about 25 km west of Barcelona (northeastern Spain). Excavations started in 1978 and are still in progress. Twenty-eight different levels have been identified, ranging from Epipalaeolithic (10 840–10 410 cal. BCE) to the Roman period. In layer 18, 80% of impressed pottery was decorated with Cardium shells. Inside one of these Cardial potteries, a conglomerate of seeds was found and radiocarbon-dated (5475–5305 cal. BCE, Blasco *et al.* 2005). Human skeletons were found in graves but not in anatomical connection owing to the fall of the entrance of the cave. Twenty-four loose teeth were selected following external preservation criteria. Dental study identified a minimum of 5 individuals (Blasco *et al.* 2005), but by comparing this information with archaeological and genetic results presented here, we were able to establish a minimum of seven individuals. The Chaves archaeological site is also a cave, located in Bastarás, Huesca province (northeastern Spain). Excavations took place from 1984 to 1992, identifying a long-time occupation of the site, spanning from the Palaeolithic (Solutrean, 19 390–20 010 cal. BCE, Montes & Utrilla 2008) to the Bronze Age. The five teeth studied belonged to three individuals, which were found in anatomical connection and come from the Cardial Neolithic level, where radiocarbon datings were directly performed on human bones (5329–4999 cal. BCE, Utrilla *et al.* 2008). Sant Pau del Camp is an open-air site found in the city of Barcelona. Excavations performed at the church of the same name revealed different occupation phases, ranging from the Early Neolithic, associated with Cardial pottery, to the Roman period. The 21 studied samples came from 12 individuals and were found in the Post-Cardial level, where human bones in anatomical connection were radiocarbon-dated (4250–3700 cal. BCE, Molist *et al.* 2008).


**Fig. 1** Archaeological sites location and population groups. The three population groups analysed here: (i) Early Neolithic samples (present study, Can Sadurní, Chaves and Sant Pau del Camp sites, white spots), (ii) Middle Neolithic samples (Sampietro *et al.* 2007; Camí de Can Grau site, black spot) and (iii) Modern samples from the same region (García *et al.* 2011, Catalonia and Aragón, shady).

Entire teeth samples, without external fissures or caries, were selected by the Population Genetics and Forensic Genetic Laboratory staff, with the exception of two bones from the 1CH0102 specimen (Chaves site), for which dental samples were not available. Whenever possible, at least two teeth or bones per individual were selected. Further information about samples is provided in Table S1 (Supporting information).

### *Ancient DNA analyses*

*Criteria of authenticity.* Genetic analyses were carried out in specialized aDNA laboratories located in the same building but with physical separation of Pre-polymerase chain reaction (PCR), PCR and Post-PCR procedures. Sample cleaning, grinding and extraction were performed in the Pre-PCR laboratory. PCRs were set up in the PCR laboratory and then amplified in a distant-separated room in which no DNA analyses were performed. Agarose gel analysis, PCR purification, cloning and sequencing were performed in a post-PCR laboratory. Pre-PCR and PCR laboratories were UV-irradiated before and after each experiment. Workbenches and laboratory equipment were regularly cleaned with 70% bleach to reduce carry-over contamination. Staff access to aDNA laboratories was restricted

to three people. Moreover, in this case, all experimental analyses were carried out by a single researcher (CG) to reduce exogenous DNA contamination. Sample cleaning and grinding, DNA extraction and PCR amplifications were carried out wearing disposable laboratory coveralls, masks, caps, glasses, shoe covers and gloves. All reagents and consumables employed were DNase and RNase free. All procedures were carried out in a laminar flow cabinet previously cleaned with bleach and UV-irradiated. Laboratory contamination was monitored with extraction blanks (one each seven samples), and at least three PCR-negative controls were included every seven samples. Only samples without any contamination in all stages were considered for further analyses (see Results).

The silica-based extraction method (Rohland & Hofreiter 2007) together with the Multiplex PCR kit (Qiagen) used to amplify aDNA in monoplex reactions proved to be a powerful tool for increasing aDNA yield and typing. Because this amplification kit has been shown to be very sensitive to contamination (data not shown), we designed a set of experiments in which noncontaminated extraction blanks (EBs), previously amplified at least twice, were re-amplified repeatedly to detect possible contamination. The overall results showed a 7% random contamination of EBs, neither related to staff nor to reagents but possibly attributed to random carry-over from floating molecules. Despite this evidence about difficulties in complete elimination of exogenous contamination at these phases, our data showed a low incidence of this problem and almost no contamination reproducibility in the same PCR set, so there was no chance of confusion with endogenous DNA data. To identify the potential sources of contamination, genetic profiles were recovered from all people involved in sample manipulation, including laboratory staff, anthropologist and archaeologists (Table S5, Supporting information). Sampling was performed by collecting blood spots on Whatman filter paper; samples were extracted with the QIAamp DNA Mini kit (Qiagen) and amplified with primers 1A and 1B under standard conditions (Table S6, Supporting information).

Results' reproducibility was assessed by setting up independent extractions and amplifications from the same skeleton. When different bones or teeth from the same individual were not available, two portions of the same tooth/bone were analysed in parallel. At least two amplifications of each mitochondrial DNA(mtDNA) region were performed on all aDNA extracts. Only consistent results among extractions and amplifications were considered. Moreover, five of 14 individuals typed were replicated by CG in an independent aDNA laboratory (LAPP, PACEA, Bordeaux 1 University—CNRS, France), using similar methodology and facilities as those described above. Consistent independent amplifications were cloned (Table S7, Supporting information), and endogenous DNA was identified taking care to follow reproducibility criteria. Staff contamination was identified by comparing the results with staff genetic profiles, and carry-over contamination by comparing the results from the same round of cleaning, DNA extraction or amplification. Moreover, frequencies of transitions (type I and II) and transversions derived from molecular damage were calculated. Authenticity of results is supported by the excess of type II transitions (CG $\rightarrow$ TA, 68%) over type I transitions (TA $\rightarrow$ CG, 28%) and transversions (4%), as already suggested in the literature (Brotherton *et al.* 2007; Gilbert *et al.* 2007). Haplogroup single nucleotide polymorphism (SNP) typing results were in concordance with HVR-I information, supporting the authenticity of the recovered haplotypes.

*Sample cleaning and grinding.* Samples were cleaned using a Sand Blaster (Dentalfarm Base 1 Plus), which allows the removal of about a millimetre of the bone/-tooth surface using aluminium oxide powder under pressure. The aim of this procedure is to clean the sample and remove contaminant DNA molecules from its outer surface. Samples were then irradiated with UV light for about 30 min per side in a laminar flow cabinet and transferred to sterile grinding vials. Grinding was performed in a Freezer Mill (SPEX Model 6700) filled with liquid nitrogen. The resulting powder was stored at −20 °C until DNA extraction was performed.

*DNA extraction.* DNA was extracted from approximately 500 mg of powdered sample following the protocol published by Rohland & Hofreiter (2007). In this protocol, DNA is absorbed to silica in the presence of high concentrations of a chaotropic salt, guanidinium thiocyanate (GuSCN).

*Mitochondrial DNA amplification.* Two overlapping fragments of the hypervariable region I (HVR-I) of the mtDNA were amplified by PCR. The primer pairs used (see Table S6 for primer sequences, Supporting information) allowed typing 294 bp (bases pairs) of the HVR-I (positions 16106–16399). PCRs were set up using the Qiagen Multiplex PCR kit in a final volume of 25 μL (12.5 μL Qiagen Multiplex PCR Master Mix, 9 μL RNAse-Free Water, primers at 2.5 mM and 3 μL DNA extract). Hot-start amplifications were carried out in a Multigene II Personal Thermal Cycler (Labnet International, Inc.), consisting of an initial denaturation at 95 °C for 15 min, 40 cycles of 30 s at 94 °C, 90 s at 55–59 °C, 90 s at 72 °C, and a 10-min final extension at

72 °C. Amplifications were checked in 2% agarose gels, and positive amplifications were purified using the QIAquick PCR Purification kit (Qiagen), obtaining a final volume of 30 μL.

*Sequencing and cloning.* Purified PCR products were directly sequenced in ABI Prism 310 Genetic Analyzer (Applied Biosystems, Life Technologies) or sent to the C.A.I. laboratory (Centro de Apoyo a la Investigación, Research Support Centre, Universidad Autónoma de Madrid, Cantoblanco, Madrid, Spain) and run in ABI Prism 3700 Genetic Analyzer (Applied Biosystems, Life Technologies). Consistent amplifications were cloned using the pGEM-T Easy Vector System (Promega) or TOPO TA Cloning kit (Invitrogen) following the manufacturer's instructions. Twenty colonies with insert were grown in a different plate. Presence of DNA insert was then double-checked by colony PCR using vector primers SP6 and T7. PCRs were performed in a final volume of 10 μL under standard conditions and reagents (Biotools B&M LAbs, S.A.). Amplifications were carried out in an Eppendorf Mastercycler PCR Thermalcycler (10-minute initial denaturation at 94 °C, 30 cycles at 94 °C for 60 s, 55 °C for 60 s and 72 °C for 60 s, and final extension at 72 °C for 10 min). Amplified DNA was then run in 2% agarose gels, and only products of right size were selected for purification. DNA was directly purified from bacterial colonies using the QIAprep Miniprep kit (Qiagen) and sent to the C.A.I. laboratory to be run in a ABI Prism 3700 Genetic Analyzer (Applied Biosystems, Life Technologies). See Table S7 (Supporting information) for cloning alignments.

*Consensus haplotype identification.* Sequencing electropherograms were read using the program Mutation Surveyor (Demo) version 3.24 (SoftGenetics, LLC). Sequences were then aligned and consensus haplotypes were established using the following criteria:

Only those haplotypes that were repeated between different samples from the same individual using independent DNA extractions and amplifications were considered as endogenous.
Consensus haplotypes had to be congruent within the two fragments and with SNP typing.
Haplotypes matching those from laboratory staff, archaeologists or anthropologists were not considered.
Miscoding lesions were identified and removed from consensus haplotypes.
Jumping PCRs and cross-contaminations were individually estimated for each sample.

For further details about consensus haplotypes, see clones alignments (Table S7, Supporting information).

*Haplogroup prediction and detection.* Haplogroup prediction was based on mutations of the HVR-I according to Richards *et al.* (2000) and van Oven & Kayser (2009). Ambiguities were resolved by comparison of consensus haplotypes with available modern mtDNA sequences collected in public databases (mtDNA manager, Lee *et al.* 2008) and publications (Richards *et al.* 2000). Haplogroups were then confirmed by the amplification of SNPs in the mtDNA coding region using specific primers (Table S6, Supporting information), following the constantly upgraded phylogeny available developed by van Oven & Kayser (2009). PCR amplification, gel electrophoresis, purification and sequencing were performed as described earlier.

### *Ancient DNA data analysis*

*Summary statistics ($F_{ST}$).* Following the recent studies of Haak *et al.* (2005) and Bramanti *et al.* (2009), we estimated pairwise genetic differentiation between three 'populations' using the $F_{ST}$ parameter (Reynolds *et al.* 1983; Slatkin 1995) considering two overlapping fragments spanning 255 bp on mitochondrial HVR-I (16126–16379) and excluding primer annealing region, using Arlequin software, version 3.5.1.2. The three population groups were as follows: (i) EN (present study, $N = 13$, Can Sadurní, Chaves and Sant Pau del Camp sites), (ii) MN (Sampietro *et al.* 2007; $N = 11$, Camí de Can Grau site) and (iii) a modern samples from the same region (García *et al.* 2011; $N = 363$). The modern data set is a pool of 'Aragón' ($N = 119$), 'Catalonia–Aragón' ($N = 164$) and 'Catalonia' ($N = 80$) populations, fully described in the Supplementary Information from García *et al.* (2011).

*Simulations and demographic models.* Simulations with aDNA data were performed using the BayeSSC program (Excoffier *et al.* 2000) available at http://www.stanford.edu/group/hadlylab/ssc/index.html.
This program uses the Bayesian version of the serial coalescent algorithm to run simulations on genealogies backward in time according to the demographic model tested. We simulated three different data sets (Early Neolithic, Middle Neolithic, Modern Catalonia and Aragón) to match the observed data set in terms of sample sizes and time, as described earlier. In this framework, calibrated years BCE have been transformed to BP by adding 1950.

The previous studies of Haak *et al.* (2005) and Bramanti *et al.* (2009) assumed one model of panmixia across wide geographical areas and for tens of thousands of years. Here, we explored three demographic models to test genetic continuity in the studied area: TPM (total panmixia model), SM (split model) and

SDGM (split with differential growth model) (Fig. 2). For all three models, we followed the general framework developed in the previous studies, where an Upper Palaeolithic population was randomly sampled from a hypothetical African source population with a constant female effective size of 5000, representing the first modern humans that settled in Europe 'out of Africa' around 45 000 BP. The first model, named total panmixia model (TPM), was the simplest scenario and represented a random mating population settled in northeastern Spain, showing genetic continuity from the Upper Palaeolithic until the present. Even though this model is not realistic, it was tested to compare this unstructured model with the other two structured scenarios implemented here. TPM has been already used by Haak *et al.* (2005) and Bramanti *et al.* (2009) to test genetic continuity in Central Europe. R. Rasteiro and L. Chikhi (submitted) have shown that it was not consistent with Central European data and that structured models explained the data better. Here, we follow R. Rasteiro and L. Chikhi (submitted) and use the same demographic models. The second model tested (split model, SM) was designed to represent a simple departure from the TPM and allow for some structure. This scenario assumed that the original populations that colonized Europe 45 000 years ago split in two populations of the same size (demes 1 and 2) that met 7500 years ago, when the Neolithic spread into this region. The two branches can be seen as representing the Palaeolithic background of northeastern Spain and the Palaeolithic population in which the Neolithic arose in Near East. The third model (split with differential growth model, SDGM) was designed to reflect a different scenario with a larger Neolithic contribution. The SDGM was based on the same assumptions as the previous one, but one of the two demes represents the Near Eastern Neolithic population that starts growing around 10 000 BP at a higher rate than the other 'Palaeolithic' deme. As in the SM, the two demes join around 7500 BP, but the Neolithic branch (deme 2) is assumed to reach a population size 20 times larger than that of the other branch (deme 1). To summarize, the SDGM and SM differ in the effective sizes of the two demes between 10 000 and 7500 BP: in the SM, demes 1 and 2 have the same effective size, while in the SDGM, the size of deme 1 is constrained to be 1/20th of deme 2 at time $t$ = 7500 BP (Fig. 2).

For all these models, we assumed a sequence length of 255 bp, a fixed mutation rate of $7.5 \times 10^{-6}$ per base pair and per generation (corresponding to $3 \times 10^{-7}$ substitution per base pair and per year, Endicott & Ho 2008), a transition over transversion bias of 0.9841, a uniform gamma distribution of mutations with a shape parameter of 0.205 (Bandelt *et al.* 2006) and 25 years per generation. The modern population effective size ($N_e$) was set to two million. This number corresponds to around one-sixth of the census size of the region sampled and represents the effective population size of mitochondrial DNA. To estimate this value, we considered that (i) the population census size of the studied area is around 12 million (Statistics National Institute, Spain, http://www.ine.es), (ii) mtDNA represents only the female population, being around one-half of the total, and (iii) just around one-third of the female population reproduces (Cela-Conde & Ayala 2007). Effective population sizes at Upper Palaeolithic (UP; 45 000 BP) and Neolithic (N; 7500 BP) periods were estimated to have been around 700 for the former and 15 000 for the latter. These values were calculated from estimated population densities, around 0.064 individuals per km$^2$ for hunter-gatherers and 20 times this value for farmers (Steele *et al.* 1998; Alroy 2001). Given the uncertainty on these values, we followed Bramanti *et al.* (2009) and used wide uniform prior distributions, spanning from 10 to 5000 for UP and from 200 to 100 000 for N, by simulating 1 million genealogies per model. Growth rates were computed on the basis of UP and N effective population sizes sampled from the priors.

