

Review

Difficulties in Kinship Analysis for Victims' Identification in Armed Conflicts

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Abstract: Regarding human identification in armed conflicts, various complications can be observed. Usually, such difficulties can be social-related, which can include the lack of access to the relative's genetic material, or the unwillingness of administrative and judicial authorities to participate in the process of identification. In the case of genetics, the analysis allows identifying the individual from a blood sample, a part of an organ, or from skeletal remains, which is why it is considered a much more extensive and effective method when compared with fingerprint techniques or odontology. However, several factors can prevent this identification, such as considerably degraded genetic material. For successful identification, it is mandatory to have access to antemortem biological samples unequivocally attributed to the individual in question, using recombinant nuclear markers, as well as using biological samples from close relatives, whether parents or sons. Nevertheless, the problems associated with armed conflicts make this type of study very difficult. In this article, we focus on the main difficulties encountered when identifying an individual victim of an armed conflict, as well as on the possibilities that exist and on viable measures that could be required to improve the identification of these victims.

Keywords: DNA; kinship analysis; victims' identification; armed conflicts; familial research



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1. Introduction

Armed conflicts and terrorist acts, characterized by hostility, aggression, and (extreme) violence (Singh et al. 2022), usually bring with them the disappearance or the profound difficulty to identify numerous persons. The situation of these individuals can be quite variable, from their disappearance in formal displacements to escape or participate in the conflict (Le and Nguyen 2020), or due to a possible arrest and/or execution, with what this implies. Other persons may have been victims of various events of the conflict itself and have perished, for example, victims of bombings, explosions, attacks with grenades, and missiles, among others.

Regardless of the reason for the individual's disappearance, the lack of news about his well-being or activity leads to a deep feeling of insecurity in the society in question, mainly among the affected relatives. The adequate recovery and identification of human remains is a necessary process during and after an armed conflict, for the reparation not only of the relatives of disappeared persons but also for the re-establishment of the community identity itself.

Armed Conflicts and Victim Identification

Since the formation of different human societies and the consequent group relationships, conflicts have always existed (Calleja 2008; Strathern and Stewart 2008; Theune

2022). For different reasons, the armed conflict has always been the (most) violent way of solving numerous problems (Fry 2008), from the conquest of territories to the resolution of “questions of honour” (Strathern and Stewart 2008; Singh et al. 2022). Although armed conflict is not a contemporary strategy for “solving problems” (Calleja 2008; Strathern and Stewart 2008; Fry 2008; Theune 2022), this article focuses on the most recognized conflicts in the 20th century, both due to the numbers of human deceased found, as well as their repercussions on their relatives (Strathern and Stewart 2008), many of them still alive. The list of armed conflicts on different continents during the 20th century is quite extensive. Below are some examples of the different armed conflicts that resulted in a truly impressive number of victims (Harbom and Wallensteen 2007; Pettersson and Wallensteen 2015; Le and Nguyen 2020), the majority of which remain unidentified.

Europe has been the scene of more than 100 conflicts and local uprisings, including two world wars. Table 1 briefly indicates some armed conflicts and the estimated number of deaths.

Table 1. Estimated number of deaths in some of the armed conflicts that devastated Europe, Asia, Africa, and America.

Armed Conflict	Date	Estimated Number of Deaths
Finnish civil war	1918	Over 33,000 (Finnish Government Project 2004)
Russian civil war	1917–1921	7,000,000–12,000,000 (Holquist 2002)
Irish civil war	1922–1923	300–400 (Durney 2011)
Austrian civil war	1934	100–1000 (Lehne and Lonnie 1985)
Spanish civil war	1936–1939	Over 300,000 (Thomas 1977; Simkin 2012; Alonso-Milan 2015; Clodfelter 2017)
Greek civil war	1945–1949	Over 150,000 (O’Balance 1966; Jones 1989; Lomperis 1996; Bercovitch and Jackson 1997)
Albanian civil war	1997	2000–3800 (Jarvis 2000)
Korean civil war	1950–1953	2,000,000–3,000,000 (Lewy 1980; Cumings 2011)
Nepalese civil war	1996–2006	12,000 (OCHA Services 2012)
Angola civil war	1961–1974	500,000–800,000 (Resource Information Center 2000)
Algeria civil war	1954–1962	150,000 (Hagelstein 2007)
Burundi civil war	1993–2005	300,000 (BBC 2008)
Rwanda civil war	1994	500,000–800,000 (Cunningham 2011)
Second Sudanese Civil War	2013–2020	1,000,000–2,500,000 (U.S. Committee for Refugees and Immigrants 2001)
Cuban Revolution	1956–1959	Over 3000 (Dixon and Sarkees 2015)
Chaco War (Paraguay–Bolivia)	1932–1935	70,000 (Eckhardt and Sivard 1987)
Brazil–Constitutionalist Revolution	1964–1985	2500–3000 (Hilton 1982)
Chile	1973–1990	Over 3000 (Truth Commission: Chile 90 n.d.)

One of the best-monitored conflicts in terms of the number of victims was the conflict in the former Yugoslavia during the 1990s. According to the International Commission for Missing Persons (ICMP 2022), it is estimated that 40,000 persons went missing or were killed as a consequence of armed conflict, abuses of human rights, and other atrocities (ICMP 2022).

