Technologies of similarities and differences: on the interdependence of nature and technology in the Human Genome Diversity Project

M'charek, A.A.

Citation for published version (APA):
Chapter 5

The Traffic in Males
and Other Stories on the Enactment of the Sexes in
Studies of Genetic Lineage

Introducing the Argument

What is genetic sex, and how is it enacted in studies of genetic lineage? These are the main questions put in this chapter. Genetic sex is hardly an issue in population studies interested in human histories. However in the laboratories one may find samples indicating male or female, and published papers contain accounts of women’s migration history and that of men. This suggests that sex does matter. But where can it be located? It will be argued that rather than a stated message in the DNA, in laboratory practices the sexes are performed as various things. However, this diversity tends to be subsumed and differences tend to be naturalised. I will examine how that is done in the context of mitochondrial DNA and Y-chromosomal research, and show that this requires a specific treatment of DNA, namely as a technology for studying the history of populations.

Human geneticists know the sexes as: XX and XY. Critics of this binary scheme, especially feminists, have argued that to state XX and XY is to fail to pay any attention to culture. My aim in this chapter is to show that neither of these approaches takes into account the practices of genetics. Difference between the sexes is neither a natural quality embodied in individuals nor a cultural additive, but is rather an affect of interfering practices where the sexes are deemed relevant. Thus rather than taking culture as the fact after the biology, I view culture as part and parcel of biology, and examine sex differences in the practices of genetics. I will view the relevance and irrelevance of the sexes in a laboratory context where experiments are conducted, and in published papers where the data is analysed and put in the context of population history and genealogy.

How 153 male samples lost their sex

On 18th January 1997 I took the night train from Amsterdam to Munich to continue my participant observation in the Laboratory for
Molecular Evolution and Human Genetics. I will refer to this lab as the P Lab. Among other things my luggage contained 153 blood samples. All the samples had been taken from males during a large-scale survey on heart disease in the 1980s. All males were 35 years of age and were living in the small Dutch town of Doetichem. I picked the samples up at the Forensic Laboratory for DNA Research in Leiden, hereafter referred to as Lab F.¹

I was taking the samples along at the request of Maris Laan, a member of the P Lab. Laan is working on a project in the field of population genetics concerned with population history and the spread of agriculture in Europe. She studies this by looking at “linkage disequilibrium”² on the X-chromosome. To avoid complexities induced by recombination she decided to look at male DNA only, since males carry only one X-chromosome and not two as females do.

When she visited me in Amsterdam in July that year she brought along half of the DNA extracted from the blood samples. We placed the two boxes in my refrigerator and the accompanying forms on my desk. The forms referred to the samples as Du208, Du209, etc., and indicated the DNA concentration of each sample. The latter was determined through mitochondrial DNA (mtDNA) PCR products, and visualised on agarose gels of which an infra-red picture was included. The samples were to be delivered to Lab F as a return favour for making the blood available.

But something strange had happened to the Dutch male blood samples which had travelled to Munich earlier that year. Not only because they had come back as DNA samples, but also because they had lost one of their qualities along the way, one of the qualities that made them move from Leiden to Munich in the first place.³ Their sex. In the forms they were referred to as “Dutch blood samples” and the DNA concentration was not determined on the basis of the X-chromosome, but on that of mtDNA amplification. The samples were still qualified as Dutch, but no longer as males. So why did these samples lose their sex or how could it be enacted?

Sex and sexual differences are not stable. As Stephan Hirshauer and Annemarie Mol have argued, they have to be performed actively.⁴ This indicates that they may become irrelevant altogether. To consider the relevance and irrelevance of the sexes, let us first have a look at feminist studies of science and then at the account introduced above.

On the Relevance and Irrelevance of the Sexes

Feminist scholars have put a great deal of effort into showing that science is, just like any other practice, sexualised. They set out to show that it was sexualised in terms of who does the research, revealing a male bias
and bringing to the surface the contributions of women in science. Others examined the language of science, providing insight into hierarchies in the designation of agency, and about biases between objects categorised as masculine and others as feminine. Again others considered scientific methods and have argued that these could be categorised as masculine. Methods were shown to establish a distinction and a hierarchy between a (masculine) subject of research, namely the scientist, and a (feminine) object of research, namely nature. These approaches lead to one basic feminist claim concerning sexual differences. "Sex" can be found everywhere. "You just have to put on gender glasses to see it," as one scholar once put it to me. Once I had been in the laboratories, however, the sexual distinction I found seemed banal, the kind of distinctions that I could have learned about in any other environment. And nothing specific to genetic sex in laboratory practices. Yet population geneticists' accounts of human history talk about men and women and their different migration histories. But where can it be located in laboratory practice and more precisely in my observations of it?

The strategy I propose and will follow here, is not that of putting on "gender glasses." For the focus is usually set somewhere else and could make oblique what is to be looked at. Moreover putting such glasses on metaphorically exposes the wearer to the danger not only of predefining - if not essentialising - the sexes and what counts as sex differences, but also of developing a blind spot for the irrelevance of the sexes. Instead I follow a strategy proposed by Annemarie Mol, a strategy of locating objects in practices. From this perspective, a universal claim, such as sexual differences are relevant in any kind of practice and thus also in genetics, gives rise to the question "where is genetic sex and how is it performed?" Another strategy suggested by Evelyn Fox Keller is that of counting, "counting past two." Keller uses this approach to draw attention to the diversity in science as well as that in "gender." This numerical and tantalising practice has an advantage that I would like to emphasise here. Not only does a commitment to counting, especially when the sum is more than two, prevent us from taking the binary scheme of biology for granted but the practice of counting also involves a risk, namely that of not finding even one. This other side of the coin of counting, "added to" the strategy of locating as suggested by Annemarie Mol, enables both the the making specific of a universal claim in locales and the revelation of practices where such a claim does not hold - in this case, practices where sex is irrelevant.

Let us now consider some of the information embodied in the story about the Dutch male samples.

As we saw in the Dutch example, samples do not travel without additional information. This does not mean, however, that all information is deemed important or is even stored in the files. The Dutch samples from
Doetinchem, taken from 35-year-old males in the context of a large-scale study of heart disease, lost many of their features in the written forms that accompanied them back to the Netherlands. The very reason why these samples travelled to Munich was because according to the genetic matrix XX-XY, males carry only one X-chromosome. Those carrying one X and not two were judged appropriate for Maris Laan’s project, in which male samples were preferred to female. Once the Doetinchem samples had established their Dutchness and maleness through the accompanying information, they became simply Dutch. And as such they were referred to in the accompanying list on their trip back to Leiden. It is remarkable that the sex of the Dutch that entered the P Lab as samples was deemed pivotal but that it no longer mattered, i.e. it lost any material reference, once these samples had left the lab. It indicates that sex may be a temporality performed in locales, and that it is not an essential feature of samples.

Taking the temporality of genetic sex into account, the aim of this chapter is to examine if and how the sexes are enacted in studies of genetic lineage. To do this we will consider three sites in studies of lineage. These sites will be referred to as the practices of establishing genetic lineage, of working with DNA in the laboratory, and of reconstructing genealogy. In a way these are the sites of theory, raw data and analysed data. It is, however, important to emphasis that differences between these sites are analytical rather than ontological. The differences do not correspond to the classic division between “hand labour” and “mental labour.” They rather point to a gradient of technologies, spaces, and problem-solving procedures that are more or less important in these three practices. Since studies of genetic lineage are dependent on lineages themselves, namely lineages between laboratories and scientific groups, let us first take a look at how that is done.

The Traffic in Males and other Gifts in Genetics

In November 1996, at a weekly Population Group Meeting of the P Lab, Maris Laan reported that she did not have enough European samples necessary for her project. Laan is in charge of all samples that enter or leave the lab and she had noted that although the Lab has many samples of populations from all over the world, there were hardly any Europeans among them. All she had found were samples of Swedes, Finns, Estonians and Samis. Confronted with this problem, especially since her project aimed at studying European population history, she raised the point during the meeting. We started to brainstorm about where the lab could ask for samples. A large amount of German DNA samples would soon be available, as a result of collaboration with a Dutch medical research group. Another
possibility, I suggested, would be to ask Lab F in Leiden for Dutch male samples. Having spent some months in this lab myself and knowing that they work with a Dutch control population for forensic purposes, I reasoned that they probably had plenty of DNA.

After the meeting I called the head of Lab F, Peter de Knijff, and it turned out that they did indeed have a large collection of Dutch male samples and were willing to share part of it with the P Lab. The arrangement was that they would give the P Lab blood samples and get back half of the DNA extracted from them.

When I visited Lab F in January to pick up the samples, de Knijff gave me some further information about them. Each sample consisted of two aliquots of blood placed in plastic tubes which together make up 10 millilitres of liquid blood per sample. This blood was "taken up" in a buffer (EDTA) to preserve its quality. They were placed in a box and put on dry ice for the forthcoming journey. De Knijff asked me to assure Pääbo and Laan that the sampled population is a good representation of the Dutch population at large, because Doetinchem shows neither a founder effect, i.e. that a limited number of individuals would account for the genetic profiles of its inhabitants, nor recent admixture, which would be reflected in the genes and distort their representativity of the Dutch.