We then compared the simulated and observed $F_{ST}$ (sim$F_{ST}$ and obs$F_{ST}$, respectively) under an approximate Bayesian computation (ABC) approach (Beaumont *et al.* 2002; Blum & François 2009), using the *abc* package (Csilléry *et al.* 2010) implemented in the R 2.10.1 version (R Development Core Team, 2009). Parameters were estimated by retaining 10 000 simulated values, associ-

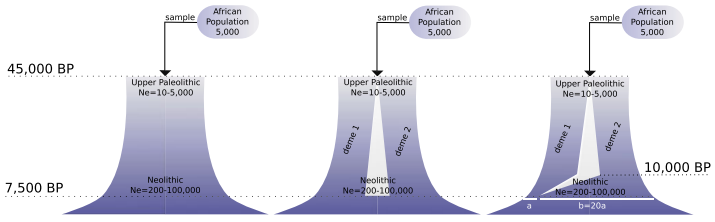



**Fig. 2** Demographic models explored. From left to right: TPM, total panmixia model; split model, SM; split with differential growth model, SDGM; $N_e$, effective population sizes ranges.

ated with the shortest Euclidean distances (tolerance = 0.01, corresponding to the 1% closest data sets). Post rejection adjustments were made using the *neuralnet* method and *logit* transformation (Blum & François 2009) available in the *abc* package (Table S4, Supporting information). On the basis of $N_e$ posterior distributions obtained with the ABC framework, we set 10 values equally distributed within the UP size range (10–5000) and 30 values for the *N* size (20 ranging between 200 and 20 000 and 10 between 20 000 and 100 000). Then, we again used BayeSSC to run 1000 simulations for each of these size combinations (10 × 30 = 300 combinations) and for each model (i.e. 900 000 simulations). Afterwards, we calculated for each parameter combination the probabilities of obtaining larger sim$F_{ST}$ values than observed (obs$F_{ST}$), under the three scenarios and plotted them using the *filled.countour* function in R version 2.10.1 (R Development Core Team, 2009), following previous studies using this approach (Bramanti *et al.* 2009; Malmström *et al.* 2009). Comparisons between sim$F_{ST}$ and obs$F_{ST}$ values within all $N$–$N_e$ values explored are shown in Fig. S1 (Supporting information). The ABC framework was also used to identify the model that best explains the observed $F_{ST}$, by computing posterior probabilities for each model (Beaumont 2008). To do this, we used the *postpr* function included in the *abc* package (Csilléry *et al.* 2010) in R version 2.10.1 (R Development Core Team, 2009), using a nonlinear conditional heteroscedastic method that applies a neural network approach (Blum & François 2009). This procedure was further validated by applying this approach to data sets for which the original model was known. We used 500 replicates within the 1 million simulated data set under each model (i.e. using the $F_{ST}$ of the replicates as pseudo-observed $F_{ST}$ values) and calculated how often the ABC procedure identified the correct model by a higher posterior probability (Table S4, Supporting information). We tested this approach when the three models were used together (i.e. choose one of three models) and in pairwise comparisons (TPM vs. SM and TPM vs. SDGM). Finally, we performed simulations by slightly changing the SM and SDGM models so as to have half of the aDNA samples in deme 1 or 2 by reducing the date at which the two demes join from 7500 to 7395 BP. Our aim was to determine whether the posterior probabilities of the structured models would change if we had had access to older aDNA samples.

## Results

### *Haplotypes and haplogroups*

We obtained consistent mitochondrial DNA results for 21 of 49 samples (42% success), corresponding to 13 of 22 individuals. The remaining 26 samples were discarded because of absence of results, lack of reproducibility or staff contamination. Samples recovered from caves (Can Sadurní and Chaves, 42% and 100% success, respectively) yielded a higher success rate than those from an open-air settlement (San Pau del Camp, 29% success), which agrees with earlier suggestions that caves offer stable temperature conditions all year round (de Torres *et al.* 2002) and protect samples from adverse climate conditions, such as rainfall (Hedges & Millard 1995).

Five different haplotypes were identified in the Can Sadurní archaeological site (Table 1). This information, combined with odontological age estimation (Table S1, Supporting information), allowed us to establish a minimum number of seven individuals, considering that samples with the same estimated age and haplotype were from the same individual. Altogether nine different haplotypes were found for the whole aDNA data set (Table 1). Most of them are currently widely distributed throughout Europe and belonged to the major European haplogroups H, K and U5. The first two haplogroups are currently distributed with high (H, ~46%) and moderate (K, ~6%) frequencies in Europe (Richards *et al.* 2000). U5 is currently present in around 9% of modern Europeans (Richards *et al.* 2000), and a recent aDNA analysis from Central European hunter-gatherers has shown that this haplogroup was present at a high frequency (64%) (Bramanti *et al.* 2009).

Three of the haplotypes were represented in more than one individual: 16224C, 16311C (one individual at each site), 16223T, 16362C (two individuals from the same site) and 16147T, 16223T, 16362C (two individuals from two different sites). These last two were assigned through SNP typing to haplogroup N*. This haplogroup was absent in MN and in the modern sample from northeastern Iberia (Table S2, Supporting information), but it has been previously detected in modern populations from the Near East and Eastern Europe (Table S3, Supporting information) and in a 25 000-year-old Cro-Magnon specimen from the Paglicci cave, southern Italy (Caramelli *et al.* 2003). However, haplogroup N* appears to be rare in modern populations as it could not be found in a combined search in most common databases (Richards *et al.* 2000; Lee *et al.* 2008; van Oven & Kayser 2009). We also identified the rare X1 branch in one Eastern Iberian Neolithic sample. Haplogroup X is found at low frequencies (2–3%) in Europeans, Near Easterners, North Africans and native Americans (Reidla *et al.* 2003). Subclade X1 has an early coalescence time (42 900 ± 11 900 BP) and is currently restricted to Northern and Eastern Africa and the Near East (Table S3, Supporting information) (Reidla *et al.*

**Table 1** Mitochondrial DNA haplotypes and haplogroups of the studied samples

| Site | Period | Date | Specimen | Haplotype | Single nucleotide polymorphisms typing | Haplogroup |
|---|---|---|---|---|---|---|
| Can Sadurní | Early Neolithic (Cardial) | 5475–5305 cal. BCE | CSA0511 | 16223T, 16362C | 5178C, 4833A, 10873T, 10398A, 10400C, 10238T | N* |
| Can Sadurní | Early Neolithic (Cardial) | 5475–5305 cal. BCE | CSA09 | 16223T, 16362C | 5178C, 4833A, 10873T, 10398A, 10400C, 10238T | N* |
| Can Sadurní | Early Neolithic (Cardial) | 5475–5305 cal. BCE | CSA152223 | 16224C, 16311C | 10550G | K |
| Can Sadurní | Early Neolithic (Cardial) | 5475–5305 cal. BCE | CSA16 | 16362C | 7028C | H |
| Can Sadurní | Early Neolithic (Cardial) | 5475–5305 cal. BCE | CSA24 | 16136C, 16192T, 16270T | 14766T, 3197C | U5 |
| Can Sadurní | Early Neolithic (Cardial) | 5475–5305 cal. BCE | CSA26 | 16183C, 16189C (het), 16223T, 16278T | 7028T, 1719G, 6371T | X1 |
| Can Sadurní | Early Neolithic (Cardial) | 5475–5305 cal. BCE | CSA29 | 16147T, 16223T, 16362C | 10398A, 10400C, 10873T, 10238T | N* |
| Chaves | Early Neolithic (Cardial) | 5329–4999 cal. BCE | 1CH0102 | 16224C, 16311C | 10550G | K |
| Chaves | Early Neolithic (Cardial) | 5329–4999 cal. BCE | 2CH0102 | CRS | 7028C | H |
| Chaves | Early Neolithic (Cardial) | 5329–4999 cal. BCE | 3CH01 | 16129A | 7028C | H |
| Sant Pau del Camp | Late Early Neolithic | 4250–3700 cal. BCE | 6SP0102 | 16224C, 16311C | 10550G | K |
| Sant Pau del Camp | Late Early Neolithic | 4250–3700 cal. BCE | 26SP0102 | 16218T, 16328A, (16362C) | 7028C | H20 |
| Sant Pau del Camp | Late Early Neolithic | 4250–3700 cal. BCE | 27SP0102 | 16147T, 16223T, 16362C | 10873T, 7028T, 10238T | N* |

In this table, mtDNA results are reported (haplotype, haplogroup and coding regions amplified), in correspondence of each specimen name, archaeological site and age.

2003). Only the branch X2 has been previously detected in ancient samples from French MN and Central European Corded Ware Culture (Haak *et al.* 2008; Deguilloux *et al.* 2011).

### *Genetic distances and simulation results*

Our EN samples were compared with published data from MN (Sampietro *et al.* 2007) and with modern populations (García *et al.* 2011) from the same geographical area. Differences in haplotype composition between EN and modern samples were reflected in the high and highly significant pairwise $F_{ST}$ value ($F_{ST} = 0.131$, $P < 10^{-5}$). A similarly high and significant value was observed between EN and MN populations ($F_{ST} = 0.101$, $P < 10^{-5}$). However, the MN and modern Iberian samples presented a lower and nonsignificant pairwise $F_{ST}$ value ($F_{ST} = 0.032$, $P = 0.072$), suggesting significant drift between the Early and Middle Neolithic but less between the latter and the present. Comparisons between sim$F_{ST}$ and obs$F_{ST}$ values are shown in Fig. 3. The left-hand panels (a.1–3) represent the comparison between MN and modern populations, whereas the central (b.1–3) and right-hand (c.1–3) panels correspond to the EN vs. modern-day and EN vs. MN samples. The top panels (a–c.1) correspond to the results under the TPM, whereas the central (a–c.2) and bottom (a–c.3) panels correspond to the SM and SDGM, respectively. The three models can explain the obs$F_{ST}$ between MN and modern-day samples for all population sizes tested (panels a.1–3 and Fig. S1, Supporting information). However, the obs$F_{ST}$ value between EN and modern populations can be only explained under small Neolithic population sizes (<1000 under the TPM and up to 2000 for SM and SDGM, panels b.1–3). When comparing EN and MN populations (panels c.1–3), it can be seen that structured models explain the observed statistics for a wider range of combinations of Neolithic effective population sizes than the unstructured model (TPM). Thus, to explain the three obs$F_{ST}$ values, the TPM only allows a very limited ranges of parameter values.

Table 2 shows the posterior probabilities for each of the three demographic models, estimated under an ABC model choice framework. The lowest posterior probability (16%) value was obtained for TPM, whereas posterior probability values were higher (>40%) for the structured models (SM and SDGM) when we compared the three models at once. When we compare the models by pairs, the posterior probabilities of the structured models are always much higher (>72%) than that of the TPM (<28%), whereas the difference between the two structured models is limited. These results clearly favour the structured models over the TPM. It is

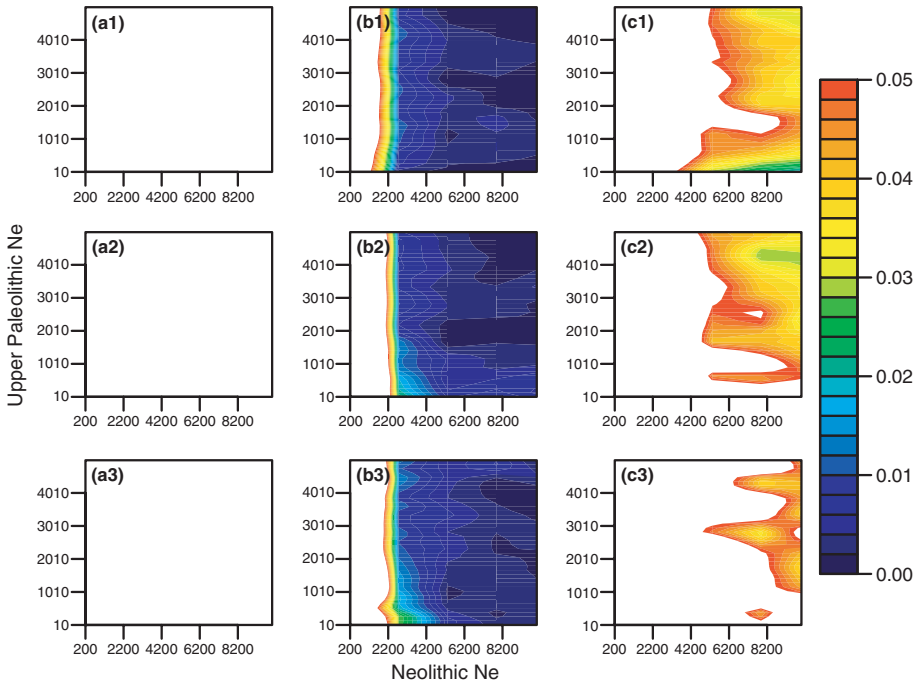



**Fig. 3** Probabilities of obtaining simulated $F_{ST}$ greater than observed. (a–c.1) TPM model, (a–c.2) SM model, (a–c.3) SDGM model, (a.1–3) MN and modern-day populations $F_{ST}$; (b.1–3) EN and modern-day populations $F_{ST}$; (c.1–3) MN and EN populations $F_{ST}$. Values higher than 0.05 are in white. $N$–$N_e$ range represented is up to 10 000. Plots with all $N$–$N_e$ values explored are available in Fig. S1 (Supporting information). UP-$N_e$, Upper Palaeolithic effective population size; $N$–$N_e$, Neolithic effective population size; TPM, total panmixia model; split model, SM; split with differential growth model, SDGM; MN, Middle Neolithic; EN, Early Neolithic.

**Table 2** Posterior probabilities of the three models (TPM, SM and SDGM)

| Models | Posterior model probabilities (%) |
|---|---|
| Total panmixia (TPM) | 16.1 |
| Split (SM) | 40.4 |
| Split with differential growth (SDGM) | 43.4 |

Posterior probabilities of the three models estimated using the approximate Bayesian computation (ABC) scheme described in the text. Posterior probabilities were obtained using the *neuralnet* method with a tolerance = 0.1, as implemented in the R *abc* package.

interesting to note that the support for the TPM is much lower than obtained in the validation under any of the models (Table S4, Supporting information), which again suggests that the TPM is a very unlikely model. Finally, when we modified the SM and SDGM to have aDNA samples in deme 1 or 2, we found that the posterior distributions for the structured models increased significantly (>93%).

## Discussion

For several decades, the Neolithic transition has been at the centre of ongoing controversies among archaeologists and geneticists, particularly since the advent of mtDNA and Y chromosome data in the 1990s (Richards *et al*. 1996; Barbujani *et al*. 1998; Semino *et al*. 2000; Chikhi *et al*. 2002; Richards 2003). Beyond the methodological disagreements among authors, it is important to note that several genetic studies, while defending one model of Neolithic spread, have acknowledged the possibility that different regions may have witnessed different processes (Barbujani & Chikhi 2006). They have thus called for regional studies to determine whether it is possible to separate demic from cultural processes using both modern and aDNA. Our study addresses exactly these issues by presenting the first aDNA evidence from the earliest Neolithic communities reaching the Iberian Peninsula.

This study demonstrates that it is possible to recover ancient endogenous DNA from temperate environments such as northeastern Iberia. Moreover, the results obtained suggest that aDNA preservation depends

more on depositional conditions than on sample age, in agreement with previous observations (Nielsen-Marsh & Hedges 2000).

Haplotype and haplogroup composition of EN and MN populations allow us to infer possible genetic relationships between populations and/or archaeological sites. For example, shared haplotypes among Can Sadurní and Sant Pau del Camp could point at a certain degree of genetic continuity during the Neolithic in northeastern Iberia. Regarding the haplogroup composition, modern and MN populations differ from EN samples, mostly due to the presence of rare haplogroups N* and X1 in the latter. These differences are reflected in high obs$F_{ST}$. Similarly, high sim$F_{ST}$ values could also be observed for a wide range of parameter values in the two structured models (SM and SDGM) but very rarely in TPM (Fig. 3). Within this range, the effect of genetic drift could have produced the loss of rare haplogroups N* and X1, as has been suggested for haplogroup N1a in Central Europe (Barbujani & Bertorelle 2001; Barbujani & Chikhi 2006; Haak *et al.* 2010; R. Rasteiro and L. Chikhi, submitted). This explains why our simulations favour the models considering previous population structure over an unstructured model (TPM) (Table 2). While the latter model is clearly too simplistic, it has been the one used in recent aDNA studies (Haak *et al.* 2005; Bramanti *et al.* 2009). Geographical and chronological origins of N* and X1 rare lineages found in Iberian EN are difficult to trace with current information. These are, however, currently present in the Near East (Table S3, Supporting information). The possibility that these haplogroups were carried by Neolithic immigrants from the Near East seems to be supported from an archaeological point of view. For example, affinities in certain burial rituals (Hodder 2007; Utrilla *et al.* 2008) have been detected along the Neolithic spread in Europe, including the Cardial culture. In this particular case, individual 1CH0102 (Chaves site) showed a Near Eastern burial ritual. Other haplogroups found at high frequency in our sample, such as H and K, could have also been introduced together with the Neolithic expansion (Barbujani *et al.* 1998; Barbujani & Chikhi 2006). Further aDNA data from Near Eastern Neolithic and Iberian Mesolithic specimens are needed, as well as a wider analysis of specimens belonging to the Cardial culture (western Italy, southern France, Mediterranean and Atlantic coast of the Iberian Peninsula) to better identify haplotypes and haplogroups that were present in different locations spanning the Near East and all of Europe. Our results allowed us to reject the TPM but did not allow us to easily separate the two structured models. We expect that the incorporation of new aDNA data from older northeastern Iberian Mesolithic and Near Eastern Neolithic sites could help distinguish them. Most of the samples used here were relatively recent in relation to the date at which demes 1 and 2 joined. However, when we modified this date to simulate the availability of more ancient aDNA, we found that it improved our ability to separate the models.

To conclude, we can tentatively propose a scenario that could explain our results, namely (i) the presence of currently rare haplogroups (N* and X1) in EN samples, (ii) high genetic drift during the period between the Early and Middle Neolithic, (iii) genetic affinities between EN and the Near East area and (iv) cultural connections with the Near East. This scenario would require that genetic drift played an important role at the beginning of the Neolithic with Near Eastern connections, hence pointing at a succession of pioneer colonization events from the Near East, which might point at other migration events along the Mediterranean, which might be identified in future studies.

## Acknowledgements

This work was supported by CGL2006-07828/BOS and CGL2009-07959 research projects (Ministry of Science and Innovation (MICINN), Spanish Government). Human resources were funded by an FPU grant (ref. AP2006-01586, Spanish Government) for C.G., a grant (ref. SFRH/BD/30821/2006, Fundação para a Ciência e Tecnologia, Portugal) for R.R. and a MICINN researcher contract 'Juan de la Cierva' for E.F. (partially supported by the European Social Fund). Replications analyses in Bordeaux have been partly supported by the CNRS (Centre National de Recherches Scientifiques). Funding was provided to LC by the ''Laboratoire d'Excellence (LABEX)'' entitled TULIP (ANR -10-LABX-41). We would like to thank all archaeologists and anthropologists who collaborated in this work: Anna Blasco, María Josefa Villalba, María Saña, Teresa Cabellos, Jordi Ruiz (Can Sadurní); Vicente Baldellou (Chaves); Josep Anfruns, Alejandro Pérez-Pérez, Mohammad Alrousan, Ferrán Estebaranz, Laura Martínez (Sant Pau del Camp). Thanks to Juan Álvarez for developping the sequence analysis program used here. The simulations were carried out in the HPC resources from CALMIP, Toulouse, France (Grant 2010-P1038).