2. Civil and Family Involvement in the Identification Process

Taking as an example the Spanish Civil War (1936–1939), considered the military preparation for the Second World War, several graves are, gradually, being studied (Baeta et al. 2019a, 2019b; C. Gomes et al. 2019a, 2019b; Palomo-Díez et al. 2019; Etxeberria et al. 2021; Herrasti et al. 2021; Palomo-Díez et al. 2021), mostly due to the concerns of the affected family members. It is estimated that the number of victims could exceed 300,000 individuals (Thomas 1977; Simkin 2012; Alonso-Milan 2015; Clodfelter 2017). The action model for the identification of human remains is based on annual governmental calls for family or historical associations so that they take the necessary steps for the identification process to begin properly (Ríos 2012; Etxeberria et al. 2021; Herrasti et al. 2021). The

financial support initially provided by families and/or associations is what often allows for the beginning of the investigation process and the victims' identification (Etxeberria et al. 2021; Herrasti et al. 2021). For example, in the case of Catalonia (Spain), the government of the Generalitat has taken the initiative to call public tenders for the identification of individuals who died in the Civil War and during the Franco Dictatorship in that specific territory (Palomo-Díez et al. 2019). An important law was published in that country in the year 2022 (Ley 20/2022, of October 19, of *Democratic Memoria*), where one of the primary objectives is to encourage the identification of individuals both during the Civil War and during the subsequent dictatorship.

3. The International Commission for Mission Persons—ICMP

The International Commission for Mission Persons, ICMP, physically situated in The Hague, Netherlands, works with different civil society organizations and governments to investigate “people who have gone missing as a result of armed conflict, human rights abuses, disasters, organized crime, irregular migration and other causes” (ICMP 2022). ICMP aims to promote legislation in affected countries and provide scientific and specialized knowledge to locate and identify victims. On the other hand, ICMP supports the process of justice, guaranteeing that governments adhere to a rule-of-law-based approach to investigating disappearances and presenting, whenever possible, evidence in criminal trials. Since its creation in 1996, the ICMP has been implicated in the excavation of more than “3000 mass and clandestine gravesites” (ICMP 2022) and has been also involved in the identification of more than 20,000 persons from different conflicts.

ICMP operates at a global level, as can be seen on its official website (<https://www.icmp.int/where-we-work/>, accessed on 16 January 2023), in different regions of Africa, Europe, the Americas, and Asia. However, for this organization to act, it is mandatory to cooperate with the governments in question, so many of the world's armed conflicts are beyond its reach. Examples include the Spanish Civil War (1936–1939), Afghanistan (from the 1970s of the 20th century), Argentina (from the 1960s to the 1980s of the 20th century), the period of armed terrorist conflict in Peru (1980–2000), Burundi (since 2015), and Nigeria (since from 2015).

4. An Attempt to Harmonize Human Identification—Interpol DVI Protocol

In 1984, in an attempt to standardize procedures for carrying out human identification in cases of disasters, Interpol published a guide for the first time—the Interpol Manual for the Identification of Victims. Through time, technological progress and the new experiences acquired by governments after numerous catastrophes led to a necessary revision of this guide. Updating and correcting the manual led to the publication in 1997 of the Disaster Victim Identification Guide. It provides practical guidance on the issue of victim identification, allowing better and faster identification in the event of an actual disaster situation. This guide has inevitably had to go through many scrutiny processes, but it continues as the central document used by Interpol member countries, and an updated version is currently available in 2018 (INTERPOL 2018). Following Interpol's recommendations, many countries should have a special national team for the disaster victim identification (DVI) process. This protocol should be activated whenever necessary, and the procedures to follow and the limitations of the centers must be known in advance. In a case of disaster, open communication must be established with local laboratories, private or not, if more resources are needed in an emergency. Additionally, an agreement between teams from different nations must exist as a way to guarantee a plan in the event of an international catastrophe (Vullo 2019).

While following the standards outlined in the guide, the organizations responsible for this identification process may encounter various obstacles in their efforts. This protocol remains without application in most cases of 20th-century civil wars, not only because of its antiquity and lack of immediacy but also because, in many cases, the governments themselves do not consider these identifications as a priority, therefore, as a catastrophe.

According to the Interpol Manual for Victims Identification, the process consists of four stages (INTERPOL 2018): (1) the examination of the crime scene; (2) recovery of postmortem (PM) data; (3) recovery of antemortem (AM) data, and (4) harmonization. The third stage, in particular, depends largely on the existence of living relatives who can provide useful information on the victim before his death (antemortem data). They do so through personal interviews and provide data such as (INTERPOL 2018; Vullo 2019):

- Biological samples for genetic identification—DNA sources can be medical samples, personal belongings, or relatives of the victim.
- Surgical data—provision of a detailed medical history.
- Dental data—including the facilitation of contact with the last dentist that the victim visited, to obtain an updated dental profile.
- Affiliation data—including the National Identity Document, driver’s wallet, passport, etc.
- Detailed physical description, including scars, moles, malformations, tattoos, etc.
- A detailed description of the clothing he was wearing at the time of his death and his personal effects (piercings, earrings, glasses, rings, etc.)

The harmonization that is performed later consists in the comparison of the antemortem with the postmortem data. In this way, a profile is made for the identification of the individual. It is possible that in some cases certain anthropological reconstructions allow for guiding toward identification, especially when there are unique physical characteristics that can lead to an unequivocal identification. If no one is looking for that specific victim, that is, if the information necessary for comparison with the postmortem data is not provided, it is most likely that person will never be identified.