From February onwards Maris and I started to extract DNA from these samples. She would refer to our laborious work as "our large-scale-extraction." Due to the large amount of blood that we had from each sample, extracting DNA from a series of thirty samples would take us two days, in which there was hardly any time left for any other work. A considerable part of this time was spent in planning the work, making sure that all the chemical solutions and equipment we needed were at hand, including enough pipettes and tubes, plastic bags for disposals, good pencils to mark the tubes according to individuals, different colours according to which step of the extraction had been performed and deciding which extraction protocol would be the most efficient. We first worked with a "phenol-chloroform" DNA extraction protocol. But Andreas Kindmark, a colleague lab member, suggested another protocol, a "sucrose gradient-high salt" extraction method. He had used the latter in a medical lab in Sweden and told us that it was not only user-friendly compared to the phenol-chloroform method but it also required fewer steps before retrieving DNA. If we were interested, he would contact his lab in Sweden and ask for the exact descriptions. Once Andreas received the protocol via e-mail Maris tried it out for three samples and found that it also yielded an amount of DNA similar to that yielded by our original method, so we changed protocol. Part of this organisational work was the booking of machines such as the centrifuge, deciding upon who does what and making sure that we protected ourselves well because we
were working with so much blood. During the first steps of the extraction, using gloves, masks and appropriate chemicals to clean our working environment was not so much to avoid contamination of the samples but of ourselves, namely to protect ourselves and others in the lab (who make use of the same environment) against contagious viruses, especially Hepatitis B. Whereas the "laminar flow cabinet" were we conducted the extractions would be packed with rather large pieces of equipment in the first stages of the extraction – bigger pipettes to pipette the clotted blood into the buffer placed in 20 millilitre tubes – towards the end of the procedure once the cell material had been separated from the DNA-containing supernatant, the equipment became smaller, the pipetting more precise, the treatment of the tubes more careful, so as not to mix the centrifuged DNA at the bottom with the soap solution at the top of a tube. Thus the logistics of doing DNA extraction, which makes up the bulk of the work, is reflected in the treatment of the samples from blood to DNA.\(^{14}\)

**Making Lineages in Genetics: An Economy of Exchange**

From the above account it is clear that doing population genetics is dependent on an economy of exchange. The exchange of samples is as valuable as the exchange of (unpublished) data, extraction protocols, or research methods.\(^ {15}\) I will first consider the samples.

There are many reasons why collections of samples can be found in this particular laboratory. Samples may be there because they are considered valuable, i.e. rare or difficult to retrieve. They may be there because the Lab happens to know the people who have them or, in many cases, because they are simply offered to the Lab. As Laan’s account showed, these gifts show a bias towards “exotic” populations or populations from regions of the world other than Europe. Other samples, as in the case of the European and some of the non-European samples, may be in the lab because geneticists who came to conduct their research in the P Lab had brought them along. In a sense, the samples just happen to be there for anybody interested in doing a project in population genetics. Samples may also enter the lab because of an express demand, originating in an ongoing project, as in the example of the Dutch male samples. The design of Laan’s project prescribed that the samples should be European and male. Consequently the samples received were selected according to sex, and their Euro-Dutch origin was assured since they were from the small town of Doetinchem and not – for example – from Amsterdam, whose population is, given its complex demographic history, much more problematic to categorise as Dutch or even European.\(^ {16}\)
The traffic in samples is very much dependent on a “gift economy.” It is dependent on lineages between labs or scientific groups. And at the same time, once samples start to move they establish and help to strengthen lineages. In the case of the Dutch samples a recently initiated collaboration facilitated the gift and the exchange of blood for DNA. The P Lab had just started to work with Y-chromosomal markers using the protocols of Lab F. According to conventions Lab F will be acknowledged in papers for the gift of samples as well as for the marker information, either in terms of a co-authorship or under the section “Acknowledgements.” Also in line with this reciprocal gifting, the head of Lab F, Peter de Knijff, was invited to the P Lab to give a seminar on Y-chromosomal research in April 1997. Moreover Lab F was able to add 153 DNA samples, instead of blood samples, to its collection. From the description above of the amount of work involved in such extraction, it is easy to see why DNA samples are preferred to blood samples. The procedure of extracting DNA also benefited from gifting protocols: the unexpected gift of an alternative extraction method from one of the lab members expedited the laborious work and proved to be friendlier to those carrying it out. The unexpected appearance of this protocol from a Swedish medical lab hints at another type of exchange between laboratories.

Lineages are not only related to the traffic in samples, technologies or scientific data, but also to the traffic in people. In addition to the exchange of samples and markers, Lab F and the P Lab have also exchanged their “in-house anthropologist,” a person who knows both labs quite well, and who contributed to a more informal traffic in Dutch samples, to information about the samples and to communication between the laboratories.17 Hence exchanging “in-house anthropologists” makes lineage as well. This position is not exceptional but applies also to other visiting researchers in the P Lab. Hence the protocol from the Swedish lab. Also other lab members establish lineage and are part of kinship relations between labs, scientific groups and countries. These lineages may be temporary, lasting mainly for the period the researcher is in the lab, or of a more durable kind. Thus genetics does not only study lineages, it is also a product of lineages, established through an exchange of people, samples, technologies and methods.

So this is how geneticists do lineage between themselves. But, how do they do genetic lineage? This is the topic dealt with in the next section, where we will be focusing on the relevance of the sexes in these studies. It will become clear that, in the practice of genetic lineage, DNA is not just the resource but is also handled as a technology, involving different systems for doing genealogy.
Archaeology of the Human Genome or How to do Genetic Lineage

"Archaeology of the Human Genome" is part of the title of a paper by Arndt von Haeseler, Antti Sajantila and Svante Pääbo. This paper – let us call it the Archaeology paper – provides a literature review and argues for the potentials of genetic data in reconstructing human history, especially when the two-sexed model of mitochondrial DNA and Y-chromosomal DNA are considered. The paper opens as follows:

Many of us, especially in our youth, are interested in the lives of our parents and immediate family; then again, as members of a particular group or population, we like to know about the life of our ancestors; finally, as members of the human race, we are fascinated with the question of human origins. [...] However, early humans left traces of their activities not only in the form of their bones and artefacts. They also passed on to us their genomes. Every genome is made up of about three billion base-pairs, several of which experience mutations in each generation, and, as the way in which these mutations accumulate in populations are influenced by how populations expand, contract, split and merge, the study of genetic variation has the potential to yield a great deal of information regarding our history.

Under the heading “A bit of theory” it goes on:

All individuals have parents, and some individuals have the same parent(s). The consequence of these trivial facts is that as genealogical lineages in a population trace back over generations, they will occasionally coalesce to common ancestors. There will be fewer and fewer ancestors as one goes back. Eventually, all female lineages will trace back through a series of consecutive mothers to one single mother and all male lineages will similarly trace back to a single father, that is, the most recent common ancestors (MRCAs) on the maternal and paternal side. [...] If the demographic history of a population is unknown, it can be reconstructed from the patterns of nucleotide substitution in the genome. DNA sequences from mitochondrial (mt) genome and those from the majority of the Y-chromosome are particularly useful as they are passed on without recombination from mother to daughter and from father to son. Consequently these sequences can be traced back directly to the genealogical maternal or paternal MRCAs. Autosomal DNA sequences, which are inherited through both males and females and occur in two copies per individual, trace back to "biparental" MRCAs that are on the average four times as old as maternal and paternal MRCAs.18

Under “The Age of the human gene pool,” the Archaeology paper indicates that these maternal and paternal MRCAs are expected to be found around 200,000 years ago. However it is not clear to geneticists and to the authors of
this paper whether our species, i.e. modern humans, originated at a time close to these MRCAs.\textsuperscript{19}

**Genealogy, Genetic Lineages and Technologies of the Sexes**

From this account about population genetics and about what it can contribute to knowledge of human history, it becomes clear that sex is relevant in studies of genetic lineages. Let us take a closer look at how exactly it is that sex matters in these studies.

Population genetics is interested in lineage and aims at reconstructing genealogical patterns. It does this by looking at similarities and differences, as a specific distribution of – for example – mutations, within and between populations. The choice for the term *archaeology* in the title of the paper quoted indicates that specific distributions of similarities and differences come with a story about the past.\textsuperscript{20} A story about populations. Similar to the treatment of archaeological artefacts as records of human history, mutations in the DNA and the way these are distributed among populations, are treated as records of population histories. Under the assumption that all populations have one origin, the differences in particular can be read as events in the past, contributing to stories about when and how populations diverged or merged, reduced in size or grew. As the quote indicates, mtDNA and major parts of the Y-chromosom e are considered very useful for studying these events. Especially because neither recombines, i.e. they are inherited unchanged from mother or father, these DNA systems represent the maternal and the paternal line of inheritance, which can be traced back to one ancestral mother and one ancestral father. Before addressing these two systems, let us take a closer look at how genealogy is practiced in studies of genetic lineage and at the relevance of sex in these studies.

The trivial fact of genealogy mentioned in the Archaeology paper, namely the fact that all individuals have parents,\textsuperscript{21} demarcates an involved relation between genealogy and genetic lineage. From a genealogical perspective going back in time means to unfold a greater complexity in biological kinship.\textsuperscript{22} It makes more and more individuals appear as part of “the family,” as ancestors of a specific individual. From the perspective of an individual, this amelioration of ancestors can be represented by the form of a V. While the intersection between the two arms of this letter indicate a contemporary moment in time where there is one individual, their divergence points deeper and deeper into history where progressively more ancestors can be located in the space between the two arms. However, the quote contends that there “will be fewer and fewer ancestors as one goes back.” This suggests that from the perspective of genetic lineage, the genealogical V
should rather be turned upside down, to become a Λ instead. At issue here is not an ever-growing family but an ever-shrinking family the further one goes back in time. But how should we understand this type of genealogy, how should we understand the occurrence of a Λ?