## References

Alroy J (2001) A multispecies overkill simulation of the end-Pleistocene megafaunal mass extinction. *Science*, **292**, 1893–1896.

Bandelt H-J, Macaulay V and Richards M (eds) (2006) Estimation of mutation rates and coalescence times: some caveats. In: *Human Mitochondrial DNA and the Evolution of* Homo sapiens, vol. 18(part 1), pp. 47–90. Springer, Berlin, Heidelberg.

Barbujani G, Bertorelle G (2001) Genetics and the population history of Europe. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 22–25.

Barbujani G, Chikhi L (2006) DNAs from the European Neolithic. *Heredity*, **97**, 84–85.

Barbujani G, Bertorelle G, Chikhi L (1998) Evidence for Paleolithic and Neolithic gene flow in Europe. *American Journal of Human Genetics*, **62**, 488–492.

Beaumont MA (2008) Joint determination of topology, divergence time, and immigration in population trees. In: *Simulation, Genetics, and Human Prehistory* (eds Matsumura S, Forster P and Renfrew C), pp. 135–154. McDonald Institute for Archaeological Research, Cambridge.

Beaumont MA, Zhang W, Balding DJ (2002) Approximate Bayesian computation in population genetics. *Genetics*, **162**, 2025–2035.

Beaumont MA, Nielsen R, Robert C *et al.* (2010) In defence of model-based inference in phylogeography. *Molecular Ecology*, **19**, 436–446.

Bernabeu J (1997) Indigenism and migrationism. The neolithisation of the Iberian peninsula. *Documenta Praehistorica*, **XXIV**, 1–17.

Blasco A, Edo M, Villalba MJ, Saña M (2005) Primeros datos sobre la utilización sepulcral de la Cueva de Can Sadurní (Begues, Baix Llobregat) en el Neolítico Cardial. In: *Actas del III Congreso del Neolítico en la Península Ibérica* (eds Arias P, Otañón R and García-Moncó C), pp. 625–634. Servicio de Publicaciones, Universidad de Cantabria, Santander.

Blum MGB, François O (2009) Non-linear regression models for approximate Bayesian computation. *Statistics and Computing*, **20**, 63–73.

Bocquet-Appel J-P, Bar-Yosef O (2008) *The Neolithic Demographic Transition and its Consequences*. Springer, Dordrecht, The Netherlands.

Bramanti B, Thomas MG, Haak W *et al.* (2009) Genetic discontinuity between local hunter-gatherers and Central Europe's first farmers. *Science*, **326**, 137–140.

Brotherton P, Endicott P, Sanchez JJ *et al.* (2007) Novel high-resolution characterization of ancient DNA reveals C > U-type base modification events as the sole cause of post mortem miscoding lesions. *Nucleic Acids Research*, **35**, 5717–5728.

Caramelli D, Lalueza-Fox C, Vernesi C *et al.* (2003) Evidence for a genetic discontinuity between Neandertals and 24,000-year-old anatomically modern Europeans. *Proceedings of the National Academy of Sciences of the United States of America*, **100**, 6593–6597.

Cela-Conde CJ, Ayala FJ (2007) *Human Evolution: Trails from the Past*, 1st edn. Oxford University Press, New York.

Chikhi L, Nichols RA, Barbujani G, Beaumont MA (2002) Y genetic data support the Neolithic demic diffusion model. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 11008–11013.

Childe VG (1964) *The Dawn of European Civilization*, 6th edn. Vintage Books, New York.

Csilléry K, Blum MGB, Gaggiotti OE, François O (2010) Approximate Bayesian computation (ABC) in practice. *Trends in Ecology & Evolution*, **25**, 410–418.

Deguilloux M-F, Soler L, Pemonge M-H, Scarre C, Joussaume R, Laporte L (2011) News from the west: ancient DNA from a French megalithic burial chamber. *American Journal of Physical Anthropology*, **144**, 108–118.

Dupanloup I, Bertorelle G, Chikhi L, Barbujani G (2004) Estimating the impact of prehistoric admixture on the genome of Europeans. *Molecular Biology and Evolution*, **21**, 1361–1372.

Endicott P, Ho SYW (2008) A Bayesian evaluation of human mitochondrial substitution rates. *American Journal of Human Genetics*, **82**, 895–902.

Excoffier L, Novembre J, Schneider S (2000) Computer note. SIMCOAL: a general coalescent program for the simulation of molecular data in interconnected populations with arbitrary demography. *Journal of Heredity*, **91**, 506–509.

Fernández J, Gómez M (2009) Climate change and population dynamics during the Late Mesolithic and the Neolithic transition in Iberia. *Documenta Praehistorica*, **XXVIII**, 67–96.

García O, Fregel R, Larruga JM *et al.* (2011) Using mitochondrial DNA to test the hypothesis of a European post-glacial human recolonization from the Franco-Cantabrian refuge. *Heredity*, **106**, 37–45.

Gilbert MTP, Binladen J, Miller W *et al.* (2007) Recharacterization of ancient DNA miscoding lesions: insights in the era of sequencing-by-synthesis. *Nucleic Acids Research*, **35**, 1–10.

Haak W, Forster P, Bramanti B *et al.* (2005) Ancient DNA from the first European farmers in 7500-year-old Neolithic sites. *Science*, **310**, 1016–1018.

Haak W, Brandt G, de Jong HN *et al.* (2008) Ancient DNA, Strontium isotopes, and osteological analyses shed light on social and kinship organization of the Later Stone Age. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 18226–18231.

Haak W, Balanovsky O, Sanchez JJ *et al.* (2010) Ancient DNA from European early Neolithic farmers reveals their near eastern affinities. *PLoS Biology*, **8**, e1000536.

Hedges REM, Millard AR (1995) Bones and groundwater: towards the modelling of diagenetic processes. *Journal of Archaeological Science*, **22**, 155–164.

Hodder I (2007) *Excavating Catalhoyuk: South, North and KOPAL Area Reports from the 1995–99 Seasons*. McDonald Institute for Archaeological Research, Cambridge.

Lee HY, Song I, Ha E, Cho S-B, Yang WI, Shin K-J (2008) mtDNAmanager: a web-based tool for the management and quality analysis of mitochondrial DNA control-region sequences. *BMC Bioinformatics*, **9**, 483.

Malmström H, Gilbert MTP, Thomas MG *et al.* (2009) Ancient DNA reveals lack of continuity between Neolithic hunter-gatherers and contemporary scandinavians. *Current Biology*, **19**, 1758–1762.

Molist M, Vicente O, Farré R (2008) El jaciment de la caserna de Sant Pau del Camp: aproximació a la caracterització d'un assentament del neolític antic. *Quarhis*, **4**, 14–24.

Montes L, Utrilla P (2008) Le Paléolithique Supérieur dans la moyenne vallée de l'Ebre. *L'Anthropologie*, **112**, 168–181.

Nielsen-Marsh CM, Hedges REM (2000) Patterns of diagenesis in bone I: the effects of site environments. *Journal of Archaeological Science*, **27**, 1139–1150.

van Oven M, Kayser M (2009) Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation. *Human Mutation*, **30**, E386–E394.

Price TD (2000) *Europe's First Farmers*. Cambridge University Press, New York.

R Development Core Team (2009) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. Available at: http://www.R-project.org.

Reidla M, Kivisild T, Metspalu E *et al*. (2003) Origin and diffusion of mtDNA haplogroup X. *The American Journal of Human Genetics*, **73**, 1178–1190.
Reynolds J, Weir BS, Cockerham CC (1983) Estimation of the coancestry coefficient: basis for a short-term genetic distance. *Genetics*, **105**, 767–779.
Richards M (2003) The Neolithic invasion of Europe. *Annual Review of Anthropology*, **32**, 135–162.
Richards M, Côrte-Real H, Forster P *et al*. (1996) Paleolithic and neolithic lineages in the European mitochondrial gene pool. *American Journal of Human Genetics*, **59**, 185–203.
Richards M, Macaulay V, Hickey E *et al*. (2000) Tracing European founder lineages in the near Eastern mtDNA pool. *American Journal of Human Genetics*, **67**, 1251–1276.
Rohland N, Hofreiter M (2007) Ancient DNA extraction from bones and teeth. *Nature Protocols*, **2**, 1756–1762.
Sampietro ML, Lao O, Caramelli D *et al*. (2007) Palaeogenetic evidence supports a dual model of Neolithic spreading into Europe. *Proceedings of the Royal Society B: Biological Sciences*, **274**, 2161–2167.
Semino O, Passarino G, Oefner PJ *et al*. (2000) The genetic legacy of paleolithic *Homo sapiens sapiens* in Extant Europeans: a Y chromosome perspective. *Science*, **290**, 1155–1159.
Slatkin M (1995) A measure of population subdivision based on microsatellite allele frequencies. *Genetics*, **139**, 457–462.
Soares P, Achilli A, Semino O *et al*. (2010) The archaeogenetics of Europe. *Current Biology*, **20**, R174–R183.
Steele J, Adams J, Sluckin T (1998) Modelling Paleoindian dispersals. *World Archaeology*, **30**, 286–305.
de Torres T, Ortiz JE, Llamas FJ, Canoira L, Julia R, García-Martínez MJ (2002) Bear dentine aspartic acid racemization analysis: a proxy for the dating of Pleistocene cave infills. *Archaeometry*, **44**, 417–426.
Utrilla P, Lorenzo JI, Baldellou V, Sopena MC, Ayuso P (2008) Enterramiento masculino en fosa, cubierto de cantos rodados, en el neolítico antiguo de la cueva de Chaves. In: *IV Congreso del Neolítico Peninsular* (eds Hernández MS, Soler JA and López JA), vol. 2, pp. 131–140. Museo Arqueológico de Alicante (MARQ), Alicante.
Zilhão J (2001) Radiocarbon evidence for maritime pioneer colonization at the origins of farming in west Mediterranean Europe. *Proceedings of the National Academy of Sciences*, **98**, 14180–14185.
Zvelebil M (2001) The agricultural transition and the origins of Neolithic society in Europe. *Documenta Praehistorica*, **XXVIII**, 1–29.

CG, EF, MT, MFD, MHP and EAP are interested in using ancient DNA genetic analyses to gain insights into archaeological and demographic aspects of ancient human populations, particularly in the genetic impact of the Neolithic. CG, RR and LC are interested in applying model-based approaches to study the demographic past of populations, using either ancient and/or modern genetic data. PU, ME and MM are interested in the archaeological and demographic Prehistory of European, Near Eastern and Iberian populations.

## Data accessibility

Sequence alignments are available in the Supporting information.

## Supporting information

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

**Fig. S1** Plots with $N$–$N_e$ values explored.

**Table S1** Samples description.

**Table S2** Haplogroup frequencies in studied populations.

**Table S3** Haplogroup N* and X1 frequencies in the Near East.

**Table S4** Posterior distribution of population sizes and power to recover the true model.

**Table S5** Staff's mtDNA.

**Table S6** Primer sequences.

**Table S7** Cloned sequences alignments.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

# C. Appendix: Sex-biased migration in the Neolithic

## C.1 Details on the simulation framework

Our simulation framework, which is implemented in Java, owes much of its conception to SPLATCHE [Currat *et al.*, 2004]. They share significant features, but are complementary due to some differences that we detail below. Recently, an improved version of SPLATCHE (SPLATCHE2 [Ray *et al.*, 2010]) was published and we refer to it when necessary.

### C.1.1 Carrying Capacity and Friction

- Carrying capacity ($K$): number of individuals (which can be either males and females) that each deme can support under a logistic model of population growth (see main text in chapter 4, for details of the equation used in this study). In SPLATCHE only genes are considered and there are no difference between males and females.
- Friction ($F$): value associated to each deme that translates how difficult it is to migrate there. Friction values are used to compute the relative number of migrants that emigrate from a particular deme to its neighbours (see below). Friction values vary between 0 (very easy to go) to 1 (impossible to colonise).

### C.1.2 Admixture

- Admixture: corresponds to migration between layers. These events can be uni-or bi-directional but only take place between demes that have the same

coordinates in the different layers. The number of individuals that can migrate, between layers, is calculated using the formula developed in Currat and Excoffier [2005].

$$N_{ij} = N_i \gamma_{ij} \times \frac{2N_i N_j}{(N_i + N_j)^2} \tag{C.1}$$

Equation C.1 gives the number of individuals that migrate from layer *i* to layer *j*. These migrants integrate the new deme and take part to the reproduction phase in their new layer. In our framework we need to provide the admixture parameters ($\gamma_{ij}$ and $\gamma_{ji}$) from layers *i* to *j*, and from layer *j* to layer *i*, respectively. In our simulations, we considered layers 1 and 2 to host the *HG* and *Farmers* populations, respectively. We only allowed migration from 1 to 2, but not the other way around ($\gamma_{21}$ = 0). It is also important to add that SPLATCHE2 allows simulating competition and admixture in two layers scenarios.

### C.1.3 Logistic growth

The fact that our framework aims at simulating in a realistic manner the movement of individuals, rather than that of genes leads to several differences with SPLATCHE and SPLATCHE2, that are briefly presented here. In SPLATCHE and SPLATCHE2, the logistic growth is computed using the following equation,

$$N_{t+1} = N_t(1 + r\frac{k - N_t}{K}) \tag{C.2}$$

where $N_t$ is the size of a population in a the deme at time *t* and *r* is the growth rate [Currat & Excoffier, 2004]. Instead of using this formula we used the equation of Maynard-Smith and Slatkin [1973]:

$$N_{t+1} = N_t \frac{1 + r}{1 + r\frac{N_t}{K}} \tag{C.3}$$

Indeed, when $N_t >> K$, $N_{t+1}$ can become negative with equation C.2, but never with equation C.3. While this situation is rare when there is only one layer, it can

happen when there is admixture between layers that have different *K* values.
Both equations ignore sex and allow the foundation of a population by one individual (either male or female) or gene, which, outside mythological oddities, is unlikely to happen in humans. This can also lead to unrealistic growth rates when a population is founded by fewer females than males. In that case, applying equations (C.2) or (C.3) would lead to unrealistically high numbers of children per female, when *K* is high.
To avoid such situations, we only allow foundation events involving at least one male and one female and we corrected the logistic growth formula in (C.3), using the females as a limiting factor for population growth.

$$N_{t+1} = 2N_{f,t}\frac{1+r}{1+r\frac{2N_{f,t}}{K}} \tag{C.4}$$

where $N_{ft}$ is the number of females at generation *t*. Equation (C.4) behaves as equation (C.3) when the number of females is equal to the number of males. Another difference in our model is that growth is not deterministic, as the number of individuals in generation *t+1* is drawn from a Poisson distribution, with mean = $N_{t+1}$, as given by equation (C.4).

### C.1.4 Short range migrations

In all our simulations, we assumed that space was divided in demes according to a typical 2D stepping-stone model [Kimura & Weiss, 1964]. Migration could only take place in four different directions at most. For simplicity these directions were named according to the four cardinal points, North (N), East (E), South (S) and West (W). For each deme, the number of individuals that will emigrate is drawn from a Poisson distribution, with mean *M*, where *M* is given by the following equation,

$$M = N_t m\frac{n_d}{4} \tag{C.5}$$

where $N_t$ is the number of individuals that occupy the deme at time *t* and *m* is the migration rate that we define (*m = 0.25*), and $n_d$ is the number of neighbouring

demes to where it is possible to send migrants. Note that $n_d$ varies from a minimum of zero for an isolated deme to a maximum of four.
The migrants are distributed stochastically among the neighbouring demes using binomial distributions (*B(P, n)*) based on the following probability:

$$P_{dir} = \frac{1 - F_{dir}}{n_d - F_t} \tag{C.6}$$

where *dir* represents the direction (i.e. N, E, S, or W), $F_{dir}$ is the friction of the deme located in the direction *dir* considered and $F_t$ is the sum of the frictions of the $n_d$ receiving demes. For instance, in the case where the four cardinal points are accessible to migrants, their numbers are distributed as follows:

$$M_N = B\left(P_{dirN}, M\right) \tag{C.7a}$$

$$M_E = B\left(\frac{P_{dirE}}{P_{dirE} + P_{dirS} + P_{dirW}}, M - M_N\right) \tag{C.7b}$$

$$M_S = B\left(\frac{P_{dirS}}{P_{dirS} + P_{dirW}}, M - M_N - M_E\right) \tag{C.7c}$$

$$M_W = M - M_N - M_E - M_S \tag{C.7d}$$

To avoid any statistical bias, the order of the migration directions (N, E, S and W) is chosen randomly, for each deme and each generation. Once these calculations are made, the sex-ratio parameter is applied to determine how many males and females migrate in the different directions, as explained in the main text. Contrary to our framework, in SPLATCHE2 stochasticity in the migration model is not available when two layers are simulated.

## C.2 Simulation framework algorithm

Next is described our framework algorithm for each simulation.