5. Types of Identification in Armed Conflicts

In the case of perished individuals, whose identification cannot be carried out by traditional methods, such as through identification documents, or in certain circumstances, finding the cadaver inside the residence, the identification procedures are normally based on three scientific methods proposed by INTERPOL, namely (from greater methodological facility and low cost, to greater methodological complexity and higher cost): fingerprint identification, dental identification, and genetic identification, as explained in the following sections.

5.1. Fingerprint Techniques

Lofoscopy is the science dedicated to human identification that is based on the study of the epidermal crests on the extremities of the fingers (dactyloscopy), palms of the hands (chiroscopy), and soles of the feet (pelmatoscopy) (Correia and Pinheiro 2013), and cheiloscopy (lip prints). Fingermarks are the impressions—generally “latent” (invisible)—which are left on smooth surfaces that are touched with bare hands. Such impressions will typically be composed of natural secretions plus contaminants derived from the environment. Various optical, physical, and chemical methods are available for the detection and recording of these impressions (Bleay et al. 2018; Lennard 2019). Fingermarks can be compared to reference fingerprints (e.g., from suspects or convicted offenders) to determine whether they may have been deposited by a particular individual. Fingerprint classification is based on the overall ridge pattern, while fingerprint identification (individualization) is based on the configuration of individual ridge features, or minutiae (Bleay et al. 2018; Lennard 2019).

The identification of fatal victims of catastrophes of different etiologies has been successfully carried out using this identification method which, among other advantages, has proven to be effective, fast, and inexpensive (Correia and Pinheiro 2013). Additionally, the existence of a fingerprints international software analysis is also an advantage, such as the Automated Fingerprint Identification System (AFIS), a computer system that allows the capture, consultation, and automatic comparison of fingerprints. At a European level, it is also worth mentioning the existence of EURODAC, an informatic system, which collects, transmits, and compares fingerprints, assisting, for example, in the examination of an

application for international protection lodged in an EU Member State by a third-country national or a stateless person. If two or more very similar individuals are present, with identical personal data, the comparison of their fingerprints allows their individualization. However, the identification of a fingerprint depends on the quantity and quality of dermopapillary traces detected at the crime scene and on the availability of fingerprints that allow unambiguous analysis, comparison, evaluation, and verification (Correia and Pinheiro 2013).

The main problem associated with the identification with dactyloscopy techniques in armed conflict, or other catastrophes scenarios, is the obligation of the human remains to have fingerprints to analyze, if possible, in a good state of conservation, and that each country has a national database to be able to perform comparisons.

5.2. Forensic Odontology

The aim of forensic odontology is the personal identification, of not only those mutilated, burnt, and decomposed, but also victims of bioterrorism and mass disasters (Forrest 2019; Jayakrishnan et al. 2021; Mohammed et al. 2022), using odontostomatological methods (Labajo and Perea 2022; Mohammed et al. 2022).

The tooth is a fundamental piece of an anthropological record and necro-identification since it has a series of characteristics that make it suitable for forensic studies, such as resistance to physical, chemical, biological, and taphonomic agents. At the site of removal of the corpse, the dentist can determine antemortem, perimortem, and postmortem odontostomatological injuries, determine postmortem dental losses (which would indicate the need to look for them in the place of discovery), classify, and individualize the located pieces, and determine bite injuries, among others (Labajo and Perea 2022).

In the laboratory, the dentist studies the jaws and/or isolated dental pieces, to subsequently perform a postmortem odontogram. With the comparison of the postmortem and antemortem odontograms, as well as the dental radiological comparison, the dentist is often able to determine the identity of the individual (Forrest 2019; Jayakrishnan et al. 2021; Labajo and Perea 2022; Mohammed et al. 2022).

One of the most problematic situations is related to charred corpses since physical destruction can be so significant that only the dental pieces are useful for the investigation of the identity (Forrest 2019; Jayakrishnan et al. 2021; Labajo and Perea 2022; Mohammed et al. 2022). On the other hand, although it could be considered an inexpensive method to identify, it always requires antemortem information to perform comparisons.

According to each case and violence degree, human remains are frequently found where it is not possible to perform identification by fingerprints or dental records, since such procedures are only possible with specific organs.

5.3. Genetic Identification in Armed Conflicts

One of the advantages of genetic identification is the possibility to be applied to most human samples. However, to identify an individual, or to attribute a sample to a specific individual, two procedures can be done. On the one hand, antemortem biological samples from the victim can be analyzed and compared with genetic profiles from the human remains. On the other hand, the profile from the human remains can be compared with biological samples from biological family members (Vullo 2019).

The different topics that involve the forensic genetics laboratory in the identification process, and the considerations in each step, can be summarized as:

- Evaluation of postmortem samples;
- Evaluation and analysis of antemortem samples;
- Family member's sample(s) selection;
- DNA extraction, quantification, and genotyping;
- Statistical evaluation.

Normally, the manipulation of biological samples for the identification of individuals from armed conflicts (and also from other major catastrophes) is carried out in laboratories

specialized in the analysis of degraded DNA. These laboratories usually have separate areas, to avoid not only external contamination but also contamination between biological samples. The areas dedicated to samples from postmortem victims are normally physically isolated, with UV sterilization mechanisms both in the work equipment and in the walls and ceiling, together with restricted access. All material used in these rooms is for single use and disposable, and all personnel working in these areas are completely protected with clothing that prevents the transfer of genetic material from the outside. Concerning the areas used for the analysis of antemortem samples of the victims, along with the next of kin, these are analyzed in areas that are also restricted, but with different characteristics, similar to those used in the forensic casuistry of samples with a high concentration of genetic material. In the case of family members, the sample normally used is saliva, due to its universal character (it exists in men and women) and painless collection through the buccal mucosa. Concerning the victim's antemortem samples, they can range from biopsies to other types of samples that can irrefutably be associated with the victim.