Although the opening sentence of the Archaeology paper evokes the idea that population genetics is interested in individuals and in where they come from, its main focus is rather groups of individuals or populations. Population genetics studies how individuals relate to each other and reconstructs the development of these relations through history. Thus the object of study, many individuals and not one, explains the Λ. The space at the bottom (the largest divergence between the two arms) stands for a group of individuals and a contemporary moment in time. But how is this possible? What is the relevance of Λ and why does not each of these individuals have his or her own V-shaped genealogy? The answer lies in how geneticists study individuals and for what purpose. Let us first look at the how question and then address the why of these studies.

Geneticists do not study all genetic material, but focus on a very limited amount of information. V is hardly ever a topic in population genetics. The purpose of reducing genetic complexity and of studying limited amounts of genetic material is to learn about the presence or absence of lineage between populations. In doing so, the further geneticists go back in time, the fewer ancestors and the more lineage they presuppose, i.e. for a specific amount of genetic information. Ultimately, so the quote indicates, this genetic information coalesces in two ancestors, a mother and a father. This suggests that lineage is a product of a genealogical V placed upside-down, through which genetic material is distributed in specific ways. If V stands for the genealogy of an individual, then Λ stands for a specific type of genealogy, one that helps establish genetic lineage. Whereas V is about how the individual is connected to predecessors, the Λ is about how individuals are connected to each other via predecessors. From this perspective, however, the fact that all individuals have parents gains importance in studies of lineage, and attributes a specific meaning to that very fact. Turned around, this fact means that all individuals are parental products. From a genetic perspective individuals are first and foremost products of sexual reproduction. All parents pass on their genetic material via sexual reproduction to individuals. But whereas in the V-type genealogy, parents themselves are seen as individuals with parents and grandparents, thus permitting the V-shape, this is not the case in the Λ-type. Parents or ancestors whose genetic material is not represented in present generations, i.e. the specific type of genetic information under consideration, are left out of the picture. This means that although sexual reproduction and “parenthood” are pivotal for studies of genetic lineages, focusing on a
limited amount of genetic information and being interested in comparing it between individuals, parents become a necessary passage point of genetic information. The passing on of genetic material indicates that presence or absence of lineage between individuals or populations is a product of sexual reproduction. However, parents are not important as individual males and females reproducing sexually but as the means of producing lineage. Genetic lineage can therefore be seen as products of a specific genealogy (A) in which a limited amount of DNA is at stake and which is based on sexual reproduction. From this we learn that in studies of genetic lineage the sexes are not relevant as male and female parents but as source of reproduction and therewith as passage points, through which genetic lineage is established.

Given this central role of sexual reproduction, it is interesting that the Archaeology paper states that mtDNA and the greater part of the Y-chromosomal DNA are especially appreciated for studying genetic lineage, because they escape recombination. From a genetic perspective recombination and sexual reproduction are interchangeable. The case of mtDNA and Y-chromosome shows that geneticists study effects of sexual reproduction, namely genetic lineage, through particles that are excluded from sexual reproduction. Thus mtDNA and Y-chromosome are deemed valuable because they are conveyed by a reproductive system through which lineage can be established. Let us briefly ponder these two systems.

Unlike the Y-chromosome, mtDNA is to be found in the cytoplasm and not in the nucleus. Situated in the cytoplasm, mtDNA is passed on via the mother only. Males and females inherit their cytoplasm and so too their mtDNAs via their mothers, i.e. via the egg cell. Males have mtDNA but do not pass it onto their offspring: only females can do that. This system of inheritance accounts for a maternal lineage. The Y-chromosome, however, shows a different pattern. Fathers do not pass on their Y-chromosomes to female offspring, but solely to male offspring. Only males carry Y-chromosomes and pass them on to males. As was seen in passage quoted above, here also it is the system of inheritance that accounts for a paternal lineage. Thus the Y-chromosome accounts for a male line of inheritance and the mtDNA for a female line. Hence the compatibility of these sexualised systems of lineage for genetics.

From the perspective of the individual, however, there are other differences between the two systems. Viewed from the mtDNA approach there is no difference between males and females. They both have mtDNA. Sex emerges only in the pattern of inheritance: males cannot pass on their mtDNAs whereas females can. But from the perspective of the Y-chromosome male and female individuals differ. Only males carry this chromosome and they pass it on solely to male progeny. Hence in the Y-chromosomal system sex is not only performed as a pattern of inheritance
but can also be located in the individual's DNA. What does this difference between the two DNA systems tell us about the relevance of sex in studies of genetic lineage? The story of mtDNA in particular indicates that geneticists are not interested in the sex of the individual. Even though males do have them, their mtDNAs are considered to be part of the female line of inheritance. Interestingly enough, the Archaeology paper even excludes males from that system. It is stated there that mtDNA is passed on "from mother to daughter." This suggests that fathers are analogously not acknowledged for the fact that they carry a Y-chromosome, but for the fact that they pass it on to their sons. Thus in practices of genetic lineage sex is performed not so much as a quality of an individual but rather as a pattern of inheritance. Hence sex is not located in the individual but in genetic kinship.26

This specific relevance of sex can be viewed further if we take the most recent common ancestors (MRCAs) into account. The paper mentions three categories of MRCAs, one single mtDNA mother, one single Y-chromosomal father, and a third type of MRCAs consisting of many single autosomal "biparents," autosomal referring to the forty-four chromosomes located in the nucleus and inherited from both parents.27 Both maternal (mtDNA) MRCA and paternal (Y-chromosome) MRCA are estimated to have occurred about 200,000 years ago;28 the "biparental" MRCAs, however, may be four times older, so the Archaeology paper suggests. This implies that from a genetic perspective our MRCAs do not necessarily have to coincide with individuals or with actual parents. From this perspective MRCAs can best be seen as partial products of genetic lineage. In line with this, DNA is handled as a variety of technologies which, together with a Λ-type genealogy, assist in producing those lineages. Moreover as I have argued in the case of genetic lineage, genetic sex is not performed as a quality of individuals but as a pattern of inheritance or – better – a technology of lineage. Similarly DNA is not so much treated as an essential feature of individuals but as a technology "embodying" different systems for producing lineage leading to different MRCAs.29 Taking the mtDNA and Y-chromosomal systems into account, this treatment of DNA can therefore be seen as a technology for producing sexualised genetic lineages.

The Ir/relevance of Sex in Laboratory Practice

In the course of my participant observation in the P Lab I was working on a project which aimed at comparing two “bottlenecks,” one in the Sinai Desert and one in Finland, by studying the Y-chromosome.30 For this purpose the Finnish population was compared to that of Sweden and the Sinai populations to those living along the Nile and in the Nile Valley in
Egypt. Mitochondrial DNA studies have shown a reduced diversity in Finns when compared to the Swedish, and the same has also been found for three Y-chromosomal markers tested. The case of the Sinai Desert looked slightly different. There mtDNA showed a great diversity as in the rest of Egypt, whereas the three Y-chromosomal markers showed reduced diversity. The P Lab was interested in testing more Y-chromosomal markers to explain this difference and to see whether that difference still holds when more Y-chromosomal markers are used. Abdel-Halim Salem, who was working on both the Finnish population and those living in the Sinai, familiarised me with the project and with the lab and gave me a brief period of training particularly in working with large numbers of samples and in preparing the reagents for the PCRs. As I arrived at the P Lab, Salem was about to finish his work there to go back to Ismaelia (Sinai). In the meantime he was travelling back and forth between Munich and Innsbruck where he was learning more about clinical genetics and diagnostics.

Before we started working with the markers, Salem drew a map of Egypt to show me where the populations along the Nile and from the Sinai are living. Discussing the faith of the populations of the Sinai, he explained to me that most of the samples we had in the lab, except for the samples he (a medical doctor) had collected himself, were assembled in the sixties by an Israeli population geneticist, Professor Batsheva Bonne-Tamir. These were serum samples and since they were so old, their quality was not always that good. The set of Y-chromosomal markers we were about to use for the Finland - Sinai project were sent to the P Lab by Lab F. Salem showed me the set of primers, the" ladder" for each marker, some control samples (tested in Leiden) and the protocol that I was already so accustomed to. I nevertheless brought in my own copy containing notes and remarks I had made earlier when I was in Leiden. We went through the protocol and talked about how to establish the PCR condition; writing the programs, making the reagents, measuring the concentration of the primers produced in the P Lab based on the Leiden primer, and testing the markers for a small number of samples from a population called Sawarka (Sinai). Once the markers appeared to work we extended our work of typing them to more individuals from that population. The strategy Salem proposed was to do one population at a time for all markers and then move on to the next.