**Algorithm C.1** Simulation framework algorithm

**foreach** *generation [1 to 1600 (total nb of generations)]* **do**
- **while** *Farmers layer expansion time (1200) is not reached* **do**
  - *In the HG layer:*
  - 1. Calculate number of individuals per deme (logistic growth);
  - **while** *the number of individuals is not reached* **do**
    - Take randomly one reproductive male and one reproductive female;
    - Their child receives randomly:
      - one sex chromosome from each parent
      - mtDNA from the mother
  - **end**
  - 2. Eliminate parental individuals;
  - 3. Calculate total number of mutations per generation and per deme;
  - **while** *the number of mutations is not reached* **do**
    - Take randomly one individual
    - add one mutation
  - **end**
  - 4. Migrations
    - (a) Calculate the total number of migrants
    - (b) Calculate number of female and male migrants
    - (c) Calculate the migration direction probability
    - (d) Send female and males individuals to each direction
- **end**
- **else**
  - **foreach** *layer [1 (HG) to 2 (Farmers)]* **do**
    - 1. Logistic growth;
    - 2. Eliminate parental individuals;
    - 3. Calculate total number of mutations per generation and per deme;
    - 4. Migrations;
  - **end**
  - 5. Unidirectional admixture (from HG layer to Farmers layer)
- **end**

**end**

## C.3 Mutation rates

The mutation rates for mtDNA and the Y-chromosome data were set to $4.5\times10^{-6}$ [Richards *et al.*, 2000] and $3\times10^{-8}$ [Xue *et al.*, 2009] per nucleotide per generation, respectively.

## C.4 Validation of the method

In order to test whether our simulation algorithm was producing reasonable results in agreement with analytical or simulation results by other programs/authors, we performed a series of simple simulations by assuming a single deme and comparing our results with those expected in a Wright-Fisher model (WFM). We simulated the genetic evolution of a population by considering one layer and one deme, with constant size, no migration and random mating. Under these conditions our model is very similar to a WFM, except that in our model we simulated actual diploid individuals rather than haploid genes, as is usually assumed. Also, since our model is stochastic, we expect some variation in the actual population size, which will never be perfectly constant, but will vary around $K$, the carrying capacity. We thus expected slight differences with the WFM. We simulated thus one deme with $K = 200$, during 2000 generations (to ensure equilibrium), and a starting population size of 100 individuals. For each individual we simulated DNA fragments with 100 bps each, for the two sex chromosomes, mtDNA and one autosome. The initial population genetic diversity was assumed to be zero. We did 1000 independent replicates of these simulations. After 2000 generations, we computed several genetic summary statistics and averages across the 1000 replicates to compare them with the expected value and those obtained using the ms [Hudson, 2002] software. The results of this simple validation showed that the average computed from our replicates was nearly-identical to the values expected for the different chromosomes (see figure S6) with different effective sizes. We should note however, that we also found that genetic diversity was slightly lower in our simulations than expected under the WFM. As noted above, this is expected for several reasons. First, the effective population size in our simulations was stochastically variable due to random variation

around $K$ and to the existence of sexes. As a consequence it is expected that the effective size should be lower than $K$, whereas it was exactly equal to $K$ in the WFM. Second, the mutation model assumed in the WFM is an infinite site model whereas in our simulations SNPs could mutate more than once. Third, since we computed our statistics using the whole population we were by definition sampling kin, which is assumed to be very unlikely in a random sample. Moreover, the coalescent assumes that the sample size is small compared to the population size, which means that it will be unlikely to sample related individuals/genes and hence is expected to produce larger diversity values. More work is needed to validate this and other complex programs.

## C.5 Supplementary Tables

**Table C.1: Sex ratio migration parameters values.** Different values were used for hunter-gatherers ($mSR_{HG}$) and farmers ($mSR_F$), generating a total of nine possible combinations for each of the five admixture values (see section 4.3.3 in chapter 4). Since the *HG* populations peopled Europe before the *Farmers* these scenarios can be seen as shifts in the patterns of residence when *HG* were replaced (with or without admixture) by the *Farmer* populations.

| Scenarios | $mSR_{HG}$ | $mSR_F$ |
|---|---|---|
| Matrilocal to matrilocal | 0.25 | 0.25 |
| Matrilocal to bilocal | 0.25 | 0.50 |
| Matrilocal to patrilocal | 0.25 | 0.75 |
| Bilocal to matrilocal | 0.50 | 0.25 |
| Bilocal to bilocal | 0.50 | 0.50 |
| Bilocal to patrilocal | 0.50 | 0.75 |
| Patrilocal to matrilocal | 0.75 | 0.25 |
| Patrilocal to bilocal | 0.75 | 0.50 |
| Patrilocal to patrilocal | 0.75 | 0.75 |

**Table C.2: Expected Heterozygosity among European populations** While the two studies used (Rosser *et al.* [2000] and Richards *et al.* [2000], for NRY and mtDNA, respectively) as real data have the same regions, sometimes wider populations were used. For example, we have Italy for NRY and the wider region Central Mediterranean area for mtDNA. These data was used for the regression lines in Fig. 4.2 and 4.3.

| Populations | NRY | mtDNA |
|---|---|---|
| Ossetia (NRY) Caucasus (mtDNA) | 0.685 | 0.828 |
| Armenia (NRY) Armenia (mtDNA) | 0.749 | 0.799 |
| Greece (NRY) East Mediterranean (mtDNA) | 0.776 | 0.747 |
| Bulgaria (NRY) South East Europe (mtDNA) | 0.740 | 0.769 |
| Romania (NRY) South East Europe (mtDNA) | 0.792 | 0.769 |
| Czech Republic (NRY) North and Central Europe (mtDNA) | 0.765 | 0.717 |
| Poland (NRY) North and Central Europe (mtDNA) | 0.639 | 0.717 |
| Germany (NRY) North and Central Europe (mtDNA) | 0.707 | 0.717 |
| Denmark (NRY) North and Central Europe (mtDNA) | 0.636 | 0.717 |
| Italy (NRY) Central Mediterranean (mtDNA) | 0.720 | 0.753 |
| Sardinia (NRY) Central Mediterranean (mtDNA) | 0.700 | 0.753 |
| Bavaria (NRY) Alps (mtDNA) | 0.693 | 0.715 |
| France (NRY) North West Europe (mtDNA) | 0.674 | 0.712 |
| Spain (NRY) West Mediterranean (mtDNA) | 0.505 | 0.704 |
| South Portugal (NRY) West Mediterranean (mtDNA) | 0.626 | 0.704 |
| North Portugal (NRY) West Mediterranean (mtDNA) | 0.573 | 0.704 |

**Table C.3: Genetic differentiation (measured by $F_{ST}$ values), among European populations.** While the two studies used (Rosser *et al.* [2000] and Richards *et al.* [2000], for NRY and mtDNA, respectively) as real data have the same regions, sometimes wider populations were used. For example, we have Italy for NRY and the wider region Central Mediterranean area for mtDNA. These data was used for the regression lines in Fig. 4.2 and 4.3.

| Populations | NRY | mtDNA |
|---|---|---|
| Ossetia (NRY) Caucasus (mtDNA) | 0.024 | 0.005 |
| Armenia (NRY) Armenia (mtDNA) | 0.005 | 0.004 |
| Greece (NRY) East Mediterranean (mtDNA) | 0.014 | 0.014 |
| Bulgaria (NRY) South East Europe (mtDNA) | 0.027 | 0.009 |
| Romania (NRY) South East Europe (mtDNA) | 0.011 | 0.009 |
| Czech Republic (NRY) North and Central Europe (mtDNA) | 0.051 | 0.022 |
| Poland (NRY) North and Central Europe (mtDNA) | 0.110 | 0.022 |
| Germany (NRY) North and Central Europe (mtDNA) | 0.064 | 0.022 |
| Denmark (NRY) North and Central Europe (mtDNA) | 0.057 | 0.022 |
| Italy (NRY) Central Mediterranean (mtDNA) | 0.028 | 0.012 |
| Sardinia (NRY) Central Mediterranean (mtDNA) | 0.053 | 0.012 |
| Bavaria (NRY) Alps (mtDNA) | 0.054 | 0.019 |
| France (NRY) North West Europe (mtDNA) | 0.056 | 0.024 |
| Spain (NRY) West Mediterranean (mtDNA) | 0.115 | 0.022 |
| South Portugal (NRY) West Mediterranean (mtDNA) | 0.068 | 0.022 |
| North Portugal (NRY) West Mediterranean (mtDNA) | 0.086 | 0.022 |

## C.6 Supplementary Figures

**Figure C.1 *(facing page)*: Genetic diversity under admixture scenarios** - This figure represents $H_e$ values that were computed for the demes located in the diagonal of the $10\times10$ lattice, between the starting deme (9_9) and the last to be colonized (0_0). The numbers represent the coordinates of the 9_9 and 0_0 demes. Since only diagonal demes were analysed they are simply represented by 9 and 0, respectively. To make the panels easier to read a line was drawn going through all demes between these two points, but their identifications are not represented. The panels are arranged in a way such that each of the columns corresponds to one value of the admixture parameter $\gamma$ (0, 0.25, 0.5, 0.75 and 1) and each of the rows corresponds to one post-marital residence system for the *Farmers* layer (the first, second and third rows correspond to scenarios where the *Farmers* are bilocal, patrilocal and matrilocal, respectively). In each panel the three possible scenarios for the *HG* layer are represented by a different colour (blue, black and green correspond to scenarios where *HG* are bilocal, patrilocal and matrilocal, respectively). In each panel the different lines correspond to different time points ($T$ = 1300, 1400, 1500 and 1600). The arrows show the overhall direction of the changes in the $H_e$ values from the older generation (1300) to the most recent one (1600). Cases where Y-chromosome and mtDNA have the same $H_e$ values would fall on the dashed line. The dotted line is the regression obtained for the real (i.e. observed) data.

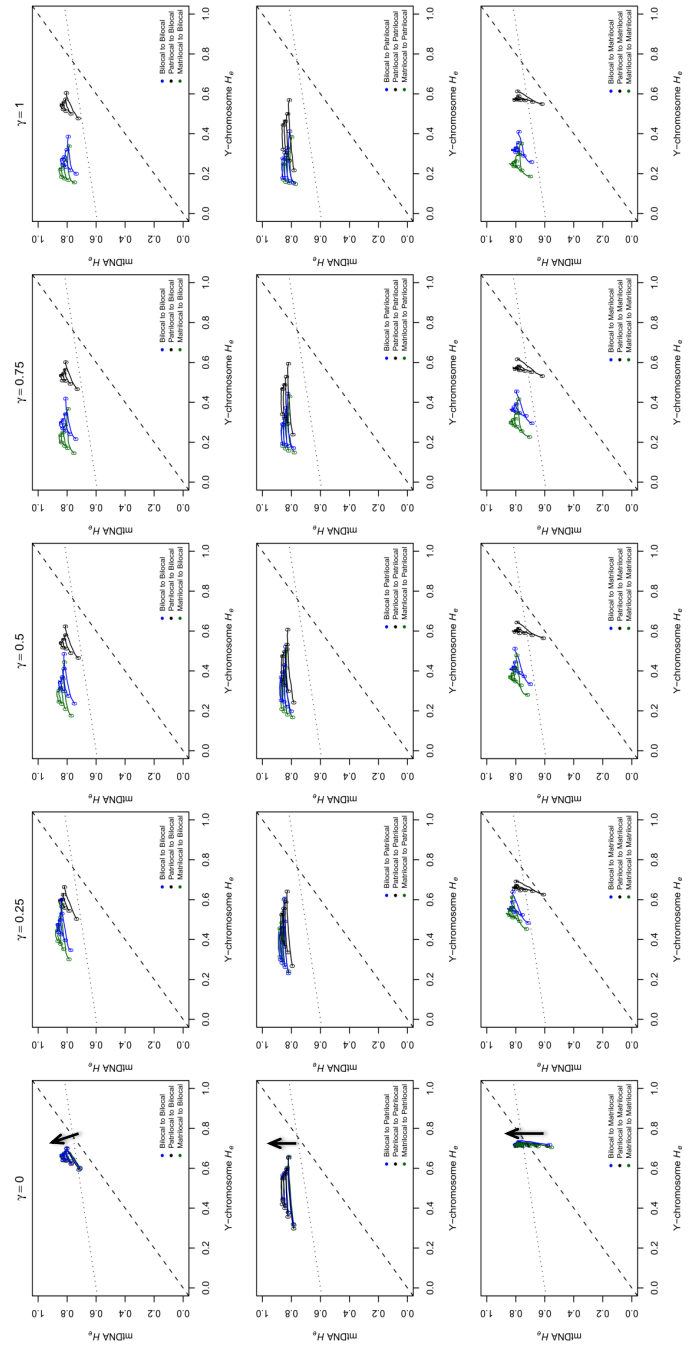

γ = 0
γ = 0.25
γ = 0.5
γ = 0.75
γ = 1
mtDNA $H_e$
Y−chromosome $H_e$
Bilocal to Bilocal
Patrilocal to Bilocal
Matrilocal to Bilocal
Bilocal to Patrilocal
Patrilocal to Patrilocal
Matrilocal to Patrilocal
Bilocal to Matrilocal
Patrilocal to Matrilocal
Matrilocal to Matrilocal

**Figure C.2 *(facing page)*: Genetic diversity in a 30×30 lattice, for patrilocal *Farmers* under admixture scenarios** - All $H_e$ values were computed for the diagonal of the 30×30 lattice, between the starting deme (29_29) and the last to be colonized (0_0). To make the figures easier to read a line was drawn going through all demes between these two points, but just the last deme is represented by 0. In each plot the three possible scenarios of the *HG* layer are represented. Each colour represents a different scenario in the *HG* layer (blue, black and green correspond to scenarios where *HG* are bilocal, patrilocal and matrilocal, respectively). Each line corresponds to a different time point ($T$ = 1400, 1500 and 1600), being the last/present-day generation (1600) a solid one. The dashed line corresponds to cases where Y-chromosome and mtDNA $H_e$ values are equal (x = y), whereas the dotted line is the regression obtained for the real observed data. The different panels correspond to different values of the admixture parameter $\gamma$ (0, 0.25, 0.5 and 0.75).

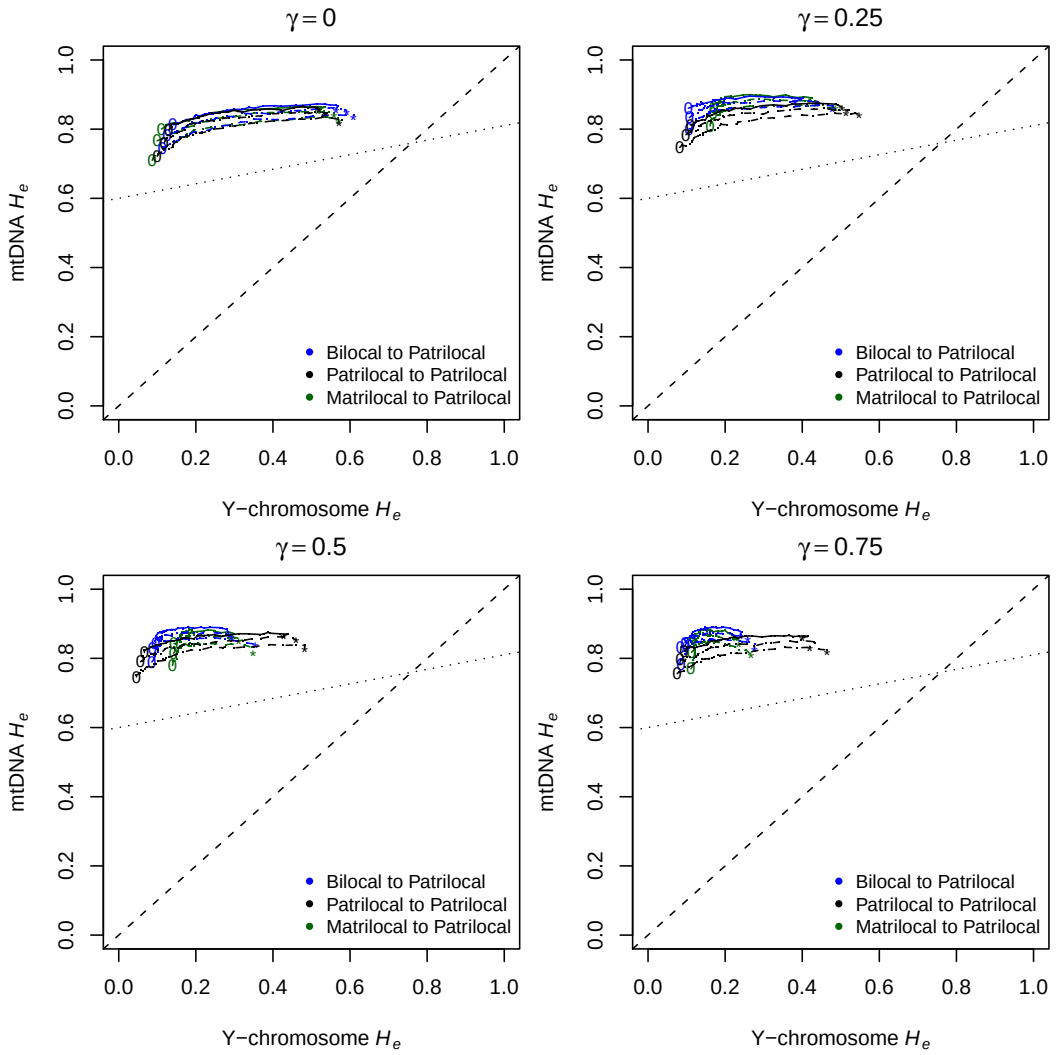

γ = 0
γ = 0.25
γ = 0.5
γ = 0.75
mtDNA $H_e$
Y−chromosome $H_e$
0.0
0.2
0.4
0.6
0.8
1.0
Bilocal to Patrilocal
Patrilocal to Patrilocal
Matrilocal to Patrilocal

**Figure C.3 *(facing page)*: Genetic differentiation in present-day populations under admixture scenarios** - This figure represents the $F_{ST}$ values in the last generation of the simulations corresponding to $T$ = 1600. All $F_{ST}$ values were computed between the starting deme (deme 9_9) and demes from the diagonal (8_8, 7_7, ..., 1_1, 0_0.). The numbers represent the coordinates of the diagonal of the $10 \times 10$ lattice, deme 8_8 being the closest to the starting deme and 0_0 the last to be colonized. Since only diagonal demes were analysed they are simply represented by 8 and 0, respectively. To make the panels easier to read a line was drawn going through all $F_{ST}$ values between these two points but the identifications of the other demes are not represented. Each colour represents a scenario (shades of grey, blue and pink correspond to scenarios where *Farmers* are bilocal, patrilocal and matrilocal, respectively). The dashed line corresponds to x = y thus to cases where Y-chromosome $F_{ST}$ values would be equal to the mtDNA $F_{ST}$ values and the dotted line is the regression obtained for the real observed data. The different panels correspond to different values of the admixture parameter $\gamma$ (0, 0.25, 0.5, 0.75 and 1).