The evolution of forensic science and, in particular, forensic genetics through DNA (deoxyribonucleic acid) analysis, has allowed many families of disappeared persons to resolve the uncertainty about the unknown whereabouts of their relatives (Palomo-Díez et al. 2019). Before the use of current genetic tools in forensic investigation, one of the elements used in human identification programs was forensic homogenetic, a technique that was applied particularly in Argentina during the 1980s and was based on the transmission of the ABO system inherited (Toscanini 2019), applied widely as Ouchterlony's test. The first DNA profile was produced for forensic purposes in 1984, and since then, forensic DNA analysis has evolved enormously, being more sensitive, accurate, cheaper, and faster. The pioneering studies were complex, costly, and lacked the scope and facilities that forensic genetics offers today. In recent years, the ability to recover and analyze low concentrations of DNA from biological material has improved forensic genetics sensibility (Toscanini 2019), both in terms of new techniques for extracting degraded DNA, and amplification, detection, and sequencing, which have become increasingly more sensitive.

The same technology that enables samples recovered from a crime scene to be compared with those from a suspect can be used to match human remains with biological relatives of missing persons (Vullo 2019). Nevertheless, studying human remains from armed conflicts or other catastrophes has associated problems that are less frequent at crime scenes. For example, the possible exposure to heavy metals and in the case of being exposed to external weather conditions, other complications can be observed, such as the acceleration of the cadaveric decomposition and skeletonization, due to extreme temperatures, excess soil humidity, acidity or basicity, among others. In general, these are factors that lead to the degradation and/or destruction of genetic material (Emmons 2015).

In the case of armed conflicts, victims' identification is usually performed using samples from close relatives, as mentioned before. Autosomal markers (Baeta et al. 2019a; C. Gomes et al. 2019b; Palomo-Díez et al. 2019; Gomes and Arroyo-Pardo 2022) are a widely used tool to "individualize the individual" in question, attributing to her/him a unique genetic profile. On the other hand, in cases where autosomal markers are not sufficient to perform a kinship analysis (Pinto et al. 2011, 2012), due to an incomplete profile or in the case of wanting to distinguish genealogies belonging to the same autosomal kinship class (Pinto et al. 2010), X-markers are also a crucial tool in solving such cases, such as X-InDels, since these markers have a high mean exclusion chance (MEC) (C. Gomes et al. 2020; I. Gomes et al. 2020), allowing to solve certain kinship questions, inaccessible with autosomal markers (C. Gomes et al. 2012, 2020; I. Gomes et al. 2020), as it was described by C. Gomes et al. (2019b) in a complex case from the Spanish Civil War. In this case, X-InDels were essential to discard the paternity hypothesis, between a presumed father and daughter. However, in the case of older armed conflicts, the existence of living close relatives is increasingly scarce, being mandatory to evaluate other types of information, such as lineage markers, despite its null power of discrimination.

5.3.1. Direct Identification

Direct identification takes place when there is a biological sample or genetic profile attributed to the victim in question. In this case, the analysis focuses on the comparison of recombinant markers, usually by routine autosomal markers, verifying that there is no difference between both profiles: antemortem and postmortem.

However, depending on the conditions of conservation of the corpse, the postmortem genetic profile may be degraded and/or partial, and may even translate into an inconclusive result. Thus, the genetic profile may be so partial that the genetic information could belong, by frequency, to any of the individuals in that population. It is in these cases that traditionally resort to the use of lineage markers to guide the investigation, since, although they do not allow identification, they allow the inclusion or exclusion of the individual from a family lineage in question.

5.3.2. Indirect Identification

The genetic identification of a cadaver to be carried out must take into account at least two factors. First, what reference samples are available, and second, whether there is genetic material available to carry out the genetic analysis (Vullo 2019). If we have access to samples from close relatives or even antemortem samples that could be from the deceased, the most used strategy is the employment of autosomal markers, since they allow the analysis of individuals regardless of their molecular sex, and, in most cases, allow for obtaining high LR values.

(a) Autosomal markers

Autosomal markers are genetic noncoding polymorphisms, located on autosomal chromosomes (from chromosome 1 to chromosome 22). In each cell, there are always two copies of each polymorphism, one on the chromosome inherited via the paternal path and the other on the maternally inherited chromosome (Figure 1). Since it is inherited both paternally and maternally, it is considered essential information for cadaveric identification, since the probability that two unrelated individuals share exactly the same set of autosomal polymorphisms, both maternally and paternally inherited, is highly unlikely. At present, the set of polymorphisms that are analyzed allows a probability of identity of 6.58×10^{-29} for the genetic kit PowerPlex[®] Fusion (Promega, Madison, WI, USA), referring to the probability of two persons sharing the same genotype by chance and not by descent.