After I had finished typing one marker (DYS 390) for all the Sawarka samples, I found only two alleles, i.e. two fragment lengths. Instead of going on to the next marker, I decided first to compare these results to another population, Jabalya. Jabalya was an exception in the Sinai. Previous studies had shown that contrary to other populations in this region, it showed no reduction in diversity on the Y-chromosome. I was of course curious as to whether that would hold for this marker as well. I was unable
to discuss the change in method with Salem since he was in Innsbruck, so I took the samples from the "minus 4" (the refrigerator) and started running the PCRs. From the 36 samples that I tested none of them showed a band on the agarose gel. I then thought: well, it may be that the bands were not very strong and that they could nevertheless be detected by the ALF™, which is a more precise visualising technology. So I booked one ALF™ for the next day. But the end result was not positive either. When Salem came back, I told him about the "Jabalya-problem." Although he was at first a little annoyed that I had changed the plans, when I showed him the collection of samples that I was typing he started to laugh and stated "now we can tell Svante that we know for sure that the samples are females." It appeared that I had been trying to type the Y-chromosomes of females. Although there were six male samples in the box (he pointed them out to me) they were, of course, among the ten samples I had not used. We walked over to another part of the lab where he showed me a file in which I could find information about Lab P's samples. It contained different kinds of information, in many cases information about sex, about when and where the samples had been collected and by whom they had been supplied to the lab. He told me that if this file did not contain information about the sex of the samples I could have a look at his personal file on the Sun computer, where he had stored his raw data, including data about the three Y-chromosomal markers that he had typed earlier. He explained that with some of the samples it was unclear whether they were male or female, and then he stated "I don't even know if all non-males are females." This is especially a problem of serum samples, because if they fail to work for nuclear DNA, you cannot determine whether this is due to deterioration of the DNA or because they are females and do not have a Y-chromosome.

Following this episode we started reorganising the samples according to sex. We first took a second collection of the Jabalya samples and separated the two sexes in the boxes and then did the same for the other populations. Then I made a list of all the Sinai samples that are known to be males and wrote this information down in my lab journal.

Technologies of DNA/Technologies of Sex

In the analyses of practices of genetic lineage sex mattered as a pattern of inheritance. It mattered in the way it helped to establish genetic lineage. However in a DNA practice, as a procedure of producing data at the bench, the sex of the individual became a significant part of studying DNA. Compared to mtDNA, studies of the Y-chromosome are rather new in the field of population genetics. MtDNA has been used extensively ever since the 1970s. The first population studies on the Y-chromosome, however,
appeared in the early 1990s, and it was only in 1995 that a number of Y-chromosomal markers were talked about as being informative for the purpose of population studies. In the P Lab the first Y-chromosomal markers were introduced in late 1995 and the lab's first paper reporting work carried out using these markers appeared in 1996. This information reflects the organisation of daily work in the P Lab and the relevance of sex in doing DNA.

In daily lab work, mtDNAs and Y-chromosomes were considered not only in the way they are passed on from individual to individual, but especially in how they were distributed to individuals. Working on the Y-chromosome, it became apparent that some individuals have a Y-chromosome, namely males, and others do not. Are these then females? Just as in the Jabalya case, the absence of Y-chromosomal alleles was read by Salem as extra information about the female sex of those samples. This allelic information contributes to a "practice of chromosomes," i.e. a practice of XX-XY. In this practice the sexes are performed as presence or absence of the Y-chromosome. Since interest centred on how Y-chromosomal information is distributed among individuals, only individuals carrying a Y-chromosome were relevant for the work and were considered males. However working with rather old samples showed that this distinction is not "natural." Absence of a Y-chromosomal allele does not necessarily mean that the individual from which the sample was taken was a female. In this case the sex of the sample is an effect of good or deteriorating DNA. In this practice the sexes are not performed as presence or absence of a Y-chromosome, but that of an allele (a DNA fragment). The visualisation of a Y-chromosomal allele in a sample would make that sample into a male sample. What is performed as sex is therefore a local and contextual laboratory product, invested by the relevance of an individual sample for a particular experiment with a particular marker. Hence in a practice of deteriorating DNA, sex is performed not as a quality of a sampled individual but as that of an individual sample. But how is this reflected in the organisation of lab work?

Labs reflect the activities carried out in such space, and the organisation of the space is often centred around such activities. For example, in the lab there are cupboards above each bench containing most of the chemical solutions needed for the specific work conducted at that specific bench and there is always a set of pipettes, pipette tips and latex gloves within easy reach. The samples are also subject to this type of organisation. There is a spatial division between individuals according to population and they are preferably stored in separate boxes, unless the number of samples is very small and then they would be pooled in one box, but stored with some space between individuals belonging to different
populations. This was so in the case of the Sinai, with new and old samples, i.e. serum and blood samples, initially being stored in separate boxes. Sex, however, did not bring about such a spatial division. Males and females were mingled and placed in the same boxes. So how should we understand this mutual relevance and irrelevance of sex differences? How should we understand the pivotal role of sex for doing Y-chromosomal DNA, and the virtual absence of sex in the organisation of work?

Whereas I had problems seeing any system in the numbers assigned to the samples (some series would have unsystematic numbers, such as "101," "7125" or "77&78," others would have a number and a letter referring to the name of the population such as "B9," "B31," or "B91," still others would have a number and two letters such as "FB25" or "MB29," indicating males or females of that same population) Salem seemed to have the relevant information at hand. Simply by looking at the containers of DNA he would indicate to me which samples were gathered when, which of the samples were male or female – and, so he told me, he even knew personally some of the people represented by the samples he had collected himself. This information was neither absent nor irrelevant, even though it was not visible to a newcomer. This also applied to information about the sexes. Having worked much longer with the samples, Salem could be said to have embodied that information. My knowledge of the samples was limited, so I had to mobilise other practices of knowing the sexes by consulting the written records, the “Sample-file” and raw data in the “Sun computer.” For Salem these practices were already part of the letters and numbers that were written on the cups. Moreover since he had been engaged in collecting some of the samples, other repertoires of enacting the sexes were at his disposal. These repertoires consisted not only of written records and previous experience in the lab but also of an anatomical way of knowing the sexes. In such a way, for example, that the presence or absence of breasts makes sex, and enters the form as such. Also his remark about knowing some of the individuals of whom we had samples indicated yet another repertoire and another practice of performing the sexes. This is a practice in which the sexes are performed as social differences between men and women and where individuals can be referred to as Mr A or Mrs B, the brother of so-and-so or the mother of this or that person, also making it easier to personalise and sexualise a DNA sample. Salem’s knowledge regarding the samples in the lab was thus based on an interference between different repertoires and different practices where the sexes were performed. I had to introduce another way of establishing the sexes, namely that of creating a visual distance between male and female samples. By making a spatial division in the boxes and drawing up a list of all the male samples in my lab journal I
created means of transforming these different ways of knowing the sexes which became pivotal parts of doing DNA.

What other information does the initial way of organising the samples contain? What does it tell us about the work involved and the relevance or irrelevance of the sexes, using those samples? As indicated above, work conducted on the Y-chromosome is rather new in population studies as well as in the P Lab. The populations we were typing for the Y-chromosome were first studied using mtDNA. Unlike the Y-chromosome, both males and females carry mtDNA. From the perspective of mtDNA the sex of the individual is not relevant. Any human sample will do, even those whose sex can no longer be determined.50 Thus the storage of samples according to population, or even according to the DNA quality of the samples, can be seen as reflecting a former practice, a practice of doing mtDNA, for which the sex of the sample was irrelevant. Although the lab was moving away from mtDNA51 and although Salem as well as other lab members had already conducted Y-chromosomal research for which sex did matter, the samples occupied “the same place” as before. The changed practice was not reflected in how the samples were organised spatially. Rather it was operative as a management of different repertoires of performing the sexes. Managing these repertoires revealed an organisation of different practices. Whereas anatomical and social practices of performing the sexes, to which Salem had access, were dominant over a practice of written records, where sexual difference did not always appear, the practice of records was dominant over my lack of knowledge about samples, but a practice of DNA deterioration as in the case of the old samples was again dominant over the very practice of records and eventually over that of anatomy and sociology, if this repertoire were no longer to hand.52 Hence while the sexes were absent in the organisation of the samples in the lab, the sex of the samples could be performed as an effect of interfering practices and as the management of different repertoires.

The analysis of laboratory work has shown the relevance and irrelevance of the sexes. Whereas sex was irrelevant in a mtDNA practice, in a Y-chromosomal DNA practice it was enacted as a quality of the individual samples. Before addressing practices of genealogy, let us take a second look at the organisation of samples in the lab. This will make clear how much the mtDNA approach was involved in the P Lab’s collections of samples and how it has affected the gifting of samples.
The Relevance of the Sexes: Sexing the Gift

The Y-chromosome typing project was proceeding slowly. I had problems establishing standard PCR conditions that would work for each sample and for each “run” equally.\textsuperscript{53} In the meantime many new samples were coming into the P Lab: from Russia, Estonia, the Middle East, Nigeria. One set of samples that came in was destined specifically for the Y-chromosome typing project. At one point I walked into the lab and found a box of DNA samples on my bench. Along with it there was a note from Svante Pääbo saying that the Bosnian samples had arrived and asking whether I could see to it that they were stored properly. I was expecting the samples since we had talked about them previously during a Population Group Meeting. There Pääbo had mentioned that we could obtain Bosnian samples from Finland and that I might want to compare these to Jabalya. As I opened the box I first checked whether all the samples that were listed were there and then started to separate the males from the females. I immediately noticed the small number of male samples among them and reasoned that this bias had to do with the origin of the samples.\textsuperscript{54} I remarked on this difference to Maris Laan, who was just loading an ALF™ situated next to my bench. Not aware that I was referring to the Bosnian samples, she answered that she was not surprised. “It’s always the case. There are always fewer males than females.” She stated further that because samples are often collected in collaboration with medical teams, women tend to participate more often than males.\textsuperscript{55}

The P Lab tried to tackle the bias towards female samples by asking explicitly for male samples, as in the case of the Dutch samples. The newly delivered samples from the Middle East were all male samples, and when the lab asked for the Russian sample they stated clearly their special interest in males.

