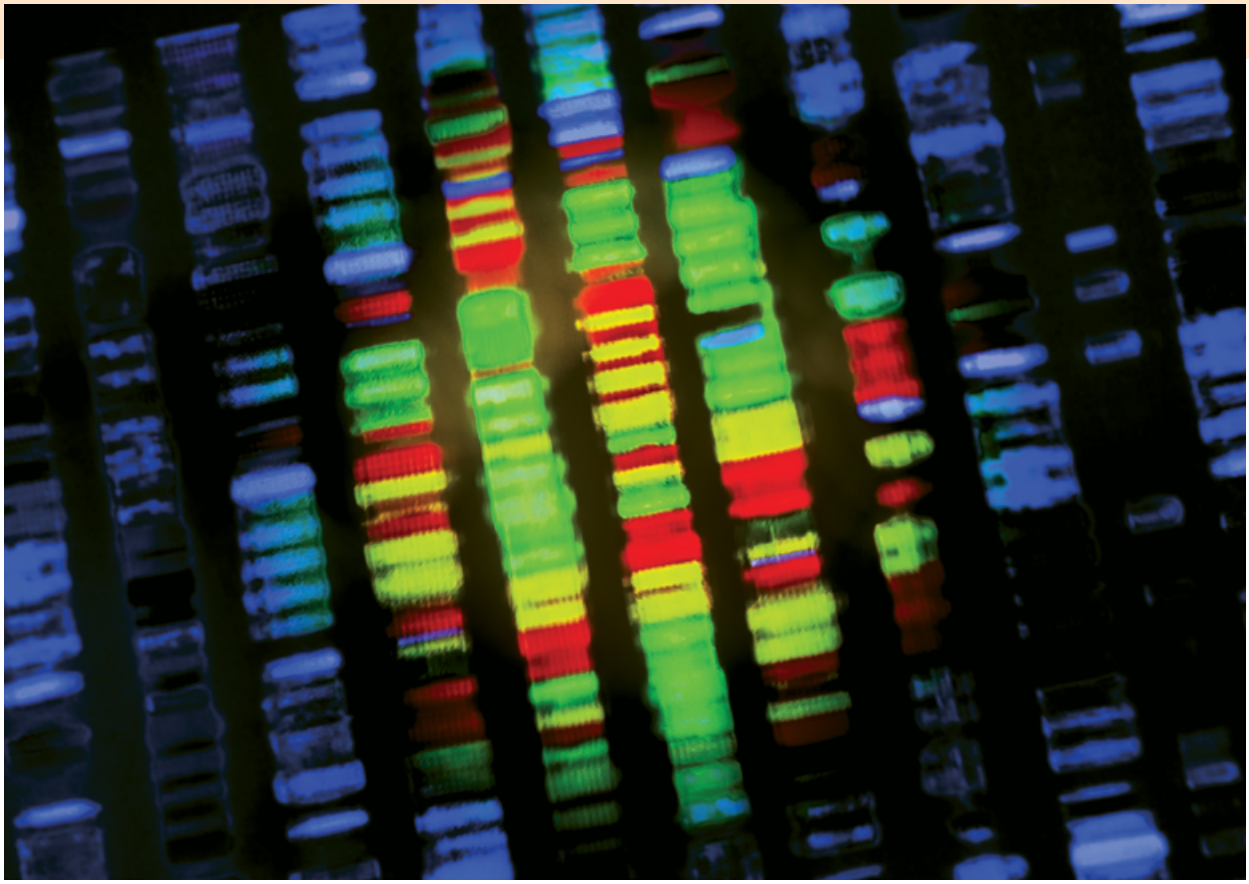
THE HIDDEN HISTORY OF THE MALTESE GENOME

By reading someone's DNA one can tell how likely they are to develop a disease or whether they are related to the person sitting next to them. By reading a nation's DNA one can understand why a population is more likely to develop a disease or how a population came to exist. ***Scott Wilcockson*** *talks to* ***Prof. Alex Felice, Dr Joseph Borg****, and* ***Clint Mizzi*** *(University of Malta) about their latest project that aims to sequence the Maltese genome and what it might reveal about the origins and health of the Maltese people. Illustrations by* ***Sonya Hallett****.*

In 1990, geneticists all over the world launched the largest biological project in history. Over the course of 13 years, the Human Genome Project sought to decipher the sequence of human DNA; the chemical code found in every cell of our bodies that contains the information to create an entire human being. The completion of this project, and the subsequent boom in the field of genetics, has turned the 21st century into the age of genetics.