The identification of the individual through close relatives (such as father, mother, and descendants) is carried out through a kinship test (Luque 2019; C. Gomes et al. 2020; I. Gomes et al. 2020). In the case that the parents' samples of the victim are available, the kinship analysis consists of maternity and/or paternity tests, according to the available individuals (Vullo 2019). A brother or sister may not share enough autosomal information with the victim (Pinto et al. 2010) to obtain a sufficiently high probabilistic value to be able to identify the individual. Indeed, for autosomal markers, two brothers/sisters or brother-sister can be like two unrelated individuals, since there is no obligation that they share identical autosomal genetic information by descent (Table 2) (Pinto et al. 2010). In the case of possible grandparents, the case is even more complex, since the information that the grandfather/grandmother transmitted to their offspring may not have been the information the deceased received (Figure 1) (Pinto et al. 2010), due to recombination processes; thus, in the case of identification, grandparents are never the first choice (Vullo 2019).

The normally accepted value beyond which the relationship is compatible with biological maternity or paternity is equal to or greater than 99.9999% (Luque 2019). A lower value normally excludes these relationships, considering that there may be up to a maximum of three mutations between the considered individuals. Table 2 shows the allele-sharing probability for some common genealogical relationships between noninbred individuals (Pinto et al. 2010).

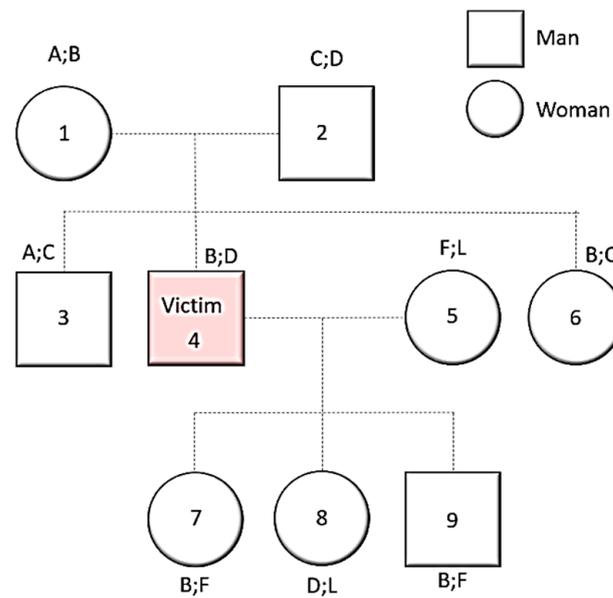


Figure 1. Autosomal marker utility in armed conflicts or other mass disaster victim identifications. The letters (A, B, C . . .) represent alleles. In the example shown, female 1 has information A and B on an autosomal chromosome pair and male 2 has information C and D for the same pair of autosomal chromosomes. Their three children in common (individuals 3, 4, and 6) receive information from their parents randomly, and, for example, individuals 3 and 4 do not share any autosomal information for that marker in question; individuals 4 and 6 share part of the information, which they received, in this case, through their mother. In this example, to identify victim 4, the most relevant autosomal information would come from the parents (individuals 1 and 2) and his offspring (individuals 7, 8, and 9). Source: Cláudia Gomes (2020), self-authored.

Table 2. Allele-sharing probabilities through identity by descent (both alleles— k_2 ; one— k_1 ; none— k_0) for some common genealogical relationships between pairs of noninbred individuals. Source: (Pinto et al. 2010).

Genealogical Relationship	Allele Sharing Partitions		
	k_2	k_1	k_0
Parent–Child	0	1	0
Full siblings	1/4	1/2	1/4
Half siblings	0	1/2	1/2
Avuncular	0	1/2	1/2
Grandparent–Grandchild	0	1/2	1/2
Double first-cousins	1/16	3/8	9/16
First Cousins	0	1/4	3/4
Double half-cousins	1/64	7/32	49/64

This computation is, in fact, an inference, since there is no direct identification between biological samples from the same individual. If the parents have, for example, two or more missing descendants, if only one corpse is found and there are no antemortem samples, it will only be possible to know that the individual was the son or daughter of those parents, not being possible to know which of the missing descendants would be. In this case, other factors could help a possible identification, such as the deceased's sex and approximate age, which is never a method as reliable as genetic identification.

(b) X-chromosomal markers

Due to its transmission properties, X-chromosome markers have emerged as an advantage in certain genealogical situations, not only completing autosomal marker information but also resolving certain kinship investigations unviable with autosomal markers (C. Gomes et al. 2020; I. Gomes et al. 2020; Gomes and Arroyo-Pardo 2022). The transmission depends on the sex of the individuals. In women's cells, there is a pair of X-chromosomes that recombine with each other, just like autosomes. As for male cells, the X-chromosome has no homologue, only recombining with the Y-chromosome at the level of the homologous PAR1 and PAR2 regions (Gomes and Arroyo-Pardo 2022). In this way, a father transmits to all his daughters a full copy of his X-chromosome, while a mother arbitrarily transmits a copy of one of her two X-chromosomes to daughters and sons, after recombination, as for autosomes.

One of the relevant points for the analysis of genetic markers located on the X-chromosome is related to the fact that it can distinguish biological relationships that are indistinguishable when analyzed with autosomal markers (Pinto et al. 2010). In a case of disaster or armed conflict where several victims of the same biological family are observed, it is not possible to distinguish, for example, the relationship between "maternal grandmother–granddaughter" and "maternal aunt–niece". In the case of three female cadavers, it is not possible to distinguish whether one of the women was the grandmother or aunt of another woman using autosomal markers. This is due to the mode of transmission of autosomal chromosomes over generations and the obligation, or not, to share identical information by descent. As mentioned before, this obligation exists only between parents and children (and identical twins). In the case of the X-chromosome, taking into account the transmission and the fact that it depends on the biological sex of the individuals, some of these indistinguishable cases when studied with X-chromosome markers can be distinguished.