Changing Practices, Making Sexes

Laan’s account of the contribution of cell material by women and men when samples are collected shows a sexual bias in the availability of samples for population studies. The joint work of geneticists and medical teams may clarify the variety in the sampled populations. However it does not necessarily explain the bias in the P Lab collection. So let us take a look at how this bias may be viewed within the context of that lab.

As I have pointed out, samples may enter the lab without a specific research objective simply by being offered to the lab or because the lab knows someone who has rare samples difficult to obtain otherwise. These samples just sit there until a project comes up in which they could become
important. Such samples may reflect the specific variety produced in fieldwork. Other samples, however, are requested because there is already a project in which they are deemed significant. Such was the case for the largest part of the samples that arrived when I was in the P Lab. And in almost all cases these were male samples. This indicates that under specific conditions it was possible for the Lab to turn around the sampling bias produced in fieldwork. Thus the ratio of male and female samples in the P Lab does not necessarily have to represent the ratio that occurs in fieldwork where samples are collected. After all, the virtual absence of European samples in the lab cannot be said to represent fieldwork either, if one considers the long-standing history of medical studies which have made available large amounts of human blood or tissue. \(^5^6\)

This shows that the scarcity in male samples had not previously been perceived as a problem. \(^5^7\) It hints at a practice in which the sexes are not actively being performed and the samples do not differ in terms of sex. And as I pointed out above, this is a mtDNA practice. \(^5^8\) Whereas male samples became important in the new projects – the Y-chromosome project and Laan’s linkage disequilibrium project which, for practical reasons, took only male samples into account – from a mtDNA point of view there is no difference between males and females. Both males and females have mtDNA and can equally be studied. The samples are simply samples and do not have a sex. The apparent scarcity in male samples hints at a changing practice. A change from a practice where there was no sex to one where the sexes are performed as males and non-males.

To relate this back to the traffic in samples as gifts between laboratories and scientific groups, one could say that the sex of the gift emerges in scientific practices in which the sexes are made relevant. The gift is not male or female by nature, but acquires this quality through ongoing projects and through the nature of experiments conducted in laboratories. \(^5^9\)

In practices of genetic lineage the sexes were enacted as patterns of inheritance. A DNA practice, however, showed a more complex picture. The sexes were either absent, as in the case of mtDNA and in the spatial organisation of the samples, \(^6^0\) or they were enacted as a variety of things, as was the case for Y-chromosomal DNA. What sex is, appeared to be a product of interfering practices such as written records, anatomy, social roles, quality of a DNA sample and laboratory practice where specific markers had already been previously applied. In the final part of this chapter let us examine a third site, concerned with making lineage and genealogy, and see how sex matters in a practice where DNA is treated as a technology to produce accounts about the past of populations.
Genealogy: Technologies of Lineage/Technology of DNA

As has been shown above there are differences between mtDNA and Y-chromosomal DNA. These differences involve not only their pattern of inheritance or the way they contribute to the production of sexualised lineages or sexualised samples but also their locus in the cell. Whereas the Y-chromosome can be found in the nucleus, the mtDNA is located in the cytoplasm that surrounds the nucleus. But there are many more. The number of Y-chromosomes and mtDNA differ per human cell. While carrying one Y-chromosome in males, a human cell may contain up to a 100,000 copies of mtDNAs. This is the reason why, compared to Y-chromosomal DNA, mtDNA is convenient to work with using old samples. Then they differ in shape and size: mtDNA is a circular genome consisting of 16.5 kilo base-pairs whereas the Y-chromosome is linear and consists of 60 million base-pairs. And more important for studies of genetic lineage is that they differ also in their mutation rate. The non-coding part of mtDNA, and this is the region of interest in these studies, mutates twenty times faster than the non-coding part of the Y-chromosome. This results in a much higher diversity in mtDNA. As stated above, mutations are read as historical events in studies of genetic lineages. They contain information not only about genetic lineage, but also about events in the history of humans or that of a population.

I will explore this through the example of scientific papers. Two P Lab publications comparing both systems will be discussed. These papers study the above-mentioned “bottlenecks” in Finnish and Sinai populations. By so doing we will see how in studies of genetic lineage DNA is handled not only as a (standardised) technology to produce sexualised lineages but also as yet another locus of sex-difference.

The papers report a reduction in Y-chromosomal diversity in the Finns and in two populations in El Sawarka and El Bayadia (north of the Sinai). The mtDNA data, however, was found to be just as diverse as in the surrounding populations of both Finland and the Sinai. The reduced diversity on the Y suggested a bottleneck, due either to a reduction in population size or to a founder effect, which means that a small number of individuals contributed to the contemporary genetic variation. The question of course was how to understand these differences in diversity. In the case of Finland it was wondered whether the reproductive success of some Finnish males over others could explain this phenomena, in the sense that a small number of males had succeeded in passing on their genetic material whereas others had failed to do so. Or should we conclude that the “colonisation” of Finland was the work of a great number of women and only a small number of men? In the case of the Sinai the results seemed to confirm notions about marriage patterns in the populations studied. It was stated that male polygamy and
cousin marriages are frequent in this part of the world and that women marrying outside would leave their groups to live in that of their husbands. Hence "the traffic in women" here is suggested to be the main source of diversity in the Sinai populations.\textsuperscript{63}

To analyse the difference between the high diversity on the mtDNA and the low diversity on the Y-chromosome further, the difference in mutation rate was considered in both papers. It was argued that the non-coding mtDNA, also called the "control region," is not only known to mutate twenty times faster than the non-coding Y-chromosomal DNA, but that this rate also varies within the control region and that certain positions within this region would mutate up to fifteen times faster than others.\textsuperscript{64} Thus in comparison to Y-chromosomal non-coding DNA some positions of the control region may mutate thirty five times faster. The control region therefore consists of "slowly evolving (nucleotide) positions" and "fast evolving (nucleotide) positions." Variation in the first would indicate events that had occurred earlier in time and the variation in the latter class of positions is considered to be more recent. Taking this information into account and by focusing on slowly evolving positions it was found that the mtDNA diversity in Finns would fall back to match the reduction found in Y-chromosomal DNA,\textsuperscript{65} whereas the mtDNA diversity in the Sinai would remain higher than that found on the Y-chromosome, and just as high as the diversity found in surrounding populations (along the Nile and in the Nile Valley). Thus for the Finnish case it was concluded that the bottleneck was a reduction in population size and that it had affected males as well as females. For the Sinai, however, the reduced diversity on the Y-chromosome could be viewed as a founder effect and confirmed that marriage patterns explain the discrepancy between Y-chromosomal and mtDNA diversity. Moreover in the Finnish case the diversity found in rapidly mutating positions of the control region was used to date the bottleneck. By assuming that those mutations occurred after the (bottleneck) event and that such mutations take place once in a thousand years, the bottleneck was estimated to have occurred 3,900 years ago. Also the results in the Sinai case indicated that "the patterns of marriage must have been upheld over substantial time"\textsuperscript{66} and that future research should make it possible to study the age of these patterns.

\textbf{Doing Genealogy: Making Sexes}

I have argued above that mtDNA and Y-chromosomal DNA provide geneticists with a two-sexed model for studying genetic lineage. Sex was considered to be especially interesting as a pattern of inheritance, namely as the way in which information on the mtDNA and on the Y-chromosome is passed on. The Sinai and Finnish papers show how DNA is handled as both
data and technology to do exactly that. And they show more. But let us first take a look at how DNA is treated as a technology.

Putting genetic data in the context of lineage is not straightforward. Because they lack recombination, both the Y-chromosome and mtDNA are viewed as “molecular clocks.”67 This “clock” notion implies that mutations found in mtDNA are assumed to occur at the same rate in each individual and the same is assumed for the Y-chromosome. Consequently the number of mutations in each of these systems is assumed to correlate to an historical time. The more mutations, the more time they took to occur. Determining the time between two mutations is a crucial part of establishing genetic lineage. As stated earlier, mtDNA in general mutates at a much higher rate than the Y-chromosome. The mtDNA clock ticks faster than the Y-chromosomal clock. Yet both types of information are treated as complementary, indicating a parallel historical event. Thus these clocks have to be calibrated in order to discern a comparable historical time. To do this, it is not enough to know about patterns of inheritance, i.e. that the Y-chromosome shows the paternal line of inheritance and the mtDNA the maternal line. Specifically the Finnish case showed that the high diversity in mtDNAs in the Finns was not taken at “face value.” The control region of the mtDNA was treated as a system consisting of different clocks indicating different time calendars. By taking into account only the slowly evolving sites of the control region (as historical records), both that information and the one found in the Y-chromosome became standardised and compatible, contributing to an account of an historical event. When the event was identified as one affecting males and females equally, the information found in the rapidly evolving sites of the control region could be applied to date that event as a point in history. So the time on the clocks had to be discerned. By presupposing the time between two mutations in the rapidly evolving sites, the time of the events of interest could be set on the complementary clocks of both mtDNA and Y-chromosome. This shows that the mtDNA system becomes a technology contributing to accounts produced about the past of a population. Hence mtDNA is handled as both a technology, i.e. a calibrating device for setting molecular clocks, and a resource containing information comparable to that found on the Y-chromosome.68

However DNA is not alone in producing accounts. From the reduction in diversity in the Y-chromosomes of the Finns it was suggested that a small number of males have contributed to contemporary genetic variation. This bias in the number of males and females was the very reason for considering the slowly evolving sites of the mtDNA.69 This indicates that the Y-chromosome came to stand for men and the mtDNA for women, both living in the same period of time. Therefore although the two systems of inheritance point back to partial ancestors that do not necessarily have to
coincide with individuals or parents (MRCAs), the presupposition of
individuals and parents is necessary for studies of genetic lineage. The
Finnish Y-chromosomal data was read as men and a reduction in diversity as
a reduction in the number of men, raising the question of whether the same
was the case for women. Hence further analyses of mtDNAs. Similarly the
Sinai reduction in diversity on the Y-chromosome was placed in the context
of social relations of marriage patterns, which should explain the lower
diversity in men as compared to women. Hence in studies of genetic lineage
sex is not only performed as a pattern of inheritance. To be able to make
sense of various types of information based on mtDNA and Y-chromosomal
data and to produce an account of human history, these particles have to
stand for woman and man who pass on their genetic material in a socio-
historical context.