The first draft of the *Human Genome* has been invaluable to researchers all over the world who sought to understand the intricacies of human biology and evolution. Another major outcome was the rapid surge in DNA sequencing technologies. The first human genome took over 200 scientists 13 years and $3 billion to complete. The newest technology, known as Next Generation Sequencing (machine-based sequencing technologies), now allows a small group of scientists to sequence one person's genome in a few weeks for around



$1,000. Such a low price has fuelled innovation—from reimagining medicine (into precision medicine that considers a person's gene variations, environment, and lifestyle) to teasing out the origins of humankind through projects like *The Cancer Genome Atlas* and the *International 1,000 Genome Project*.

While useful, the first draft of the human genome does not paint a complete picture of every person alive today. While 99.9% of the sequence of every human's DNA is the same, the 0.1% which is slightly different (called variations or mutations) makes us unique. Borg explained, 'all of our traits, such as eye colour and height, boil down to small variations in our DNA sequence. Importantly, diseases are also attributed to [gene] mutations and variants.' While every person is genetically different, so are large groups of populations. Caucasians have particular DNA variations that make them unique from East Asian populations and vice versa.

Thus, the current data on the human genome falls short when one attempts to study a specific population's genetics. Researcher Clint Mizzi explains, 'there have been a number of [genome sequencing] projects but how many Maltese people were included? […] Populations from different countries have different variants that appear in different percentages of the population, thus some [gene variants] may be found mainly in the Maltese population [while] others are absent.' This is why many countries worldwide are initiating their own genome projects. Now Malta has entered the foray with the *Maltese Genome Project* and a partial genome has already been completed.

A GENOME FOR THE PEOPLE

The three-year *Maltese Genome Project* was launched in 2015, based on nearly 25 years of human genomic research in Malta. It will map the genomes of around 4,000 Maltese people, or 1% of the population, in order to obtain an averaged or referenced Maltese genome sequence. This means that the end result will not be the sequence of any one person's genome but a representative example of the entire Maltese population.

Having this kind of information will be invaluable to geneticists and clinicians to diagnose rare diseases and investigate new therapies. Borg describes how 'if they embark on their own genetics project and uncover a mutation […], instead of having no idea how frequently it occurs [in the

Maltese population] or what it does, they will now have a reference they can look to.' This knowledge will vastly improve the understanding of how particular gene variants affect the Maltese population when studying disease mechanisms.

FORGETTING PAST TECHNOLOGY?

Malta has a long history of genetics research. Older genetics technologies were less focused and much more labour intensive. They looked at one gene at a time, forcing the researcher to choose particular genes, possibly missing the gene linked to a disease or condition. Modern whole genome sequencing (next generation sequencing) is fast, relatively inexpensive, and allows researchers to look at every single gene and all the DNA in between.

So does this mean that next generation sequencing technology will signal the end for old technologies? On the contrary, Borg explains that 'we are at a stage where we usually sequence the whole genome [...] but if we can obtain enough data [about the Maltese population], researchers no longer need to sequence everything. Once we have the thousands of unique and non-unique [gene] variants, a researcher can study a Malta-specific [range of genes] that can be more precise and less time consuming.' Far from replacing the old technology, whole genome sequencing can work alongside it to streamline research. '[Genome sequencing] will help direct research to specific genes,' Borg explains, '[so a scientist can] tailor design experiments rather than exploring [...] work that might be futile, which can be very frustrating.'