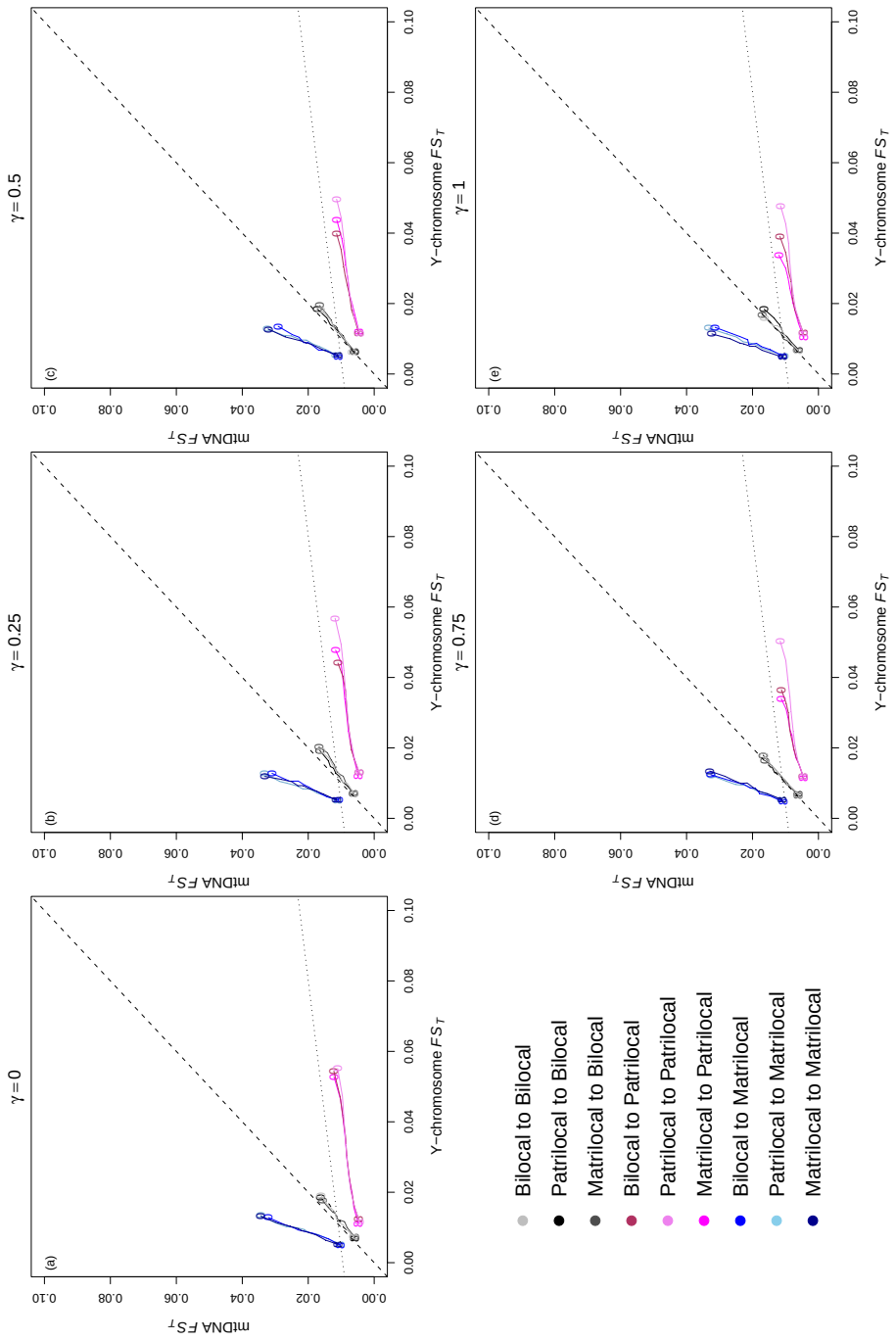

γ = 0
γ = 0.25
γ = 0.5
γ = 0.75
γ = 1
(a)
(b)
(c)
(d)
(e)
mtDNA $FS_T$
Y−chromosome $FS_T$
0.00
0.02
0.04
0.06
0.08
0.10
Bilocal to Bilocal
Patrilocal to Bilocal
Matrilocal to Bilocal
Bilocal to Patrilocal
Patrilocal to Patrilocal
Matrilocal to Patrilocal
Bilocal to Matrilocal
Patrilocal to Matrilocal
Matrilocal to Matrilocal

**Figure C.4 *(facing page)*: Genetic differentiation under admixture scenarios** - All $F_{ST}$ values were computed between the starting deme (9_9) and demes from the diagonal (8_8, 7_7, ..., 1_1, 0_0). The numbers represent the coordinates of the diagonal of the $10\times10$ lattice, deme 8_8 being the closest to the starting deme and 0_0 the last to be colonized. Since only diagonal demes were analysed they are simply represented by their row number (8 and 0, respectively). To make the panels easier to read a line was drawn going through all demes between these two points, but the other demes identifications are not represented. The panels are arranged in a way such that each of the five columns corresponds to one value of the admixture parameter $\gamma$ (0, 0.25, 0.5, 0.75 and 1) and each of the three rows corresponds to one post-marital residence system in the *Farmers* layer (the first, second and third rows correspond to scenarios where the *Farmers* are bilocal, patrilocal and matrilocal, respectively). In each plot the three possible scenarios of the *HG* layer are represented. Each colour represents a different scenario in the *HG* layer (blue, black and green correspond to scenarios where *HG* are bilocal, patrilocal and matrilocal, respectively).In each panel the different lines correspond to a different time point ($T$ = 1300, 1400, 1500 and 1600). The arrows show the changes in the $F_{ST}$ values from the older generation (1300) to the most recent one (1600). The dashed line corresponds to cases where Y-chromosome $F_{ST}$ values equal to the mtDNA $F_{ST}$ values and the dotted line is the regression obtained for the real (i.e. observed) data.

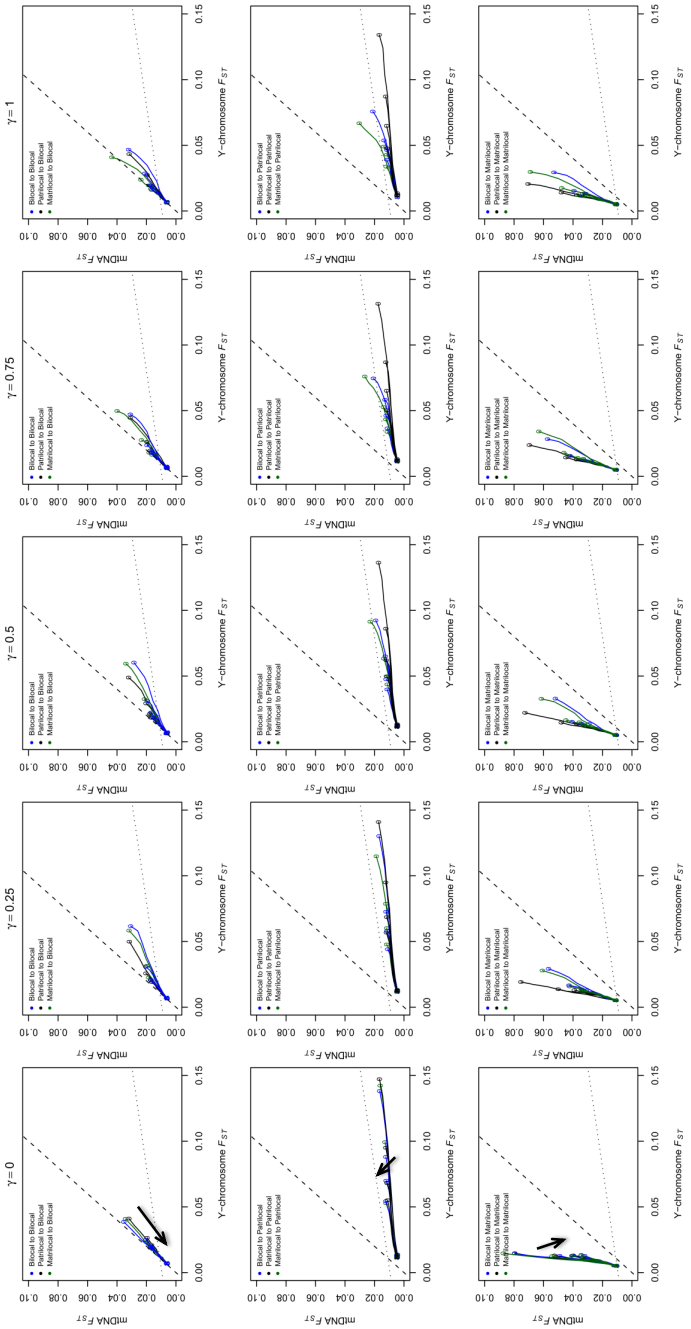
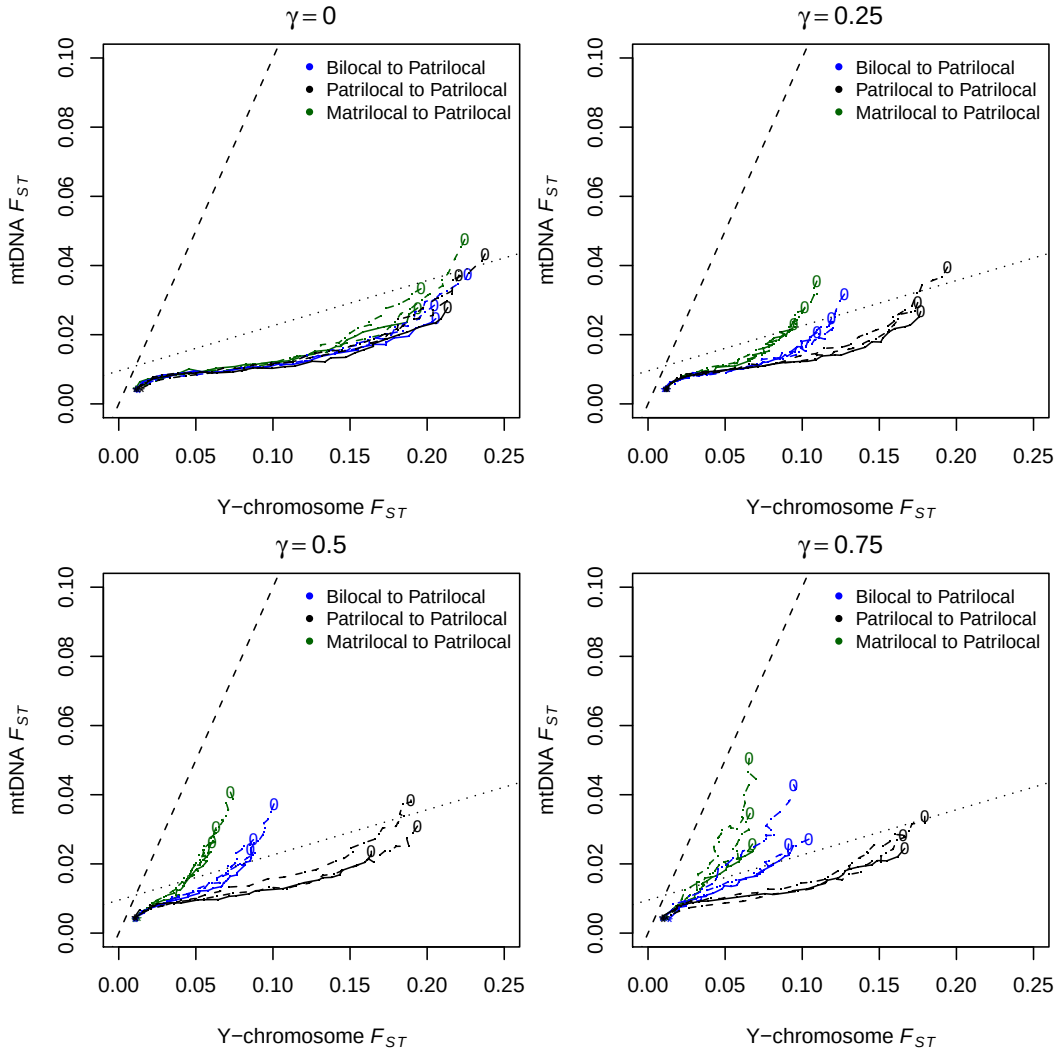

γ = 1
γ = 0.75
γ = 0.5
γ = 0.25
γ = 0
Bilocal to Bilocal
Patrilocal to Bilocal
Matrilocal to Bilocal
Bilocal to Patrilocal
Patrilocal to Patrilocal
Matrilocal to Patrilocal
Bilocal to Matrilocal
Patrilocal to Matrilocal
Matrilocal to Matrilocal
mtDNA $F_{ST}$
Y-chromosome $F_{ST}$
0.00
0.02
0.04
0.06
0.08
0.10
0.05
0.15

**Figure C.5 *(facing page)*: Genetic differentiation in a 30×30 lattice, for patrilocal *Farmers* under admixture scenarios** - All $F_{ST}$ values were computed values for the diagonal of the 30×30 lattice, between the starting deme (deme 29_29) and demes from the diagonal (28_28, 27_27, ..., 1_1, 0_0). To make the figures easier to read a line was drawn going through all demes between these two points, but just the last deme to be colonized is represented by 0. In each plot the three possible scenarios of the *HG* layer are represented. Each colour represents a different scenario in the *HG* layer (blue, black and green correspond to scenarios where *HG* are bilocal, patrilocal and matrilocal, respectively). Each line corresponds to a different time point ($T$ = 1400, 1500 and 1600), being the last/present-day generation (1600) a solid one. The dashed line corresponds to cases where Y-chromosome and mtDNA values are equal, whereas the dotted line is the regression obtained for the real observed data. The different panels correspond to different values of the admixture parameter $\gamma$ (0, 0.25, 0.5 and 0.75).