Another advantage of the analysis of markers located on the X-chromosome, mainly X-InDels (C. Gomes et al. 2019b, 2020) and X-SNPs, is the fact that it permits obtaining probabilistic values that allow for excluding or accepting certain biological relationships (C. Gomes et al. 2019b) when the analysis of autosomal markers proves to be insufficient (Pinto et al. 2010).

In the case of the study of genetic markers located on the X-chromosome, the identification of a deceased through relatives will depend, as already mentioned, on the usefulness of the relative for this study. If a victim is a man and the available family members are his father and son, then there is no possibility of carrying out a study of X-chromosome markers (Figure 2). In the case of the mother or daughters, the X-chromosome study may be as informative as the analysis of autosomal markers. In routine forensics, autosomal markers continue to be the commonly used tool; however, the probabilistic kinship value can be improved by studying X-chromosome markers. In case the nuclear information is irremediably degraded, the most frequent routine is mitochondrial DNA (mtDNA) analysis.

(c) Lineage markers

(c₁) mtDNA

When the nuclear information is not informative, depending on the case in question and when individuals are related by maternal side, mtDNA is traditionally used (Baeta et al. 2019b; Marshall et al. 2019; Mienkerd et al. 2019; C. Gomes et al. 2019a; I. Gomes et al. 2020; Palomo-Díez and López-Parra 2022). Transmitted by the maternal side, without recombination, between mothers and all their offspring, whether male or female (Figure 3), the SNPs from the mtDNA allow, in the event of a match, to associate the victim with a particular family. It does not allow the identification in its strict sense, it has a null power of discrimination, since any member of this family through the mother's side shares this genetic information. Still, associated with other information (anthropological and historical, among others) it allows for determining a unique profile of the individual, leading to a later identification (C. Gomes et al. 2019a, 2019b; Palomo-Díez et al. 2019; Palomo-Díez and

López-Parra 2022). The problem arises if brothers, siblings, or other kinship by the maternal side, must be identified using this type of genetic information. In this case, only with the mtDNA information, it can only be said that the individuals belong to the same maternal family, being impossible to distinguish between them based only on the mtDNA. The great advantage this lineage marker presents is related to the fact that genetic information from distant relatives can be used. In fact, in the case of armed conflicts, these data can be crucial, since often the family members who request the analysis are either elderly (in the case of old armed conflicts), or they are not close and maybe cousins, great-grandchildren, nephews, and grandchildren, among others (Figure 3). Another fundamental point to take into account is the high number of mtDNA copies in each cell, which increases the probability of obtaining mitochondrial genetic information in biological samples in a high state of degradation and decomposition.

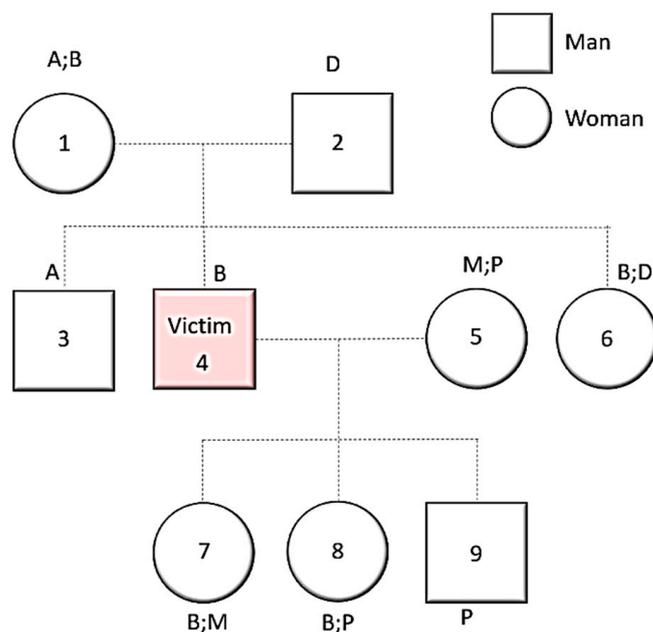


Figure 2. X-chromosome marker utility in armed conflicts or other mass disaster victim identifications. All letters (A, B, D, . . .) represent letters. In cases where genetic markers of the X-chromosome are analyzed, it is necessary to pay attention to the sex of the individuals, since the usefulness of this chromosome is directly related to its mode of transmission. All females have two X-chromosomes; each man has only one. Females 1 and 5 transmit their information to their offspring randomly. Individuals 2 and 4 only transmit their information to their daughters, and always the same information since they have only one X-chromosome. For the possible identification of victim 4, only individuals 1 (mother) and 7 and 8 (daughters) would be useful. Concerning individuals 3 and 6, they are not especially useful, since for each analyzed marker they could or could not share genetic information with their brother, the victim, depending on what they inherited from their mother. Source: Cláudia Gomes (2020), self-authored.