CONVERTING PEOPLE INTO BIG DATA

Getting hold of a person's DNA is quite easy: a cheek swab or some blood is all you need. Once the DNA has been prepared—which involves cutting it up into tiny fragments—it is placed in one end of a DNA sequencing machine and left to run. These machines essentially make a copy of the DNA fragments and monitor which molecules are added in sequence to the growing chain of new DNA. This then allows you to determine the original sequence. Then it is someone else's job to make sense of what comes out the other end. Say 'hello' to the bioinformaticians!

Clint Mizzi is a bioinformatician at the University of Malta working on the *Maltese Genome Project*. He explains that 'bioinformatics encompasses multiple disciplines [...] involving an understanding of biology, computer science, mathematics, statistics, and some engineering. We apply mathematical sciences to biological data.' A single person can equate to 200–400 gigabits of raw data and Mizzi needs to make sense of it.

Once Mizzi has the DNA sequence fragments he aligns them to a reference genome to match everything up like a giant jigsaw puzzle. By comparing the genomes, any variations in the DNA sequence specific to the Maltese population can be singled out.

With this information, the researchers can then focus their efforts on the specific gene variants or mutations that are affecting the Maltese population. Mizzi stresses 'that bioinformatics does not stand alone. [...] The machines are not 100% perfect, although there are a number of [methods we use] to minimise the errors. [...] So it is important to go back to the laboratory to confirm results and do experimental functional studies.' It is imperative to check that the variants or mutations have an effect on our biology. By working together, researchers from different fields are putting this knowledge to good use.

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The age of genetics heralds a bright future for our understanding of human physiology and what treatment is best when our genes turn against us. But the field of genetics is not only about working towards a better future. Hidden deep within our DNA are clues of our distant past. By reading the Maltese genome one can understand the origins of the contemporary Maltese population and the evolutionary forces that shaped their genome.

Lebanese DNA contributed less than 5% to today's Maltese DNA

MALTESE ORIGINS

The Mediterranean has enjoyed a turbulent past with more civilisations and empires rising and falling than a year's hot dinners. This question of 'where did the Maltese come from?' has been debated for centuries. To understand how this modern nation arose needs a bit of history.

A long time ago in South-East Africa, the environment was just right for the beginning of humankind. Modern humans (*Homo sapiens*) entered the world stage around 200,000 years ago—exact dates are still unknown. Evolutionary genetics studies that look into our distant past rely on two genetic markers.

The first is mitochondrial DNA. This DNA is distinct from the rest of our DNA found in the cell nucleus. Mitochondrial DNA is found in small energy producing factories known as mitochondria—if they stop working, death follows quickly. These are inherited only from one's mother and only transmitted through daughters. By looking at specific parts of the mitochondrial DNA (known as haplogroups, that remain largely unchanged over time so are shared worldwide) researchers are able to trace ancestry through the female lineage.

The second is the Y chromosome. Human DNA is broken up into 46 chunks known as chromosomes, with each parent contributing half. Gender is determined by two chromosomes known as X and Y. XX makes a female, XY makes a male. The combination depends on one's father. The Y chromosome also has haplogroups, making it a useful genetic marker for evolutionary studies on men's origins.

THE VOYAGE OF HUMANITY

Around 80,000 years ago humans embarked on the most important journey in humanity's history. They left Africa. From the analysis of mitochondrial DNA, humans 'exited from East Africa as a small group of male and female modern humans,' explains Prof. Alex Felice. Two splinter groups of the Eastern African population, known as M and N, moved into the Middle East and made their first steps toward global colonisation.

By 40,000 years ago, humans had entered Continental Europe. Felice explains that 'over a relatively short period of time [...] humans replaced the pre-existing humanoids, mostly Neanderthals in Europe, due to some kind of Darwinian advantage.' Some cross-breeding took place between the two humanoids but gradually *Homo sapiens* took over the planet (except Antarctica). Malta was only colonised around 7,000 years ago.

THE FIRST PEOPLE OF MALTA

The first humans in Malta are presumed to have been Sicilian farmers, who brought cattle and crops over that changed the Maltese landscape. After more than a millennium, the culture of this people took an interesting turn. They built over 30 temple complexes, the oldest free-standing stone structures in the world. This Temple Period saw the rise of a complex civilisation with a ritualistic and artistic

culture (see *Death of the Temple People* in **THINK**, Issue 10, pp. 34–41).