γ = 0
γ = 0.25
γ = 0.5
γ = 0.75
Bilocal to Patrilocal
Patrilocal to Patrilocal
Matrilocal to Patrilocal
mtDNA $F_{ST}$
Y−chromosome $F_{ST}$
0.00
0.02
0.04
0.06
0.08
0.10
0.05
0.15
0.20
0.25

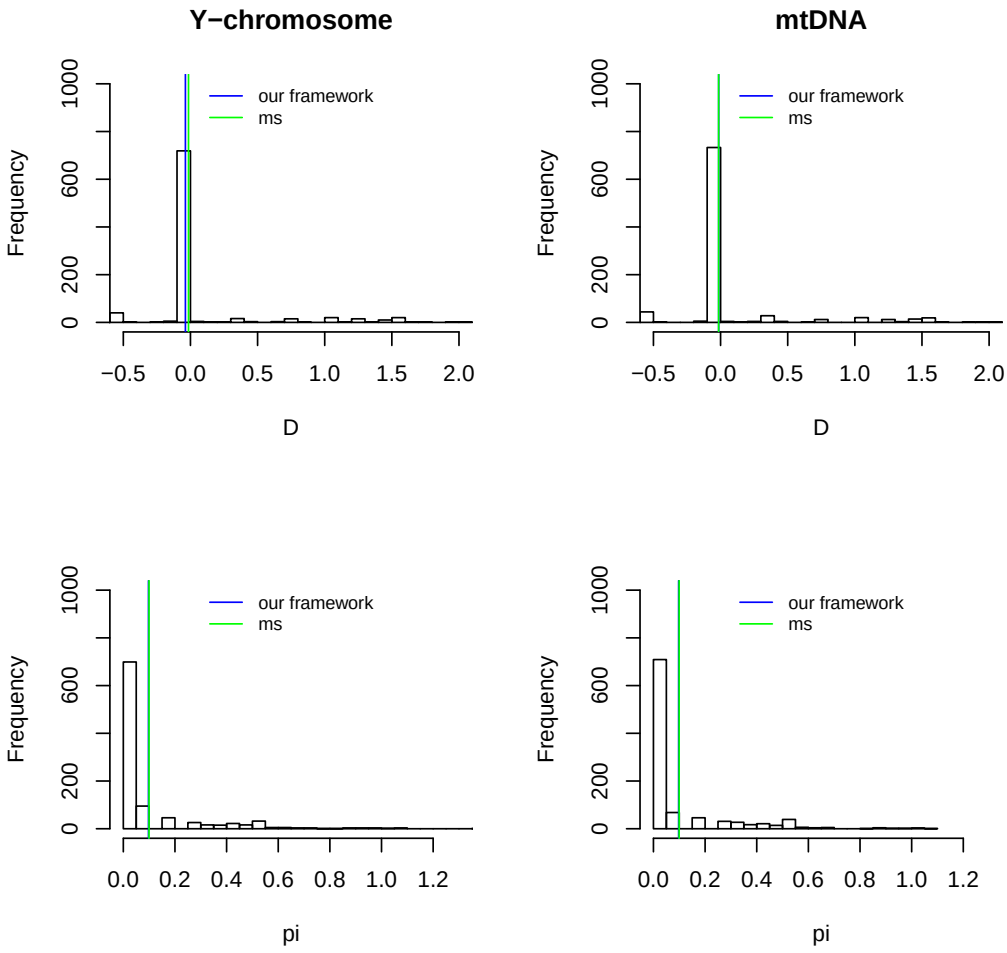
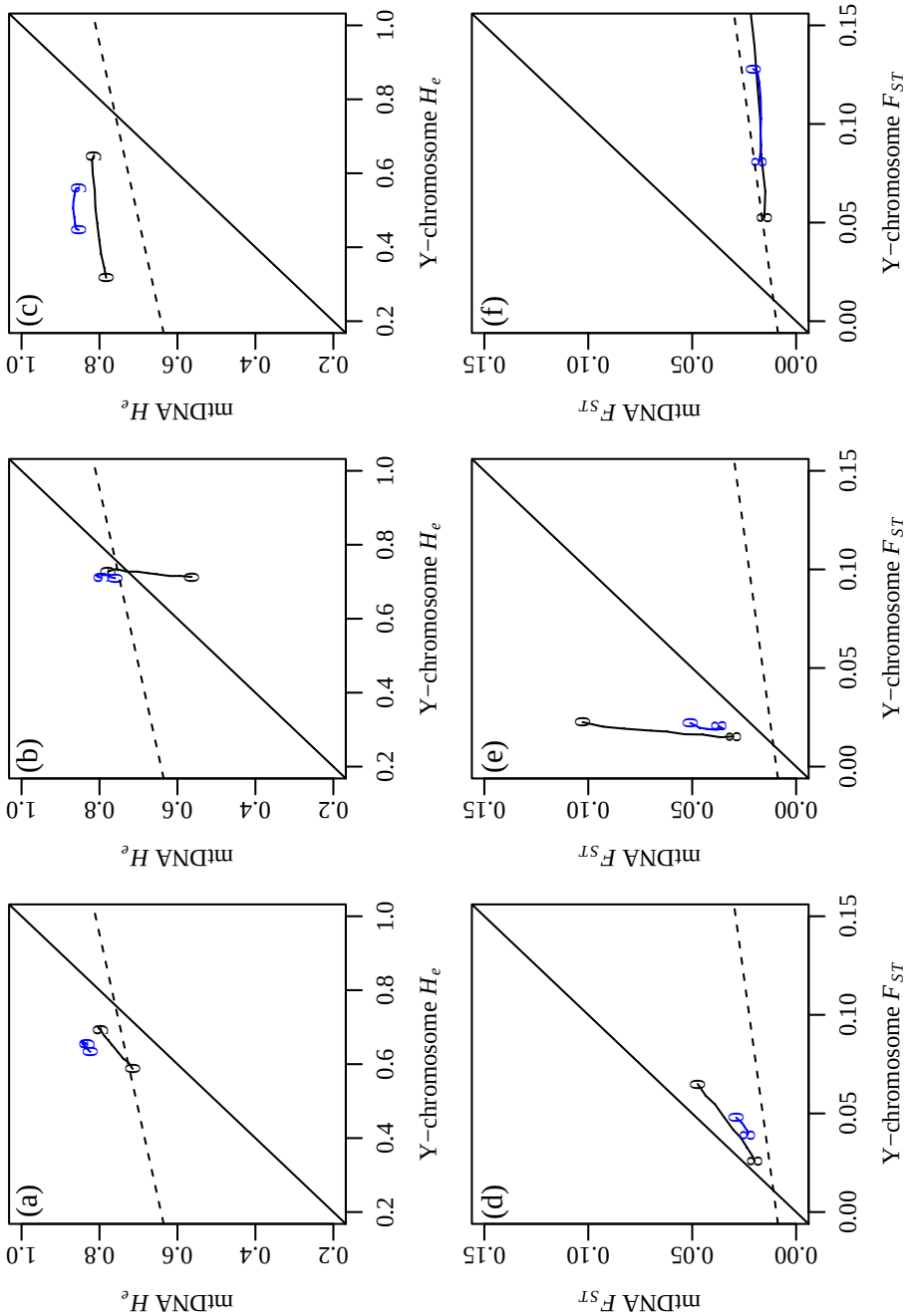



**Figure C.6: Framework validation** - This figure represents the Tajima D and $\pi$ distributions in the sampled DNA sequences across the 1000 independent simulations for the Y-chromosome and the mtDNA sequences. The green and blue vertical lines correspond to the average value for the simulations under a Wright-Fisher model using the framework presented in this study and ms, respectively (when the values are almost the same just one colour is visible in the plot). Similar and good results were also found for autosomes and X-chromosome and for segregation sites statistics.

**Figure C.7** ***(facing page)*****: Genetic diversity and differentiation in the no admixture scenarios, with a sampling scheme** - All the panels are similar to the ones of figure 4.2 and the same axes limits were used. However, a sampling scheme was used where 170 individuals were sampled for the mtDNA and 100 for the NRY. The solid line corresponds to cases where Y-chromosome and mtDNA values are equal, whereas the dashed line is the regression obtained for the real observed data.


(a)
(b)
(c)
(d)
(e)
(f)
mtDNA $H_e$
mtDNA $F_{ST}$
Y−chromosome $H_e$
Y−chromosome $F_{ST}$
0.2
0.4
0.6
0.8
1.0
0.00
0.05
0.10
0.15

# D. Appendix: SINS user guide

*The main purpose of this document is to quickly present the main functionalities of SINS. First, we present the demographic and genetic models implemented and then detail the input/output of the program. Finally we describe SINS-stat, a companion program developed to analyse the SINS outputs and explain how to run both programs.*

## D.1 General Introduction

SINS (for Simulating INdividuals in Space) is a new forward and individual-based spatial simulation approach that incorporates both geographical and demographic data, as well as several types of genetic markers. The general principle is very similar to that followed by the SPLATCHE and SPLATCHE2 software [Currat *et al.*, 2004; Ray *et al.*, 2010]. However, SINS is not based on the coalescent, but uses an individual-, rather than gene-, based forward simulation framework where the demographic and genetic simulations are carried simultaneously. While this makes our program computationally slower, it has several advantages:

- possible to track ancestral information;
- follow evolutionary processes through time and space, and not just the outcome;
- follow *multilocus* genotypes within individuals;
- model complex realistic biological demographic events easier (like variation in male and female migration rates) than in a coalescent framework.

## D.2 Demographic model

Space is assumed to be divided in demes according to a typical 2D stepping-stone model [Kimura & Weiss, 1964]. SINS allows to simulate different "layers", in the same geographical space as in Currat and Excoffier [Currat & Excoffier, 2004, 2005]. Each deme can exchange migrants, at a certain rate *m*, with up to four neighbours depending on its geographical location relative to the edges. Each deme is characterized by carrying capacity (*K*) and friction (*F*) parameters, which define the maximum population size and the difficulty to move into that deme ($0 \leq F \leq 1$), respectively. Carrying capacity and friction values can be different among demes and layers and can change with time. Density is logistically regulated within each deme, with intrinsic *K* and growth rate (*r*). Interaction between layers can be done either by competition and/or admixture.
For each generation the demographic events occur by the following order:

1. Logistic growth with/without competition
2. Migration (i.e. within layers)
3. Admixture (i.e. between layers)

Both competition and admixture are only modelled if more than one layer is simulated.

### D.2.1 Logistic Growth

SINS computes a corrected version of the Maynard-Smith and Slatkin [1973] equation for logistic growth (equation D.1), using the females as a limiting factor for population growth:

$$N_{t+1} = 2N_{f,t}\frac{1+r}{1+r\frac{2N_{f,t}}{K}}, \tag{D.1}$$

where $N_{t+1}$ is the total population size at generation *t+1*, $N_{f,t}$ is the number of reproductive females in the deme, at generation *t*, *r* is the growth rate and *K* is

the carrying capacity. In addition, foundation events are only allowed if they involve at least one male and one female. Growth is not deterministic, as the number of individuals in generation *t+1* is drawn from a Poisson distribution, with mean = $N_{t+1}$, as given by equation D.1.

### D.2.2 Migration

#### *D.2.2.1 Number of migrants*

Migration can only take place in four different directions at most. For each deme, the number of individuals that will emigrate is drawn from a Poisson distribution, with mean *M*, where *M* is given by the following equation,

$$M = N_t m \frac{n_d}{4} \tag{D.2}$$

where $N_t$ is the number of individuals that occupy the deme at time *t*, *m* is the migration rate and $n_d$ is the number of neighbouring demes to where it is possible to send migrants. Note that $n_d$ varies from a minimum of zero for an isolated deme to a maximum of four.

The migrants are distributed stochastically among the neighbouring demes using binomial distributions ($B(P, n)$) based on the following probability:

$$P_{dir} = \frac{1 - F_{dir}}{n_d - F_t} \tag{D.3}$$

where *dir* represents the direction, $F_{dir}$ is the friction of the deme located in the direction *dir* considered and $F_t$ is the sum of the frictions of the $n_d$ receiving demes. To avoid any statistical bias, the order of the migration directions is chosen randomly, for each deme and each generation.

#### *D.2.2.2 Sex-biased migration*

Once the number of migrants is calculated, for each direction, a sex-ratio parameter is applied to determine how many males and females will migrate in the different directions. The $mSR$ parameter (equationD.4) allows the user to vary male ($m_m$) and female ($m_f$) migration rates and simulate sex-biased migration.

$$mSR = \frac{m_f}{m_m + m_f} \tag{D.4}$$

Thus, if $mSR$ values are:

- $= 0.5$, the probability of males and females migrate is similar;
- $> 0.5$, females migrate more;
- $< 0.5$, males migrate more.

### D.2.3 Interaction between layers

*Both admixture and competition can be uni-or bi-directional, but only take place between demes that have the same coordinates in the different layers.*

#### *D.2.3.1 Competition*

The Lotka-Volterra model [Lotka, 1932; Volterra, 1931] is used to incorporate competition on logistic growth (equation D.5), such that for each layer $i$, the user can define several terms $\alpha_{ij}$ that give the pressure exerted by each layer $j$ over layer $i$. Thus, the size of deme $i$ at time $t+1$ is calculated as:

$$N_{i,t+1} = 2N_{f,i,t}\frac{1 + r_i}{1 + \sum_{j=1}^{nlayer} r_i \alpha_{ij} \frac{2N_{f,i,t}}{K_i}}, \tag{D.5}$$

where $N_{f,i,t}$ and $N_{f,j,t}$ are the number of females in the deme, at time $t$, in population $i$ and $j$, respectively.

#### *D.2.3.2 Admixture*

The number of individuals that can migrate between layers, is calculated using the formula developed in Currat and Excoffier [Currat & Excoffier, 2005]:

$$N_{ij,t} = N_{i,t}\gamma_{ij} \times \frac{2N_{i,t}N_{j,t}}{(N_{i,t} + N_{j,t})^2}. \tag{D.6}$$

where $N_{ij,t}$ is the number of individuals that migrate from layer $i$ to layer $j$, $N_{i,t}$ and $N_{j,t}$ are the number of individuals in layers $i$ and $j$, respectively and $\gamma_{ij}$ is an "admixture" or "interbreeding" parameter. These migrants integrate the new deme and take part in the reproduction phase in their new layer. The user needs to provide the admixture parameters ($\gamma_{ij}$ and $\gamma_{ji}$) from layers $i$ to $j$, and from layer $j$ to layer $i$, respectively. A value of zero indicates that the two layers do not admix, whereas a value of one means that the two layers exchange migrants as if they were in random mating [Currat & Excoffier, 2005].

## D.3 Genetic Model

SINS's genetic model is built in a forward framework and can simulate several types of molecular markers (sequences, SNPs and microsatellites). This is done defining genetic objects and assigning them to each individual. An individual is characterized by different genetic objects:

- Sex chromosomes define that the sex of the individual (XX or XY, for female or male respectively);
- mtDNA;
- *n* independent non recombining *loci* (two sequences with the same length), that for simplicity we will refer as "autosomes".

At the moment, the main assumptions of the genetic model of SINS are based on the Wright-Fisher model [Fisher, 1922; Wright, 1931]:

- non-overlapping generations (once the new generation is created, the parental

one is “eliminated”);
- no selection;
- random mating.

### D.3.1 Reproduction

Random mating is assumed by default and individuals are generated as described in algorithm D.1. However, it is possible to simulate the variance in the reproductive success of individuals, by giving as input the percentage of reproductive females and males (see section D.4.1).

**Algorithm D.1** Generating individuals

Calculate new number of individuals per deme (logistic growth)
**while** *the number of individuals is not reached* **do**
- Take randomly one reproductive male and one reproductive female of previous generation;
- Their child receives randomly:
  - one sex chromosome from each parent
  - mtDNA from the mother
  - for each "autosome", one sequence from each parent

**end**

### D.3.2 Mutation model

Once the new generation is created, equation D.7 is applied to calculate the total number of mutations, per deme and generations ($N_{mutation}$).

$$N_{mutation} \sim Poisson(N_{ind} \times \mu \times N_{marker}) \tag{D.7}$$

where $N_{ind}$ is the number of individuals in the deme, $\mu$ the mutation rate and $N_{marker}$ the length of the marker (number of SNPs or length of the sequence). Then, for each mutation an individual is randomly chosen to have that mutation (mutate from 0 to 1 or vice-versa). Microsatellites are simulated under a stepwise

mutation model (SMM).

## D.4 SINS organization and Settings

Due to the individual-based and forward nature of the simulation framework, both demographic and genetic parameters are simulated at the same time (Fig. D.1).

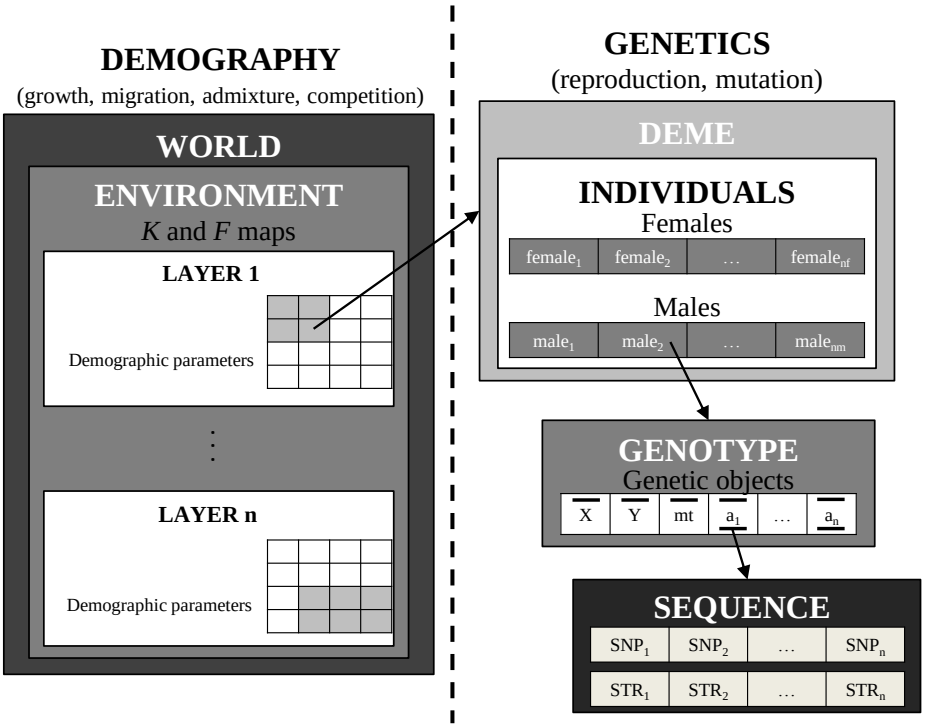



**Figure D.1: SINS organization** - Representation of the several demographic and genetic classes and objects that that constitute SINS and the correspondent demographic and genetic events.

Figure D.2 represents the inputs and outputs of SINS. To launch a simulation SINS needs several user-defined settings. They are basically contained in four major classes: world, environment, genetics and layer parameters. Once a simulation

is finished the output is divided in two main set of files corresponding to the demographic and genetic data. In the following sections, we describe in detail the structure of the input and output files.