In the case of mtDNA, the probabilistic calculation is not carried out in the same way as with nuclear markers (autosomal and X-chromosome). In this case, the calculation is based on the frequency of that lineage in a specific population—the less frequent, the greater the probability that the victim came from a particular family. This type of inference is particularly complex in populations with a high inbreeding index, as the lineages will be overrepresented. Large mtDNA databases such as EMPOP (<https://empop.online/>) are particularly useful, not only because they provide populational or metapopulational data and their respective frequencies, but also allow for detecting and determining the “rarity” of certain lineages.

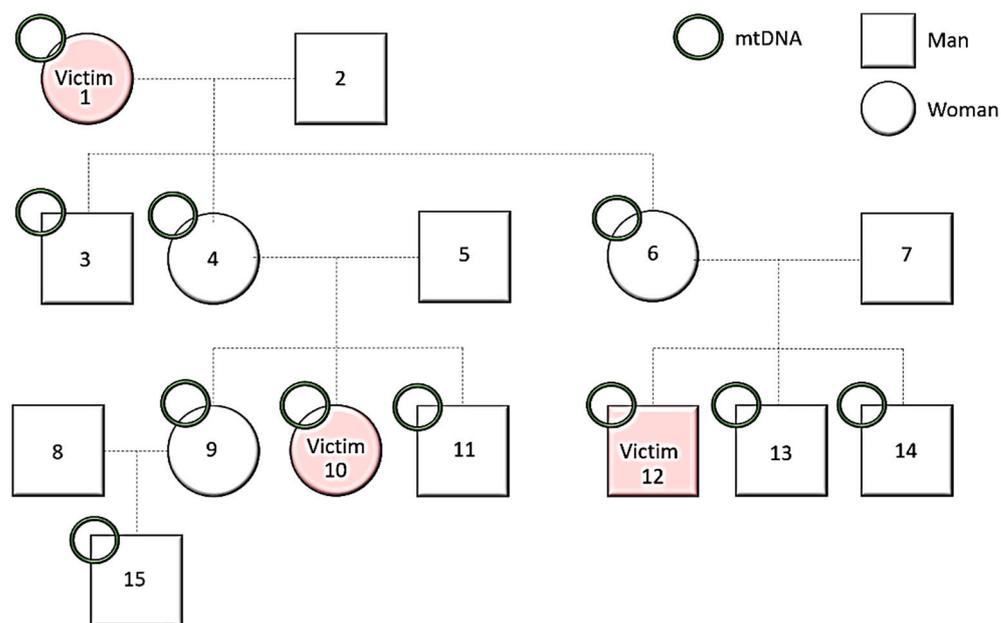


Figure 3. MtDNA utility in armed conflicts or other mass disaster victim identifications. Female 1 transmits to all her offspring (3, 4, and 6) a copy of her mtDNA, usually without any changes. Subsequently, her daughters (4 and 6) pass on to their offspring the same genetic information regarding mtDNA. In this way, individuals 1, 3, 4, 6, 9, 10, 11, 12, 13, 14, and 15 share the same mtDNA. Thus, to help in the victim identification process (1, 10, and 12), any of the previously mentioned individuals could be used, comparing their mtDNA information with the information obtained from the victim. However, if victims or human remains are observed together, for example, in a mass grave, it would not be possible to distinguish the three individuals from each other using only mtDNA information. Source: Cláudia Gomes (2020), self-authored.

(c₂) Y-Chromosome

In the case of kinship by the paternal side, the most employed lineage marker is the Y-chromosome. In this case, this information is transmitted from father to son. As observed for mtDNA, the Y-chromosome does not allow for individualization, allowing each male to be associated with a specific family (paternal lineage) (Figure 4) (Palomo-Díez and López-Parra 2022).

Additionally, in this case, the statistical calculation is carried out by calculating the frequency of that lineage in the population in question. Given the importance of the use of Y-STR in forensic analysis, extensive population databases of haplotype frequencies are necessary to allow for calculating the probability of coincidence between two genetic profiles, and an analysis of the possible population substructure, both highly dependent on the amount of information available. In addition to the existence of a large number of publications with the participation of numerous laboratories around the world (for example, Chen et al. 2018; Caputo et al. 2019; Jankova et al. 2019; Ambrosio et al. 2020; Claerhout et al. 2020; Hakim et al. 2020; Adnan et al. 2020; Bini et al. 2021; Fang et al. 2021; Babić Jordamović et al. 2021; Neyra-Rivera et al. 2021; Adnan et al. 2022; Albarzinji et al. 2022; Ashirbekov et al. 2022; Dooley et al. 2022; Mihajlovic et al. 2022; Pamjav et al. 2022; Tätte et al. 2022; Ashirbekov et al. 2023; Lee et al. 2023), an online database was created, the YHRD—Y chromosome haplotype reference database (<https://yhrd.org/>) containing haplotype profiles of the markers most used in forensic genetics, for a high number of populations. In addition to making it possible to search for haplotypic profiles in various populations, the YHRD contains relevant information regarding Y-STRs for forensic use. For the same population, it is common to observe that there are data for different blocks of Y-STR markers, so for groups of markers included in more recent commercial kits, the databases are usually represented by a smaller number of individuals. Thus, in

estimating the haplotype frequency, many laboratories choose to use a smaller number of markers that enables them to use a larger database. According to (SWGDAM n.d.) guidelines (SWGDAM—Interpretation Guidelines for Y chromosome STR testing), in such situations it is acceptable to perform database searches using a reduced set of loci, in an attempt to obtain the maximum potential for discrimination between our sample and the profiles in the population database; that is, the set of markers that, even obtaining a greater number of coincidences, generates an acceptable lower haplotype frequency, owing to the increase in the population size of the subset of the database used to perform the comparison (Pereira and Gusmão 2019). In the case of past armed conflicts, there are no data on these populations, and current data must be used. On the other hand, another problem is related to the geographic region where the armed conflict occurs, since most scientific studies in the area of population genetics usually focus on European and North American populations, with most of the remaining populations either not studied or underrepresented.