For one and a half millennia the Temple People flourished, leaving behind their distinctive mark on the Maltese and Gozitan landscape. However, their departure left us with Malta's greatest mystery: why did they suddenly disappear around 2500 BC? A number of theories have been proposed, including environmental stress and their own religious fervour. The real reason is being unravelled by the FRAGSUS project involving archaeologists, biologists, engineers, and others from the Universities of Cambridge, Belfast, and Malta.

Events like this seem to echo throughout Malta's history. 'The archaeological record is such that [humanity's presence in Malta] is like that of the dinosaurs. The Temple People were here but they seem to have been replaced by others,' comments Felice. For the next four millennia Malta constantly changed hands, closely following the rise and fall of the great Mediterranean Empires. 'It is not correct to say that the island was completely uninhabited; there is not a very good record, but in principle there was not a substantial population [...]. It was probably a mixture of the main populations of the time,' he continues.

The Temple People were replaced by Bronze Age settlers. Then came the Phoenicians around 700 BC, followed by the Carthaginian Empire in 332 BC, then the Romans during the First Punic War in 218 BC. Malta's population was thought to be very small, Felice adds how there was 'maybe a small urban presence in modern Mdina and [a few other places], but apparently only a couple thousand at most.'

DESCENT FROM PHOENICIA OR SICILY?

In 2004, a *National Geographic* magazine interview sparked exciting revelations on the origins of the Maltese people. Early results of a Y chromosome study showed that 50% of Maltese men are of Phoenician origin. In 2008 the study was published in *The American Journal of Human Genetics*. The researchers looked for Phoenician DNA in modern day colonial areas based on haplogroups in modern day Lebanese people. Late Stone Age farmers in Greece, Crete, and Southern Italy had the same piece of DNA. The Maltese population did too, but this small genetic footprint could have been left behind by others like the Stone Age ancestors. The methodology of this study turned out to be flawed. Maltese history does not reflect a large Phoenician population that could have lasted till today

So where do the contemporary Maltese come from? Research carried out in Malta points to just a few hundred miles north. A study published in the *Annals of Human Genetics* in 2004, on which Felice collaborated, looked at Y chromosome haplogroups found throughout the Mediterranean and identified common population groups. 'Data on Mitochondrial DNA [from the ongoing *Maltese Genome Project*] is also nearly complete but what we have also points in the same direction [as the previous study]: that most contemporary Maltese males and females can trace their ancestry to Sicily and [Southern]

Italy around 1,000 years ago,' reveals Felice. Middle Eastern DNA, including Lebanese DNA, contributed less than 5% to today's Maltese DNA.

NORMAN DOMINION

History reflects the DNA evidence. The decline of the Roman Empire was followed by Arab rule of the Islands for at least two centuries from around AD 870. First under the Aghlabid Emirate and then the Fatimid Caliphate. Malta was either uninhabited or there were very few people. The turn of the first millennium brought a documented influx of people from Arab-ruled Sicily.

At the turn of the 11th century a new set of players entered the game. Adventurers from Northern France had gained a foothold in Southern Italy and sought to expel the Arab and Byzantine occupiers. By 1091, Count Roger I landed in Malta and established Norman rule.

Malta continued to be governed by the Arab administration until 1127 when Count Roger II of Sicily, the son of Roger I, finally displaced the Arab governors and established complete Norman dominion. Over the next few centuries, the Maltese population grew with an influx of Sicilian and Norman settlers. Felice explains 'there was [still] a strong Arab subculture in Sicily and Southern Italy [...]. If you go to the small villages outside [Sicilian] towns today they speak very differently to modern Italians, not too different from what we call Maltese. These [people] began to re-inhabit Malta, although there were only around 20,000 people up to AD 1500.' Once again, Malta was colonised by Sicilians who gradually latinised the island and brought their unique Siculo-Arabic language that evolved into modern Maltese.