Despite all the differences between mtDNA and Y-chromosome, in the
context of genealogy the two systems are considered comparable because
both are passed on without recombination. They are also deemed compatible
because they point back to human ancestors in both mtDNA and Y-
chromosome who are supposed to have lived at the same historical time. And
then they are also considered to be complementary because they tell two
parallel human histories, that of men and women. They are considered to be
"molecular clocks," each ticking, i.e. mutating, equally rapidly in human
individuals. From the Sinai-Finnish example it became clear that these clocks
can be set in such ways as to give the same time whenever you look back
into history, revealing men and women.

To Conclude

In genetics XX and XY are just two of the various way of knowing the
sexes. Throughout this chapter I have located the sexes in practices of
generic lineage, I have traced the relevance and irrelevance of the sexes and
have examined how the sexes are enacted in such practices. The analyses
make clear that genetic sex is a doing, and that the various ways of
performing the sexes, the various ways of doing genetic sex in practices,
goes well beyond an identity that can be located in the individual – or the
DNA for that matter. Sex is just one form of doing genetic lineage, and in
itself it consists of many things. To be sure, genetic sex is not a list of
references to an individual. Such a list would rather point to practices of
doing genetic lineage. Thus to study the sexes and the differences between
them is to study the practices in which they are performed.

In studies of genetic lineage geneticists aim at giving an account of
human history based on the DNA. In these studies DNA is treated both as a
resource for learning about similarities and differences and as a technology
to establish lineage. I have shown that in a practice of mtDNA and of Y-chromosomal DNA, the handling of DNA as a technology helps to establish sexualised lineages. This, in its turn, affects the treatment of DNA as resource. DNA samples, as in the case of the P Lab, were no longer just population samples but were enacted as male and female samples. Moreover given the aim of studying the history of populations, the handling of DNA as a technology assisted the naturalisation of sexual differences. Differences and similarities in the DNA could thus be read back onto the history of populations, producing men and women. This indicates that studying the history of humans via the DNA subsumes the diversity in practice of doing genetic lineage and the various ways of performing the sexes in laboratories.

Might this then lead to the conclusion that in the end genetics does the same old thing: that it makes “biological” categories, and that feminists should keep an eye on how men and women are done outside this field? The aim of this chapter, however, was to show that genetic sex and sexual differences do not exist by themselves but are enabled by technologies. These very technologies affect not only our ways of knowing genealogy, lineage, parenthood and individuality but they also affect the practice of genetics itself. They thus affect what the sexes are made to be. The heterogeneity of scientific practice examined here may give hints about how to do biology according to the nature of scientific practice.
Acknowledgement
I would like to thank all members of the Laboratory for Evolution and Human Genetics (Munich). I thank Svante Pääbo for welcoming me into his lab, Mark Stoneking for in-depth contributions in the Population Group meetings. Abdel Halim Salem and Maris Laan especially are thanked for their help, advice and good company. All the lab members are thanked for being helpful, open and interested in an interdisciplinary study of their conduct and they are also thanked for making the lab a place I kept coming back to, long after I had finished my fieldwork. I would therefore like to mention the people working in the lab during my fieldwork: Valentin Börner, Alex Greenwood, Michael Käser, Christian Kilger, Andreas Kindmark, Matthias Krings, Sonia Meyer, Hans Zichtler, Helga, Gertraud, Walter, Frau Krella, and the other lab members. The Lab also had many visiting scientists from whom I learned a great deal, and among them I would like especially to thank Batsheva Bonne-Tamir for an exceptional seminar and for the very good time we had during her visit to Munich and mine to Tel Aviv. I thank Annemarie Mol for both moral and intellectual support and for characteristically pointed comments and practical solutions. Ruth Benschop, Brenda Diergaarden, Olaf Posselt, Hans-Jörg Rheinberger, and Frans Willem Korsten are thanked for generous comments full of insight. This chapter has also benefited from comments and suggestions made by members of the Belle van Zuylen Institute and the participants at the ASCA course “Presentation Skills” (1999) given by Frans Willem Korsten. Finally I thank the Deutscher Akademischer Austauschdienst (DAAD) and the Netherlands Organization for Scientific Research (NWO) who kindly supported my research in Munich.

Notes to Chapter 5

1. In colloquial speech between geneticists the laboratories are usually addressed as the Pääbo Lab and the Forensic Lab, hence my choice to refer to them as the P Lab and Lab F.
2. Linkage disequilibrium is the non-random association of alleles on chromosomes, i.e. the phenomenon that different alleles or genes are linked in their pattern of inheritance. A popular example of this is that of hair and eye colour and the specific combinations in which they are inherited. It should be noted that Maris Laan’s project did not aim such phenotypic traits but at what she calls “anonymous loci” and their frequencies in different populations; see Maris Laan and Svante Pääbo, “Demographic History and Linkage Disequilibrium in Human Populations,” Nature Genetics 17 (1997):
435-438. Note that the X-chromosome does not escape recombination in general, because it may just as well stem from a female individual, but Maris Laan looked at markers in the isomers where the chances of recombination are the lowest.


5. Both feminisms and feminist studies of the sciences constitute a large and contested terrain. I can hardly do justice to the diversity within these domains nor acknowledge the many inspiring work of different kinds, such as that of Evelyn Fox Keller or that of Sally Hacker, Donna Haraway, Judith Butler and Annemarie Mol, by talking simply, as if it were that, about feminist scholars. Nor can I do justice to the elaborate studies contributed to the field of science studies by feminist scholars and feminists in general. For early contributions to the latter see, for example, two edited issues; Ruth Bleier, ed., Feminist Approaches to Science (New York, Oxford: Pergamon Press, 1988), Jan Harding, ed., Perspectives on gender and Science (London, New York: The Falmer Press, 1986). For an insightful study on feminisms (liberal, Marxist, radical and socialist feminism), see Alison M. Jaggar, Feminist Politics and Human Nature (Sussex, New Jersey: The Harvester Press, Rowman & Allanheld Publishers, 1983). My interest in genetics is very much indebted to and inspired by the work of Evelyn Fox Keller. Sally Hacker taught me that one should engage in science and technology to make a political difference, Donna Haraway’s work gave me the promise of combining socialist, feminist and anti-racist politics with academic work and doing science studies, Annemarie Mol’s and Judith Butler’s works showed that there were other ways of theorising sex or gender, making it possible for me to relate to it. Moreover from Annemarie Mol’s work I learned to focus on processes of doing science and their normative involvement in scientific objects. Sally Hacker, Pleasure, Power, and Technology: Some Tales of Gender, Engineering and the Cooperative Workplace (London, Sydney: Unwin Hyman, 1989), Evelyn Fox Keller, Reflections on Gender and Science (New haven: Yale University Press, 1986), idem, Secrets of Life, Secrets of Death: Essays on Language, Gender and Science (New York: Routledge, 1992), idem, “Nature, Nurture, and the Human Genome Project,”

6. My use of "sex" and not "gender" is motivated. Even though the English language puts some constraints on its use. I apply it not only to destabilise the seemingly neat distinction between sex (as being biology) and gender (as being culture), but also in accord with my claim that culture is part and parcel of genetic practices. In her study on primatology, Donna Haraway states the following with regard to the distinction between sex and gender: "The boundary between sex and gender is the boundary between animal and human, a very potent optical illusion and technical achievement”(Donna J. Haraway, “Primatology Is Politics by Other Means,” in *Feminist Approach to Science*, ed. Ruth Bleir [New York, Oxford: Pergamon Press, 1988], 77-118, at pp. 95). For an elaboration on sex and gender and especially on its use in Dutch feminist studies, see Annemarie Mol, "Dit Geslacht Dat Zoveel Is: Een Conversatie Tussen een Onbekend Aantal Onbekenden van Wie Slechts ÉÉn zich Bekend Zal Maken," *Tijdschrift voor Genderstudies* 1


8. Keller’s elegant paper on the issue of gender and science tries to go beyond, or to find diversity between, the feminine-masculine-dichotomy. It should be noted that Keller does not elaborate on this aspect of counting, namely that there might as well not be anything there to count. In her paper Keller engages in a discussion about biological discourse, but along with many feminist scholars she does not address the issue of biological sex. The latter is taken as the unproblematic and not so relevant fact in the complex configuration of cultural sex determination, namely gender; Evelyn Fox Keller, “How Gender Matters, or, Why It’s so Hard for Us to Count Past Two,” in Perspectives on Gender and Science, ed. Jan Harding (East Sussex, Philadelphia: The Falmer Press, 1986), 168-183. For work that does take the matter of biology seriously, see the work of Donna Haraway and that of Annemarie Mol quoted (above, n. 5 & 6); on the materiality of sex-difference, see also Nelly Oudshoorn, Beyond the Natural Body: An Archaeology of Sex Hormones (London, New York: Routledge, 1994); for criticism of the stability of biological sex, see also Judith Butler, Bodies that matter: On the discursive Limits of “Sex” (London, New York: Routlegde, 1993), Stephan Hirshauer, “Performing Sexes and Genders in Medical Practices,” in Differences in Medicine: Unraveling Practices, Techniques, and Bodies, ed. Marc Berg, and Annemarie Mol (Durham, London: Duke University Press, 1998), 13-27.
9. Judith Butler proposes the notion of “matter” as an alternative to notions of construction. I follow this notion, which she defines it as “a process of materialization that stabilizes over time to produce the effect of boundaries, fixing, and surface we call matter,” Judith Butler, Bodies That Matter (above, n. 8), pp. 9 (italic in original). The notion of “matter” is akin to that of “performance” which I am more inclined to use, specifically because the latter focuses more on the process of performing an object rather than the end-product. Moreover, I hesitate to apply “matter” because of the understanding it conveys. Although for Butler it means both, matter is commonly understood as “to matter,” i.e. to be important rather than “to become matter,” i.e. to become material. Haraway would call this effect crusted language, see Amâde M’charek and Els Rommes, “Herkouwende Bewegingen: Een Verslag van een Zomerschool met Donna Haraway,” *Tijdschrift voor Genderstudies*, Vol. 3 (1998), 61-66. On performativity, see below, n. 10.