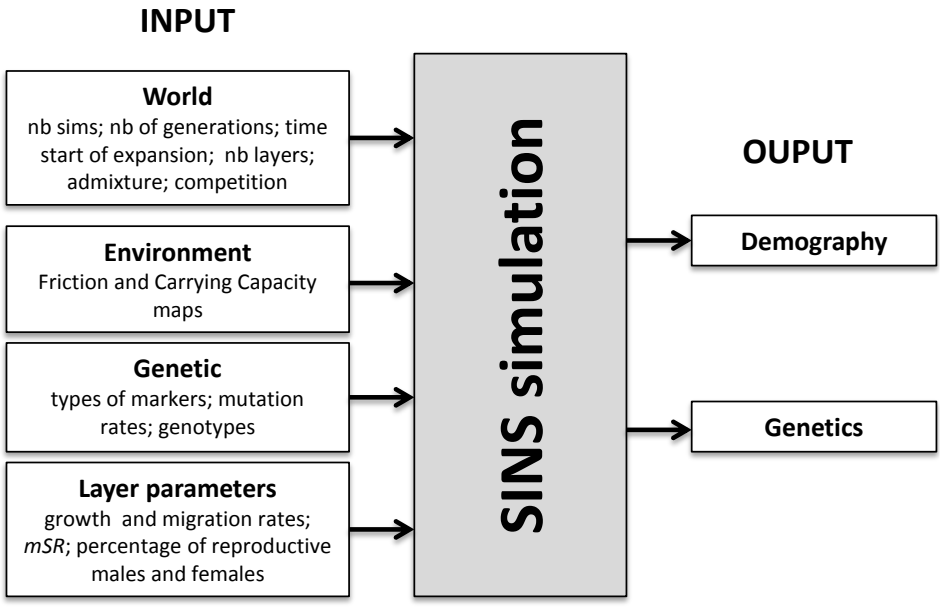



**Figure D.2: Inputs and outputs of SINS** - Representation of the several demographic and genetic parameters that are needed to launch SINS and its outputs.

*IMPORTANT: the input and output files are all in text format.*

### D.4.1 SINS Inputs

Figure D.3 shows the structure of the input files and folders. Sequentially, each box corresponds to a folder (with their name in bold), that in turn have sub-folders and/or text files. The input folder can contain several different <name of project> folders,

for each of the scenarios you want to simulate. In turn, the <name of project> folder has several sub-folders corresponding to different types of parameters called environment, genetic and layer, and two text files (world and output) that contain specific settings for the simulations.

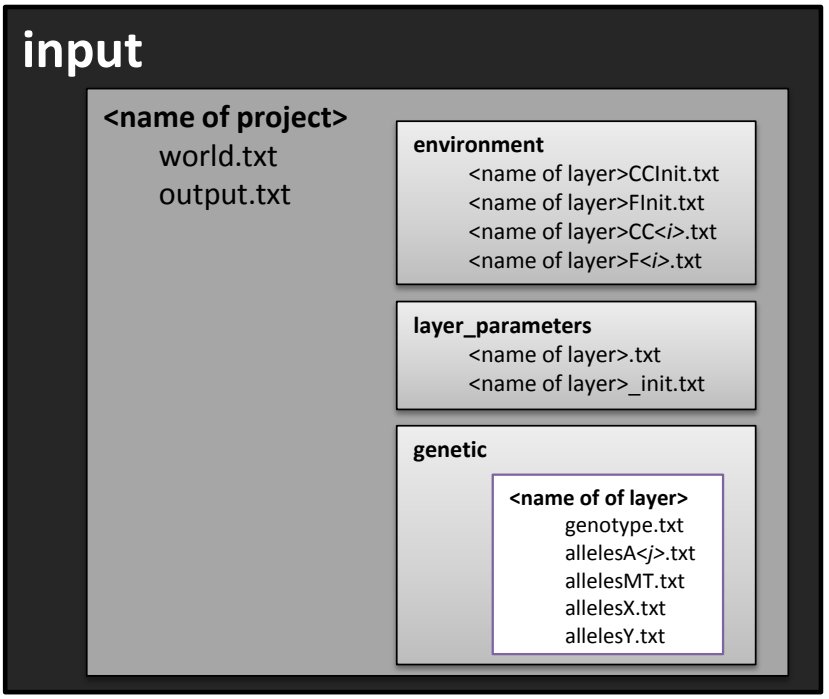



**Figure D.3: Input files and folders** - Organization of the input files and folders needed to launch SINS. Note that the names that appear between < > are labels set by the user (*i* = [1: total number of environmental changes] and *j* = [1: total number of "autosomes"]).

### *D.4.1.1 World and output files*

SINS requires two text files (world.txt and output.txt). The world.txt file has some of the main parameters defining the world to be simulated (number of simulations,

number of generations and number of layers) (Fig. D.4 and D.5). This file has a specific layout and both the order and number of parameters are important (algorithm D.2). The number of parameters depends on (i) the number of layers and on (ii) the number of environmental fluctuations, i.e. how many times the user wants to change the *K* and *F* maps.

**Algorithm D.2** How to create a world.txt file

numberOfSimulations `# Total number of simulations`
numberOfGenerations `# Total number of generations to be simulated`
numberOfLayers `# Total number of layers to be simulated`
**for** *l=0 to l=(numberOfLayers-1)* **do**
- layerName<l> `# Name layer l`
- expansionTime<l> `# Start of expansion time of layer l (in generations)`

**end**
**for** *i=0 to i=(numberOfLayers-1)* **do**
  **for** *j=0 to j=(numberOfLayers-1)* **do**
    - admixture<ij> `# admixture parameter (`$\gamma_{ij}$`), from layer i to layer j`

  **end**

**end**
**for** *i=0 to i=(numberOfLayers-1)* **do**
  **for** *j=0 to j=(numberOfLayers-1)* **do**
    - competition<ij> `# competition parameter (`$\alpha_{ij}$`), pressure exerted by layer j on layer i`

  **end**

**end**
numberOfEnvVarEvents `# Number of environmental variation events (number of times the` *K* `and` *F* `maps) are changed`
**for** *e=1 to e=numberOfEnvVarEvents* **do**
- EnvVarTime<e> `# Environmental variation events time (in generations)`

**end**

**Note:** Comments for each parameter appear after the # sign.

Figures D.4 and D.5 show simple examples of world.txt files for one and two layers, respectively.

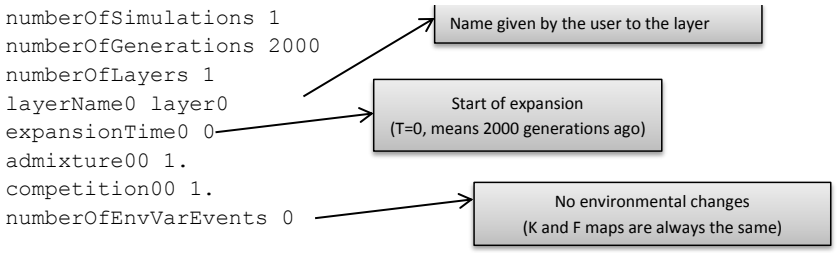



**Figure D.4: Example of a world.txt file, for a one-layer scenario** - In this example, SINS will simulate one layer (named layer0), that started to expand at time 0 and will run for 2000 generations. In addition, no environmental events are going to be simulated, i.e., the simulations will use always the same initial $K$ and $F$ maps, given in the appropriate folder. Because it is just one layer, there is no interaction between layers and the admixture and competition parameters are set to one, i.e. SINS uses a simple logistic growth.

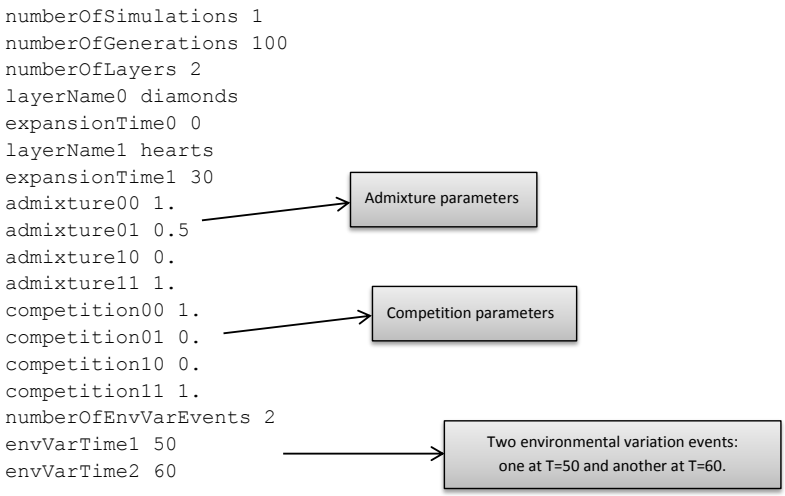



**Figure D.5: Example of a world.txt file, for a two-layer scenario** - In this example, SINS will simulate two layers, named diamonds and hearts, that started to expand at time 0 and 30, respectively, for 100 generations (the second layer hearts was empty until generation 30) In addition, two environmental events are going to be simulated, i.e. the simulations will change $K$ and $F$ maps at generations 50 and 60.This means that the user has defined three different set of $K$ and $F$ maps, that are located in the appropriated folders. In this example, admixture is unidirectional and the admixture parameter from layer diamonds to hearts is set to 0.5, whereas admixture in the other direction is set to zero. There is no competition between layers. Within layers, the admixture and competition layers are set to one (i.e. no competition and full admixture).

The output.txt file (Fig. D.6)is used by SINS to determine the type of output the user wishes. This is where the user defines the time intervals to record the genetic and demographic data and if the demographic data is recorded or not.

In both output.txt and world.txt files, each line corresponds to one parameter. Each line has a label, separated from the parameter value by a space. **This layout must be maintained.**

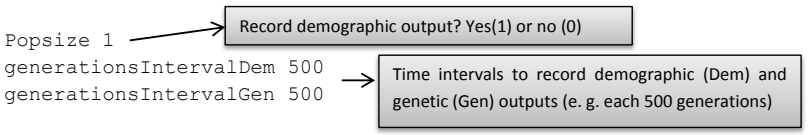



**Figure D.6: Example of an output.txt** - In this example, the demographic output, together with the genetic output, are recorded every 500 generations.

*IMPORTANT: the layer name specified in the world.txt file must be used to define the environmental, genetic and layer parameters. It is important to always use the same layer names.*

### *D.4.1.2 Environment folder*

The environment folder contains the *K* and *F* maps, for each layer and each environmental variation event. The maps are in a rectangular matrix format.
At the start of expansion or colonization of a layer, the files named <name of layer>CCInit.txt and <name of layer>FInit.txt were used by SINS to define *K* and *F* maps, respectively. If environmental variation events are defined in the world.txt file, additional set of *K* and *F* maps are required for each event. These maps are in files named <name of layer>CC<$i$>.txt and <name of layer>F<$i$>.txt, where $i$ = [1 to total number of environmental variation events].
Thus, for the example in Fig. D.5, the following files are required in the environmental folder:

- layer0 *K* maps: diamondsCCInit.txt; diamondsCC1.txt; diamondsCC2.txt
- layer0 *F* maps: diamondsFInit.txt; diamondsF1.txt; diamondsF2.txt
- layer1 *K* maps: heartsCCInit.txt; heartsCC1.txt; heartsCC2.txt
- layer1 *F* maps: heartsFInit.txt; heartsF1.txt; heartsF2.txt

### *D.4.1.3 Genetic Folder*

This folder contains subfolders named according to the <name of layer> defined in the world.txt file, each with a genotype.txt file (see algorithm D.3). The genotype.txt

file contains information about the genetic markers to be simulated. Optionally, files can be provideed by the user specifying the initial allele frequencies of the markers simulated (see Fig. D.3). When no allele frequency file is given (algorithm D.3 and Fig. D.7) the simulations will start with diversity zero.

**Algorithm D.3** How to create a genotype.txt file

Xlength `#length of X chromosome marker`
typeX `#type of X chromosome marker`
Ylength `#length of Y-chromosome marker`
typeY `#type of Y-chromosome marker`
mtDNAlength `#length of mtDNA`
typeMT `#type of mtDNA marker`
nbAutosomes `#total number of autosomes`
**for** *a=1 to a=numberAutosomes* **do**
- A<a>length `#length of autosome <a> marker`
- typeA<a> `#type of autosome <a> marker`

**end**
XmutationRate `#X chromosome mutation rate`
YmutationRate `#Y-chromosome mutation rate`
mtDNAmutationRate `# mtDNA mutation rate`
**for** *a=1 to a=numberAutosome* **do**
- A<a>mutationRate `#autosome<a> mutation rate`

**end**

**Note:** Comments for each parameter appear after the # sign.

The allele frequencies files are written in the format presented in Fig. D.8. The first line corresponds to the total number of alleles/haplotypes and the following lines to the frequencies of each one. Thus, the first number corresponds to the frequency itself and the followings to the allele/haplotype with a length equal to the one defined in the genotype.txt file.

```
Xlength 1
typeX microsat
Ylength 1
typeY microsat
mtDNAlength 10
typeMT seqSNP
nbAutosomes 2
A1length 1
typeA1 microsat
A2length 1
typeA2 microsat
XmutationRate 0.01
YmutationRate 0.03
mtDNAmutationRate 0.00005
A1mutationRate 0.05
A2mutationRate 0.05
```

Corresponds to the number of microsatellites or SNPs

Mutation rates for each marker

**Figure D.7: Example of a genotype.txt file** - This where the user defines the type of markers (*microsat* or *seqSNP* for microsatellites and sequences or SNPs, respectively), for each *locus*, their length and mutation rates. In this example, SINS will simulate one microsattelite for two "autosomes", for the X and Y-chromosomes and a sequence of length ten for mtDNA

(a)

```
nbAllelesMT 3
0.3 0 0 0 0 0 1 0 0 1 0
0.6 0 0 0 0 1 1 0 0 0 0
0.1 1 1 1 0 0 1 0 0 1 0
```

(b)

```
nbAllelesA2 1
1. 30
```

**Figure D.8: Allele files** - Example of allele frequencies files for **(a)** SNPs and **(b)** microsatellites. In **(a)**, is represented an allelesMT.txt file with three different alleles, with frequencies 0.3, 0.6 and 0.1, respectively. Note that the length of the sequence is 10, as it must to be equal to the value defined in the genotype.txt file (see Fig. D.7). The allelesA2.txt file represented in **(b)**, has just one allele with frequency and length one.

The genetic make-up of founding populations can be taken from pre-specified allele frequencies, which can thus be obtained from observed or simulated data. When several layers are simulated, it is also possible to found a new population by sampling the corresponding deme from another layer (see section D.4.1.4).

In SINS, SNPs and sequences are represented (Fig. D.8a) using a binary notation (0 and 1 for ancestral and derived mutation, respectively). Microsatellites are modelled adding or subtracting one repeat, under SMM. Thus, the size of the repeat does not matter to the program and the user must take in account this issue. While it is not important for the analysis, in order not to have negative values in the microsatellites the user can start with a higher value of repeats (see Fig. D.8b).

### *D.4.1.4 Layer parameters folder*

This where the layers' parameters(growth and migration rates, mSR and percentage of reproductive males and females) are defined (<name of layer>.txt file, see Fig. D.9) and how a layer is founded when more than one layer is simulated (settling of the layer).

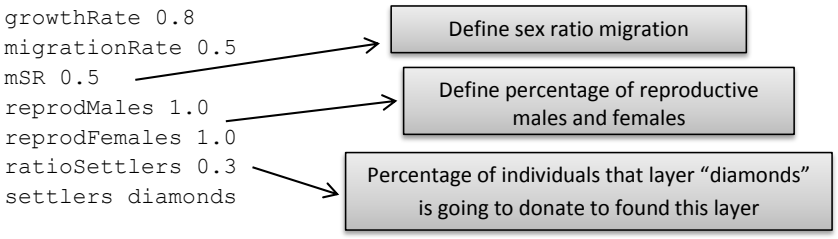



**Figure D.9: Example of a <name of layer>.txt** - In this file, the parameters of the layer are defined by the user. Note that the label of the file should correspond to the label defined in the world.txt file

SINS can found a layer by a sampling individuals from another one. In that case,

the user should set the percentage of individuals that are going to move and the name of the layer of origin (Figure 9), using the $ratioSettlers$ parameter. If on the other hand, the user does not want a founding event from another layer the $ratioSettlers$ must be zero and the program will use the genetic input defined in the genetic folder.
In the <name of layer>_init.txt file, the starting demes of expansion are going to be defined. This file has a rectangular matrix similar to the ones in the *K* maps files in the environment folder. However, all demes must have values equal to zero, except the ones where the founding events start. Thus, if the $ratioSettlers > 0$ the source demes just need a number different than zero. If the $ratioSettlers = 0$, then the user should choose a start deme size ($\leqslant K$), remembering that the initial genetic diversity is going to be taken from the input in the genetic folder.

*Once the input files are correctly written and placed, you are ready to run SINS (see section D.7).*

### D.4.2 SINS Outputs

SINS produces demographic and genetic outputs which can be tailored to the user's wishes. All outputs are recorded inside the results folder. If the this folder does not exist, SINS automatically creates it. SINS creates automatically a <name of project> subfolder, with the label used in the input folder (Fig. D.10).Each simulation has its own folder.

#### *D.4.2.1 Demographic output*

The demographic output is a single text file, named dem.txt (Fig. D.11). This file contains the number of individuals recorded for each deme, layer and generation time. Depending on the user's input files options, these numbers can be saved at pre-specified generations (for instance, every 10, 50 or 500 generations) or for all

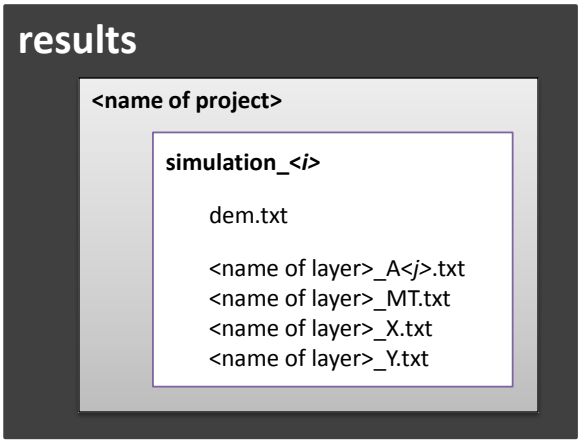



**Figure D.10: Output folders and files generated by SINS** - SINS generates a results folder, which contains a subfolder per scenario (project) simulated. SINS uses the labels that were defined by the user ($i$ = [1: total number of simulations] and $j$ = [1: total number of autosomes])

generations. Beware that this file can be huge.