THE PRICE OF PROSPERITY

'So this is the [genetic and historical] data on the recent origins of the contemporary Maltese. This is important for a number of reasons. Firstly it addresses questions such as: Who am I? Where am I going? Where did I come from?' Felice observes, adding, though, that 'there are also important questions [for Malta today] regarding public health' that must be asked. For millennia ships have dropped anchor along the Maltese shore and the ripples can still be felt today.

> *Who am I? Where am I going? Where did I come from?*

The current population of Malta stands at just over 420,000 and originates from a small population that settled here after the first millennium. Felice explains, 'these [people] were visited by small groups, military details of young men who stayed for a short time [...] and left genetic memories in the form of gene variants and mutations [...].' This, he notes, is what we now recognise as Founder Effects. As the small population expanded over the centuries, these newly introduced Founder Mutations became widespread across the population for better or worse.

The history of Malta continued to become more and more interesting with various groups and nations visiting over the centuries, which provided ample opportunities for these Founder Mutations to arrive and mix with genomes from distant countries. Felice describes two major events that occurred after 1500: 'first was the arrival of the Order of the Knights of St John and second, as in the rest of Europe, this was the beginning of a certain degree of public hygiene and prosperity [...]. The populations of Europe and Malta started to grow exponentially. It was during this time that rare diseases accumulated'.

In 1528 the population of Malta was estimated at 12,000 with 5,000 residing in Gozo. Within 10 years, the estimate had almost doubled to 22,000 in Malta and 6,500 in Gozo, including the Knights. Despite sieges and depopulations of Gozo, by 1814 the Island's population boomed to 41,000.

EXPERIMENTING WITH NEW MASTERS

Nothing lasts forever; the Knights had fallen out of favour with the Maltese toward the end of the 18th century due to the opulence of Grandmaster Pinto's reign. On 9 July, 1798 Napoleon Bonaparte landed in Malta and, by the 12th of the month, Malta was added to the French Empire.

Despite the Maltese collective memory of their evil French overlords, rule under France was not all that bad. Napoleon planned the building of hospitals and invested in education. Unfortunately, the new rules did not sit well with the clergy who stood to lose their significant power over the Maltese. So they initiated a rebellion. The Maltese were induced to revolt 82 days after accepting French rule (see *Malta: Stockholm Syndrome* in **THINK**, Issue 13, pp. 48–55).

The two-year-long siege resulted in great suffering. Malta's population plummeted by 18.7% around this time from 114,000 to 93,000 due to war, famine, and disease. By 1800, the French relented and the Maltese won their freedom back. Without

Malta being involved in the negotiations, it was handed back to the Knights with the British acting as protectorate. The British amalgamated Malta into their empire. The Maltese were deemed incapable of governing themselves leaving Malta to enjoy 164 years of British rule.