10. I use “temporality” to indicate temporary nature. In this chapter I use performance and enactment interchangeably. The notion of performance or enactment was introduced by the sociologist Erving Goffman to examine how people stage identities or social roles. The philosopher J. L. Austin has theorised the performativity of language - “the performativity of utterance” - , namely the effect of content and context of words in a specific setting. In the social studies of science, and especially in ethnographic studies, this notion is applied to indicate that objects emerge in networks consisting of people, technologies, language and other things, and to show objects are “staged” in scientific practice. Moreover, the concept of performance emphasises the non-stable character of such objects as well as their dependence on locales in which they are actively performed. On the notion of performativity in the social studies of science, see for example Annemarie Mol, “Pathology and the Clinic: An Ethnography of Two Atheroscleroses,” in *Intersections: Living and Working with the New Medical Technology*, ed. M. Lock, Alberto Cambrisio, and Allan Young (Cambridge: Cambridge University Press, 2000), John Law, “On the Subject of the Object: Narrative, Technology, and Interpellation,” *Configurations* 8 (2000): 1-29; Erwin Goffman, *Encounters: Two Studies in the Sociology of Interaction* (Harmondsworth: Penguin University Books, 1961), pp. 71-134, J. L. Austin, *How to do Things with Words* (Cambridge, Massachusetts: Harvard University Press, 1962). On performativity of sex, see Stephan Hirschauer and Annemarie Mol, “Shifting Sexes, Moving Stories” (above, n. 4), Hirschauer, “Sexes and Genders” (above, n. 8), Butler, *Gender Trouble* (above, n. 5), idem, *Bodies that Matter* (above, n. 8). On (inter)sex(uality) as an achievement and passing, i.e. an active performance and a management of


12. The various projects conducted in the P Lab are subdivided into five groups. For example the different projects concerned with human population histories are part of the Population Group. All groups meet on a weekly basis with the professor of the P Lab to discuss the individual projects and how they are progressing.

13. This large amount of samples was in fact available as blood samples in the P Lab. But as the HIV research group of professor Jaap Goudsmitt (Academic Medical Centre, Department of Human Retrovirology) in Amsterdam became interested in these samples for family studies and since the samples consisted of so called trios, i.e. father, mother and child, the HIV lab offered to do the extraction and share the DNA with the P Lab, which saved an enormous amount of work.

study in a chemistry lab/class where students carried out their experiments. Schreck er assisted a disabled student (partially paralysed) in this class. This study shows that laboratory work is embodied and circumstantially contingent, and that experiments are conducted “in the spatiality and arrangement of the lab-table’s display.”

15. The importance of this “gift economy” becomes apparent at conference meetings. Especially during coffee or lunch breaks or during dinners scientists would establish collaboration of various kinds. Furthermore the acknowledgement section in published papers could also be read as a tribute to the various gifts, in the form either of samples or other material, comment and feedback comments, statistical analyses and various forms of assistance and so on.

16. For different versions of population in laboratory practice, including Dutchness, see chapter 2.

17. The exchange economy between the P Lab and Lab F was initiated during the conference Genetic Variation Europe: Genetic Markers (Barcelona November 1995). Peter de Knijff, the head of Lab F, and myself attended this conference and met Svante Pääbo, the head of the P Lab, and Antti Sajantila. There collaborations on the Y-chromosome as well as on my own research project were discussed and established; for elaboration on collaborations amongst geneticists, specifically with reference to Y-chromosomal markers, see chapter 3.


19. Ibid., p. 137

20. The reference to archaeology points to a heated debate between disciplines, namely between genetics and palaeontology. The battle is about human origin, and about which discipline has the best access to it. An example of such a debate is that between the paleontologists Alan Thorne and Milford Wolpoff on the one hand, and Alan Wilson and Rebecca Cann on the other. The issue is not only which sources provide the best entry into human history and origin but also how the spread of humans around the world came about; the multiregional theory versus the African origin theory; see Alan G. Thorne and Milford H. Wolpoff, “The Multiregional Evolution of Humans,” Scientific American, no. April (1992): 28-33; Allan C. Wilson and Rebecca Cann, “The Recent African Genesis of Humans,” Scientific American, April (1992): 22-27. Also, in an interview Mark Stoneking, a population geneticist talked about this ongoing debate and made clear the privileged view of genetics as follows: “We geneticists know that our genes must have had ancestors, but palaeontologists can only hope that their fossils
had descendants” (Interview with Prof. Mark Stoneking, at The Laboratory for Evolution and Human Genetics in Munich, March 11, 1997).

21. Note that I do not wish to discuss different ideas about parenthood or practices of parenthood as such, but rather how parenthood contributes to versions of the sexes in studies of genetic lineage. Anthropologists of different kinds have addressed the issue of kinship and reproduction. Traditionally anthropologists have taken kinship to be that which comes “‘after the fact of nature.’” “It is important to realize at the outset that, while the biologist studies kinship in the physical sense, for the social anthropologists kinship is not biology, but a particular social or cultural interpretation of the biological universals just mentioned,” (Robert Parkin, *Kinship: An Introduction to the Basic Concepts* [Oxford: Blackwell Publishers Ltd, 1997], pp. 3). Marilyn Strathern, more attune with the approach on kinship I wish to explore here, looks at similarities and dissimilarities between nature and culture. She takes kinship as a hybrid connecting these technically assisted domains and as a product of individuality and diversity, see Marilyn Strathern, *Reproducing The Future: Anthropology, Kinship and the New Reproductive Technologies* (Manchester: Manchester University Press, 1992), idem, *After Nature: English Kinship in the Late Twentieth Century* (Cambridge: Cambridge University Press, 1992).


23. It should be mentioned that genetic lineage in a population refers to frequencies and to the distributions of variation (in alleles), where a Gauss curve stands for lineage and a skewed curve indicates the absence of lineage. It goes beyond the scope of my argument here to address these statistical technicalities of genetic lineage.


25. See Chapter 4 for a discussion of the controversy over bi-parental inheritance of mtDNA.

26. Similarly Annemarie Mol argues that certain strands of the life sciences do not sex the individual body but its characteristics. The sexed body emerges, for example in anatomy, only in relation to other bodies assisted by
statistics, as being feminine or masculine; Mol, “Wie Weet Wat een Vrouw Is” (above, n. 5), p. 20.

27. Note that another category of MRCAs is not considered, namely the X-chromosomal MRCA. The X-chromosome is not an autosomal but a sex chromosome, and it is bi-parentally inherited in females in contrast to its pattern of inheritance in males.

28. Geneticists would always point out that these estimates are rough. Modern humans are estimated to have emerged in Africa about 100,000 – 200,000 years ago. Whereas this dating would coincide with estimates for mtDNA, and this is not surprising because the emergence of modern humans was based on mtDNA data, the dating of Y-chromosomal DNA seems to be a little older, namely 270,000 years ago; see for mtDNA estimations, Arndt von Haeseler, Sajantila, and Pääbo, “The Genetic Archaeology” (above, n. 18), p. 135, for both estimates, see Svante Pääbo, “The Y-chromosome and the Origin of All of Us (Men),” *Science* 268, no. 26 May (1995): 1141-1142, and for Y-chromosomal DNA estimates, see Robert L. Dorit, Hiroshi Akashi, and Walter Gilbert, “Absence of Polymorphism at the ZFY Locus on the Human Y-chromosome,” *Science* 268, no. 26 May (1995): 1183-1185.

29. For an analysis of how both DNA and the cell are treated as technologies, see Hans-Jörg Rheinberger, “Von der Zelle zum Gen: Repräsentationen der Molekularbiologie,” in *Räume des Wissens: Vertretung, Codierung, Spur*, ed. Hans-Jörg Reinberger, Michael Hagner, and Betina Wahringer-Schmidt (Berlin: Akademie Verlag, 1997), 265-279. In this paper Rheinberger states the following: “Diese Enzyme und sonstigen gereinigten Moleküle stellen eine Art ‘weicher’ Technologie dar, eine molekulare Technologie, die der Lebensprozeß selbst über eine Periode von Milliarden Jahren entwickelt hat” (ibid., p. 275).