#### *D.4.2.2 Genetic output*

For each layer, the genetic outputs are divided by chromosome/*locus* (D.10). For each *locus* one file is created with the genotypes of all individuals for all demes and time steps pre-specified by the user (Fig.D.12). Each individual is identified by its layer, deme, sex and time step. Moreover, the parents of each individual are also recorded, together with their original birth deme. Both the individuals' and parents' labels are built with a S_I_L_R_C structure:

- S: sex of the individual (M or F for male or females, respectively);
- I: individual index given by the program to each individual in a deme;

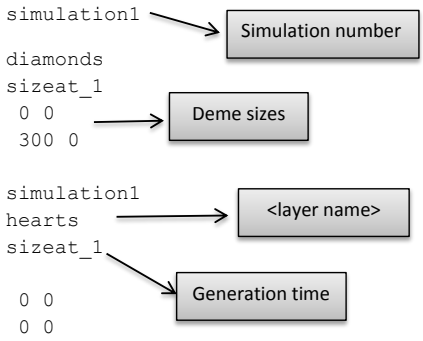



**Figure D.11: Demography output** - Example of a dem.txt file. In this file, the demographic output is recorded, for each simulation, layer and generation interval that was set by the user. In this example, a 2×2 matrix is simulated for two layers. Thus at generation 1 (sizeat_1), *diamonds* layer have 300 individuals at the bottom-left corner deme of the matrix (this is the founding deme), whereas the *hearts* layer is empty.

- L: layer of birth. From 0 to the [total number of layers -1];
- R and C: coordinates of the deme of birth (row and column of matrices, respectively).

| generation | deme | Individuals | Parents | genotype |
|---|---|---|---|---|
| 10 | 0 0 | F_1_0_0_0 | F_369_0_0_0 M_40_0_0_0 | 300 |
| 10 | 0 0 | F_2_0_0_0 | F_306_0_0_0 M_40_0_0_0 | 301 |
| 10 | 0 0 | M_3_0_0_0 | F_33_0_0_0 M_61_0_0_0 | 299 |
| 10 | 0 0 | M_4_0_0_0 | F_0_0_0_0 M_303_0_0_0 | 300 |
| 10 | 0 0 | M_5_0_0_0 | F_433_0_1_0 M_61_0_0_0 | 301 |
| 10 | 0 0 | M_6_0_0_0 | F_65_0_0_0 M_150_0_0_0 | 300 |
| 10 | 1 1 | F_415_0_1_1 | F_46_0_1_1 M_60_0_1_1 | 300 |
| 10 | 1 1 | F_416_0_1_1 | F_268_0_1_1 M_236_0_1_1 | 302 |

**Figure D.12: Genetic output** -

The genetic output files are organized as exemplified in Fig. D.12. In the first column is the generation time, the second and third columns correspond to the deme coordinates (row and column of the *K* and *F* matrices, respectively). The fourth, fifth and sixth columns correspond to the individuals and their mother and father labels, respectively, followed by the individual genotype.

*IMPORTANT: The numbering of both layers and deme coordinates starts at zero.*

## D.5 SINS-stat: sampling and genetic analysis

A companion package (SINS-stat) is also available to sample individuals from specified demes and layers and to compute single locus population genetic statistics (Fig. D.13).

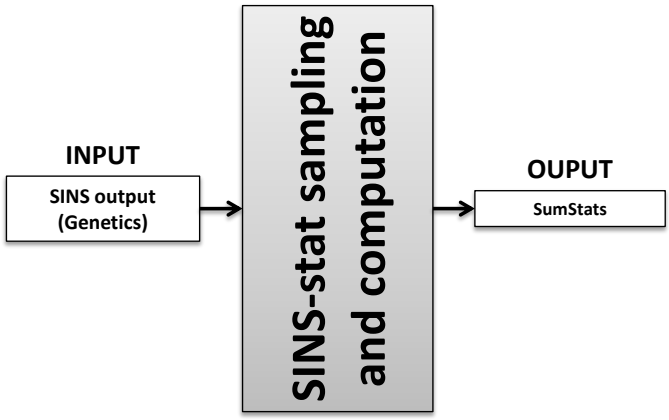



**Figure D.13: SINS-stat** - Representation of a SINS-stat sampling and computation.

### D.5.1 SINS-stat inputs

Similarly to SINS, SINS-stat requires a specific organization of the input files and folders that should be created by the user. Fig. D.14 shows this structure, with each box corresponding to a folder (with their name in bold), that in turn have other folders and/or text files. The general SINS-stat folder (stats) can contain several different <name of project> folders, for each of the scenarios simulated by SINS. In turn, the <name of project> folder has an input subfolder, which holds a generations.txt and sampling files (Fig. D.14).

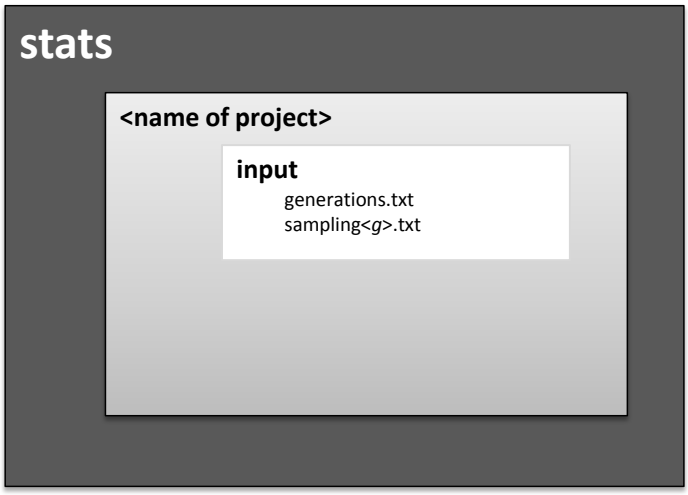



**Figure D.14: SINS-stat input folders and files** - Note that the names that appear between < > are labels set by the user (*g* = generation time(s) defined in generations.txt)

In the generations.txt file, the user defines the generation time(s) from where the samples should be taken. Then, for each generation from which samples are required, a corresponding sampling<*g*>.txt file must be created., which will define the samples. In this file, the samples are defined by their layer name, deme coordi-

nates, and by the number of individuals the user wants to sample from: i) female and ii) male X-chromosomes, iii) Y-chromosome, iv) mtDNA and v) autosomes. The autosomes are the last to be defined and they appear in the same order as in the genetic input files in SINS (from $A_1$ until $A_n$, with $n$ as the total number of autosomes). The sampling<*g*>.txt file must always have this organization. The number of individuals to be sampled can go from 1 to the total number of individuals (max) that occupy the specified deme, at a certain time.

```
Layer demeR demeC XFem XMale Y MtDNA A1 ... An
hearts 0 0 1 1 1 1 max max
hearts 0 1 1 1 1 1 max max
diamonds 0 0 1 1 1 1 max max
diamonds 0 1 1 1 1 1 max max
```

**Figure D.15: Layout of sampling<*g*>.txt** - In this example, for generation *g*, the *hearts* and *diamonds* layers are sampled in demes 0_0 and 1_1 (*g* = generation time(s) defined in generations.txt file).

### D.5.2 SINS-stats summary statistics and outputs

For each SINS simulation, SINS-stat creates a new folder named after the SINS simulation folder (see Fig. D.16) and estimate the summary statistics described below. In each SINS-stat simulation folder several files are created for each summary statistic and *locus*.
Thus, from a data set of diploid or haploid genetic markers, SINS-stat calculates the following summary statistics:

- Allele frequency estimated per *locus* and sample and overall;
- Allelic richness [Foulley & Ollivier, 2006] estimated per *locus* and sample and overall;
- Tajima's D [Tajima, 1989] estimated per *locus* and sample;
- Observed heterozygosity ($H_o$) estimated per *locus* and sample and overall;

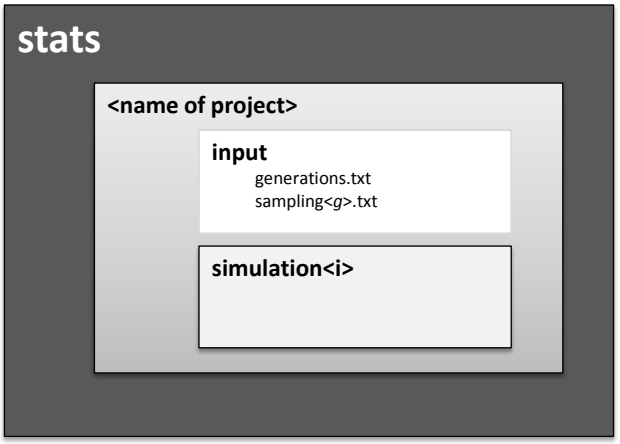



**Figure D.16: SINS-stat output** - SINS-stat creates a simulation folder, with summary statistics files, for each of the SINS simulations (*i* = [1: total number of simulations])

- Nei's [1978] estimators of genetic diversity ($H_e$) and differentiation ($G_{ST}$), estimated per *locus* and sample and overall;
- Weir and Cockerham's [1984] Capf ($F_{IT}$), $\theta$ ($F_{ST}$) and smallf ($F_{IS}$) estimated per *locus* and sample.

## D.6 SINS and SINS-stat Implementation and Installation

SINS and SINS-stat are written in Java and require JRE 1.6 (Java Runtime Environment) to be installed. Because they are written in Java both programs are portable and run on any Operating System (Linux, Windows and Mac). One archive is available to download (SINS1.zip). The SINS1 archive has two jar files (a kind of JAVA executable) named SINS.jar and SINS-stat.jar, together with several input examples.

## D.7 Running SINS and SINS-stat

Both SINS and SINS-stat are command-line programs. To launch a set of SINS simulations or SINS-stat, open a terminal in the SINS folder where the input and jar files are located. Then enter:

- java -jar SINS.jar <name of project>
- java -jar SINS-stat.jar <name of project> <number of simulations>

For e.g., to run simulations for a scenario named one_layer in SINS:

- java -jar SINS.jar one_layer

For e.g., to analyse the SINS's one_layer output in SINS-stat (1000 simulations) :

- java -jar SINS-stat.jar one_layer 1000

*IMPORTANT: jar files, input, results and stat folders, must always be placed in the same folder.*

# E. References to Appendices

## E.1 References


BRAMANTI, B., THOMAS, M.G., HAAK, W., UNTERLAENDER, M., JORES, P., TAMBETS, K., ANTANAITIS-JACOBS, I., HAIDLE, M.N., JANKAUSKAS, R., KIND, C.J., LUETH, F., TERBERGER, T., HILLER, J., MATSUMURA, S., FORSTER, P. & BURGER, J. (2009). Genetic discontinuity between local hunter-gatherers and central Europe's first farmers. *Science*, **326**, 137–140.

CURRAT, M. & EXCOFFIER, L. (2004). Modern humans did not admix with Neanderthals during their range expansion into Europe. *PLoS Biol*, **2**, e421.

CURRAT, M. & EXCOFFIER, L. (2005). The effect of the Neolithic expansion on European molecular diversity. *Proc R Soc B*, **272**, 679–688.

CURRAT, M., RAY, N. & EXCOFIER, L. (2004). SPLATCHE: a program to simulate genetic diversity taking into account environmental heterogeneity. *Mol Ecol Notes*, **4**, 139–142.

FISHER, R.A. (1922). On the dominance ratio. *Proc R Soc Edin*, **42**, 321–341.

FOULLEY, J.L. & OLLIVIER, L. (2006). Estimating allelic richness and its diversity. *Livest Sci*, **101**, 150–158.

GILLESPIE, J.H. (2004). *Population Genetics : A Concise Guide*. Princeton University Press, The Johns Hopkins University Press.

HAAK, W., BALANOVSKY, O., SANCHEZ, J.J., KOSHEL, S., ZAPOROZHCHENKO, V., ADLER, C.J., DER SARKISSIAN, C.S.I., BRANDT, G., SCHWARZ, C., NICKLISCH, N., DRESELY, V., FRITSCH, B., BALANOVSKA, E., VILLEMS, R., MELLER, H., ALT, K.W. & AND, A.C. (2010). Ancient DNA from european early neolithic farmers reveals their Near Eastern affinities. *PLoS Biol*, **8**, e1000536.

HUDSON, R.R. (2002). Generating samples under a Wright-Fisher neutral model of genetic variation. *Bioinformatics*, **18**, 337–8.

KIMURA, M. & WEISS, G.H. (1964). The stepping stone model of population structure and the decrease of genetic correlation with distance. *Genetics*, **49**, 561–76.

LOTKA, A.J. (1932). The growth of mixed populations : two species competing for a com-

mon food supply. *Journal of Washington Academy of Sciences*, **22**, 461–469.

MAYNARD-SMITH, J. & SLATKIN, M. (1973). The stability of predator-prey systems. *Ecology*, **54**, 384–391.

NEI, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, **89**, 583–590.

PINHASI, R., FORT, J. & AMMERMAN, A.J. (2005). Tracing the origin and spread of agriculture in Europe. *PLoS Biol*, **3**, e410.

RAY, N., CURRAT, M., FOLL, M. & EXCOFFIER, L. (2010). SPLATCHE2: a spatially explicit simulation framework for complex demography, genetic admixture and recombination. *Bioinformatics*, **26**, 2993–2994.

RICHARDS, M., MACAULAY, V., HICKEY, E., VEGA, E., SYKES, B., GUIDA, V., RENGO, C., SELLITTO, D., CRUCIANI, F., KIVISILD, T., VILLEMS, R., THOMAS, M., RYCHKOV, S., RYCHKOV, O., RYCHKOV, Y., GÖLGE, M., DIMITROV, D., HILL, E., BRADLEY, D., ROMANO, V., CALÌ, F., VONA, G., DEMAINE, A., PAPIHA, S., TRIANTAPHYLLIDIS, C., STEFANESCU, G., HATINA, J., BELLEDI, M., RIENZO, A.D., NOVELLETTO, A., OPPENHEIM, A., NØRBY, S., AL-ZAHERI, N., SANTACHIARA-BENERECETTI, S., SCOZARI, R., TORRONI, A. & BANDELT, H.J. (2000). Tracing european founder lineages in the Near Eastern mtDNA pool. *Am J Hum Genet*, **67**, 1251–1276.

ROSSER, Z.H., ZERJAL, T., HURLES, M.E., ADOJAAN, M., ALAVANTIC, D., AMORIM, A., AMOS, W., ARMENTEROS, M., ARROYO, E., BARBUJANI, G., BECKMAN, G., BECKMAN, L., BERTRANPETIT, J., BOSCH, E., BRADLEY, D.G., BREDE, G., COOPER, G., CÔRTE-REAL, H.B., DE KNIJFF, P., DECORTE, R., DUBROVA, Y.E., EVGRAFOV, O., GILISSEN, A., GLISIC, S., GÖLGE, M., HILL, E.W., JEZIOROWSKA, A., KALAYDJIEVA, L., KAYSER, M., KIVISILD, T., KRAVCHENKO, S.A., KRUMINA, A., KUCINSKAS, V., LAVINHA, J., LIVSHITS, L.A., MALASPINA, P., MARIA, S., MCELREAVEY, K., MEITINGER, T.A., MIKELSAAR, A.V., MITCHELL, R.J., NAFA, K., NICHOLSON, J., NØRBY, S., PANDYA, A., PARIK, J., PATSALIS, P.C., PEREIRA, L., PETERLIN, B., PIELBERG, G., PRATA, M.J., PREVIDERÉ, C., ROEWER, L., ROOTSI, S., RUBINSZTEIN, D.C., SAILLARD, J., SANTOS, F.R., STEFANESCU, G., SYKES, B.C., TOLUN, A., VILLEMS, R., TYLER-SMITH, C. & JOBLING, M.A. (2000). Y-chromosomal diversity in Europe is clinal and influenced primarily by geography, rather than by language. *Am J Hum Genet*, **67**, 1526–1543.

TAJIMA, F. (1989). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*, **123**, 585–595.

VOLTERRA, V. (1931). *Variations and fluctuations of the numbers of individuals in animal species living together*, 409–448. McGraw-Hill, New York.

WATTERSON, G.A. (1975). On the number of segregating sites in genetical models without recombination. *Theor Popul Biol*, **7**, 256–276.

WEIR, B.S. & COCKERHAM, C.C. (1984). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Evolution*, **38**, 1358–1370.

WRIGHT, S. (1931). Evolution in Mendelian Populations. *Genetics*, **16**, 97–159.

XUE, Y., WANG, Q., LONG, Q., NG, B.L., SWERDLOW, H., BURTON, J., SKUCE, C., TAYLOR, R., ABDELLAH, Z., ZHAO, Y., , MACARTHUR, D.G., QUAIL, M.A., CARTER, N.P., YANG, H. & TYLER-SMITH, C. (2009). Human Y chromosome base-substitution mutation rate measured by direct sequencing in a deep-rooting pedigree. *Curr Biol*, **19**, 1453–7.

ITQB-UNL | Av. da República, 2780-157 Oeiras, Portugal
Tel (+351) 214 469 100
Fax (+351) 214 411 277

**www.itqb.unl.pt**