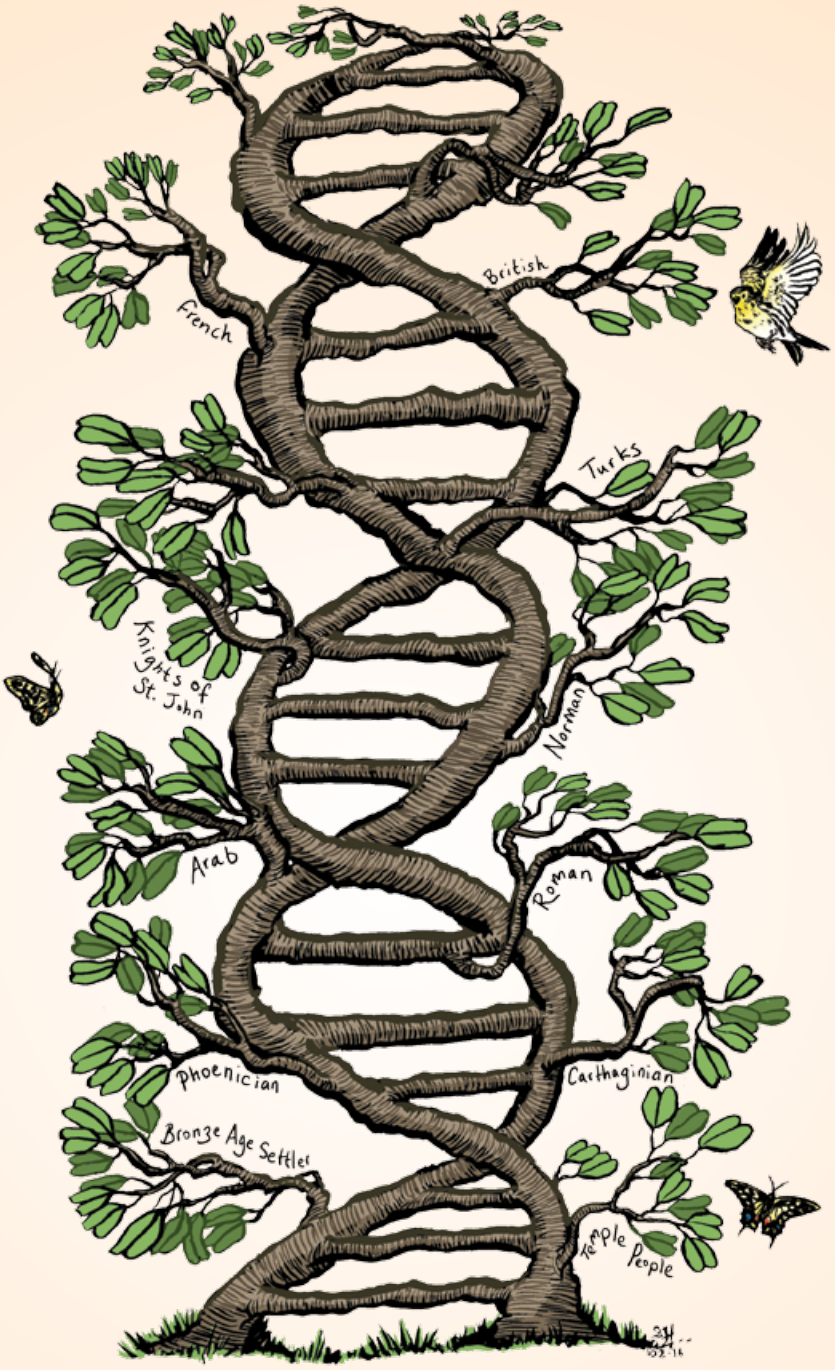
BOTTLENECKS AND FOUNDER EFFECTS

Events over the last millennia have shaped the modern Maltese people. The rise and fall in population numbers created genetic bottlenecks. These events impacted genetic diversity so much that rare DNA mutations became common spreading disease.

The problem is evident today. 'There are a number of mutations that give rise to rare diseases, those [found] in less than one in 10,000 people. [...] So, there is this genetic burden,' explains Felice. 'In the 1990s we set up, with the Department of Health and the late Dr Joe Louis Grech, the Laboratory of Molecular Genetics [at the University of Malta] and the Thalassaemia Clinic at St Luke's Hospital, now at Mater Dei Hospital, and we began to identify some of these mutations.' Interestingly, the research on these disease-causing mutations supports the Y chromosome and mitochondrial DNA studies carried out in Malta: most of the Islands' genetic mutations are shared with Sicily and Southern Italy.

Some mutations that cause rare diseases are disproportionately high in the Maltese population and include gangliosidosis, coeliac disease, and blood disorders like thalassaemia. One study in 2007 by Felice and his team focused on a mutation in the *SPR* gene that leads to a rare disorder known as Segawa's Disease, a motor neuron disorder with some similarities to Parkinson's Disease. A single mutation in the SPR gene was found in a high proportion of the population. Because of this discovery, babies are diagnosed at birth and treated immediately preventing severe disability.

Genetics is making great strides. Felice adds that 'because of the efficiency and costs of the new technology, over the next few years research and diagnostics shall be moving to whole genome sequencing.' With the Maltese Genome in hand, researchers will be able to figure out how to treat diseases widespread locally while helping others worldwide. Researchers will generate a complete picture of where the Maltese came from and who they are today.



The study's co-principal investigators are Prof. Alex Felice and Dr Joseph Borg with Clint Mizzi and Dr Nikolai Pace as close associates.