30. A bottleneck refers to a reduction in genetic diversity explained, for example, by a reduction in population size.


32. Whereas Lab F, where I was trained to conduct these experiments, works with standardised reagents, primers, and PCR programmes, in the P Lab there are no standard protocols and all ingredients needed for a PCR reaction are prepared in the lab. In Lab F reagents, the polymerase enzyme and primers are ordered by default at pharmaceutical companies. Also in this lab I was used to working with a small number of samples, which has an impact on the procedure of “setting up” a PCR and reduces the risk of making mistakes.
33. Professor Bonne-Tamir visited the P Lab when I was there. She gave a seminar in which she addressed the issue of sampling, how it was conducted in 1967 and showed slides of some of the populations as well as the scientists.

34. Blood serum is the fluid which precipitates when blood clots.

35. Primers are necessary for the copying or cloning of DNA fragments using PCR. They make up the beginning and end of such a fragment and are synthetically produced. The ladder could be seen as an extra sample which works as reference because it contains all possible variations that could occur in such a DNA fragment and therefore helps to estimate the size of the fragments in the samples under study. Some ladders are called universal. They do not contain the fragment sizes that can be found for a specific marker, but standard ones, such as 50 bp, 100 bp, 150 bp, etc. These ladders, also called sizers, serve as molecular weight “markers” for the latter, see Daniel L. Hartl, *Essential Genetics* (Sudbury: Jones and Bartlett Publishers, 1995), p. 379; see also chapter 3, where I describe PCR and the ingredients necessary for it more extensively.

36. For allele, see Chapter 3.

37. The origin story of Jabalya states that it was founded in the 17th century as a monastery by Christian monks. Its population became intermixed both due to pilgrimages and due to the fact that Jabalya was traditionally a passage point for those wishing to cross the desert.

38. PCR, Polymerase Chain Reaction, is a DNA fragment copying technology using a thermostable enzyme. This procedure not only produces more DNA, making it easier to study, but a chemical group (such as a fluorescent group) is also attached to the copies in order to visualise them using ultra violet or laser beams, see Chapter 3.

39. ALF™ and agarose gel assist the visualisation of DNA. They render visible the marker fragments of interest. Both are based on electrophoresis. When an electromagnetic field is applied to the DNA fragment, which has a plus and a minus pole, it is enabled to “migrate” over the gel. The time a fragment needs to migrate is related to the “molecular weight,” the length of such a fragment. Typically the visualised alleles via agarose are referred to as “bands” and on ALF™ as “peaks,” indicating what can be seen or – better – how the alleles are represented.

40. Again I would like to emphasise that the distinction between doing genetic lineage and doing DNA is not ontological. Genetic lineage is involved in daily lab work, hence my move from Sawarka to Jabalya. More in general, analysing the data may include doing more DNA, looking at more samples, consulting the data of colleague labs, etc.


43. The viability of these markers was acknowledged in a round-table discussion at the conference *Human Genome Variation in Europe: DNA Markers* (Barcelona, November 1995); see for an elaboration Chapter 3.


45. On the locally invested nature of genetic markers and an elaboration on how they are involved in producing similarities and differences, see chapter 3.


47. The P Lab does not assign new numbers to samples that come into the lab. Whatever number the samples have, this is how they are stored. It is a practical method because most of the samples are collected by other laboratories or scientific groups. Keeping records of who supplied the samples and when, and adopting their nomenclature offers a way of communicating what is and is not already in the lab when new deliveries come in.

48. For such interferences, see Mol, "Wie weet wat een Vrouw is" (above, n. 5).

49. Interference is a Donna Haraway term. For an elaboration on interfering practices, see Law, "the Subject of the Object" (above, n. 10). On the relevance of breasts in medical practices of trans-sexuality, see Hirschauer, "Sexes and Genders" (above, n.8).

50. Old samples work better for mtDNA than for nuclear DNA. The reason for this is that each human cell contains about 100,000 copies of the same mtDNA and only one copy of nuclear DNA, the latter being divided over the
46 chromosomes. If DNA starts to deteriorate there is a fair chance that there are still some mtDNAs left useful for study, which cannot be said to be the case for nuclear DNA.

51. Prof. Svante Pääbo, interview held at The Laboratory for Evolution and Human Genetics in Munich, February 4, 1997.

52. On lack of stability of a person’s sex and the need for it to be actively performed to keep it, see Hirschauer and Mol, “Shifting Sexes, Moving Stories” (above, n. 4). On how performing atherosclerosis needs to be revitalised again and again in order not to disappear, see Mol, “Pathology and the Clinic” (above, n. 10).

53. On problems and frustrations of beginners working with routine technologies; see Kathleen Jordan and Michael Lynch, “The Sociology of a Genetic Engineering Technique: Rituals and Rationality in the Performance of the “Plasmid Prep”,” in The Right Tool For the Job: At Work in Twentieth-Century Life Sciences, ed. Adele E. Clarke and Joan Fujimura (Princeton, New Jersey: Princeton University Press, 1992), 77-114. Also when I was talking about these PCR problems with a lab member, Valentin Börner, I explained that my PCRs seemed to work at random. Valentin helped me to change the protocols for some markers, and told me that sometimes there seems to be more to experiments than rationalised approaches. Although one can cut experiments into distinctive parts, written down step by step in a lab journal, one sometimes simply does not succeed in repeating them. Experiments at times appear to him as unique achievements in which “something else” is very much involved. He referred in this context to the “The Puppet Theatre” in which rationalisation of body movements and gestures as singular achievements are portrayed, see Heinrich von Kleist, “Über Das Marionettentheater,” in Sämtliche Werke und Briefe, Band II, ed. Heinrich von Kleist (Darmstadt: ?, 1993), 338-345.

54. The Bosnian samples were collected for a forensic study aiming at identifying the victims of the war massacre, i.e. the corpses found in the mass graves. It was not clear whether the samples we received in the lab were from the dead victims or their relatives.

55. For example when professor Bonne-Tamir gave a presentation about how she and her team collected the samples in the Sinai Desert, she explained that they would first have a long talk or negotiation with representatives of these tribes who would then give them a list of individuals they could sample.

For example, the geneticists Mark Jobling and Chris Tyler-Smith, both conducting work on the Y-chromosomes, urge geneticist engaged in collecting samples within the context of the Human Genome Diversity Project to take notice of this bias and to include adequate numbers of male samples; Mark A. Jobling and Chris Tyler-Smith, “Fathers and Sons: The Y Chromosome and Human Evolution,” TIG 11, no. November (1995): 449-456, p. 455.

See; Annti Sajantila et al., “Genes and Languages in Europe: An Analysis of Mitochondrial Lineages,” Genome Research 5 (1995): 42-52. Matthias Krings has been studying ancient mtDNA in samples from Egypt (unpublished data) and Abdel-Halim Salem studied contemporary populations in the Nile Valley and in the Sinai; data partially unpublished, partially published in Salem et al, “Genetics of Traditional Living” (above, n. 44).

Similarly Marilyn Strathern has argued the following on the gender of the gift: “However, one cannot read such gender ascriptions off in advance, not even when women appear to be the very items gifted. It does not follow that “women” only carry with them a “female” identity. The basis for classification does not inhere in the objects themselves but in how they are transacted and to what ends. The action is the gendered activity” (Strathern, The Gender of the Gift [above, n. 11], p. xi).

The absence of the sexes is not exceptional. This would also hold for the twenty-two autosomal, i.e. non-sex, chromosomes. Since all human individuals carry a set of two for each of these chromosomes, sex does not matter there either.

This is one of the reasons why the Y-chromosome had been insufficiently studied. It proved to be difficult to find “polymorphisms” on the Y-chromosomes and those found were not deemed suitable for studies of lineage and evolution. For a good and comprehensive review of Y-chromosomal studies; see Jobling and Tyler-Smith, “Fathers and Sons” (above, n. 57).

Salem et al., “The Genetics of Traditional Living” (above, n. 44), Sajantila et al., “Paternal and Maternal DNA Lineages” (above, n. 31).

In this respect Luca Cavalli-Sforza stated the following in a lecture: “the Y-chromosome is highly geographically clustered, compared to mtDNA. MtDNA is far less clustered. There are two reasons for that. One is that there are so many mutations on mtDNA and two that women tend to migrate more than men.” He added jokingly: “La Donna e Mobile,” referring to the opera of Giuseppe Verdi, Rigoletto (1851). The lecture was given at the 5th Annual
Meeting of the Society for Molecular Biology and Evolution, Garmisch-Partenkirchen, Germany, 1 - 4 of June 1997.

64. This phenomenon is also known to be the case for Y-chromosomal non-coding DNA, but is not addressed in these P Lab publications. On various different mutation rates on the Y, see Jobling and Tyler-Smith, “Fathers and Sons” (above, n. 57), p. 450. For different mutation rates in the control region (mtDNA) see, John Wakely, “Substitution Rate Variation Among Sites in Hypervariable Region I of Human Mitochondrial DNA,” *Journal of Molecular Evolution* 37 (1993): 613-623, Masami Hasegawa et al., “Towards a More Accurate Time Scale for the Human Mitochondrial DNA Tree.” *Journal of Molecular Evolution* 37 (1993): 347-354, especially 350.

65. This is the case if only positions that change once in 13,000 years are considered, Sajantila et al., “Paternal and Maternal DNA Lineages” (above, n. 31).


67. For an elaboration of the concept of the molecular clock, see chapter 4.

68. This hybrid feature of mtDNA, being technology and resource, is not exceptional. I have shown this to be the case for genetic markers and for the Anderson sequence, see chapter 3 and chapter 4.