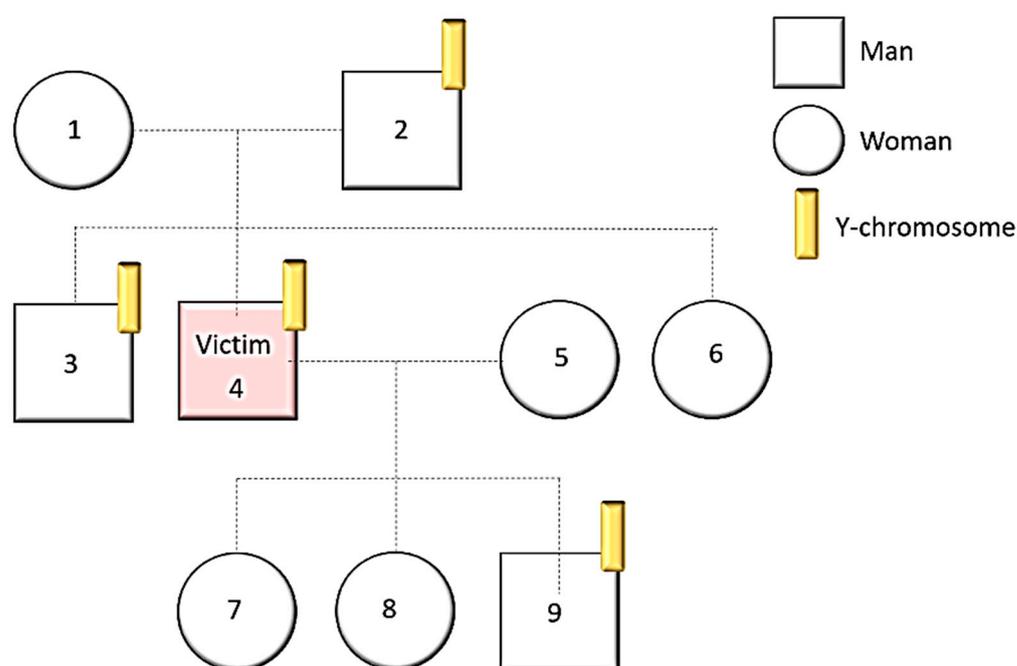


Figure 4. Y-chromosome utility in armed conflicts or other mass disaster victim identifications. Male 2 transmits his Y-chromosome to his male offspring, usually without mutations. In this way, individuals 2, 3, 4, and 9 have the same male lineage information. To guide the identification of the victim, it would be possible to use the data present on the Y-chromosome of individuals 2, 3, and 9. Source: Cláudia Gomes (2020), self-authored.

5.3.3. New Trends in Forensic Genetics

In recent years, the number of laboratories in the field of forensic genetics that are investigating and beginning to use massively parallel sequencing (MPS) technologies has increased considerably, for the analysis of markers classically used in this field (control region of mtDNA and STRs), and in the study of other markers of potential application for the determination of identity, ancestry, and/or phenotype (such as SNPs, InDel markers, or complete mtDNA sequencing) (Alonso et al. 2018). One of the advantages of this type of platform is the ability to incorporate into a single workflow the simultaneous analysis of hundreds or thousands of different DNA markers, and the determination of variations at the sequence level. An example of the application of MPS in the field of armed conflicts is the research published by Pajnič et al. (2020), where they analyze 10 people by massive sequencing, which they consider to have been the “largest Second World War family massacre in Slovenia”.

One of the most analyzed polymorphisms with MPS techniques is single nucleotide polymorphism (SNP), although its use predates the use of massive sequencing in the field of genetics. There are several advantages to using SNP polymorphisms in forensic genetics, such as obtaining genotypes from small amplicons (less than 100 base pairs), especially useful in cases of degraded and fragmented DNA: they have a very low mutation rate and they can provide information on biogeographic ancestry, parentage, and visible external characteristics (Budowle and Daal 2008; Ziętkiewicz et al. 2012). In addition, they can be reliably genotyped in multiplex reactions, reducing the amount of sample and processing time. This is especially useful in cases of armed conflicts, where biological samples are usually presented in a highly degraded state (Kidd et al. 2006). The analysis of a large panel of SNPs can provide a high power of discrimination, similar or proportional to the number of STRs usually genotyped. The use of SNPs in the field of forensic investigation in armed conflicts allows, for example, the determination of visible external characteristics (EVC), such as eye or hair color, as shown in the study by Pajnič (2021).

6. Difficulties in Victims' Genetic Identification in Armed Conflicts

In some cases of victims' identification after armed conflicts, the judicial and administrative authorities are not in charge of the organization, control, or provision of human resources. Instead, it transfers these tasks to civil organizations (Etxeberria et al. 2021; Herrasti et al. 2021), commonly known as the "Association of Victims and Relatives of X conflict", and/or families. This conduct adds difficulties to the process, owing to the associations' and/or families' total lack of knowledge about the steps to be taken to start. As previously mentioned, an example of this situation is the extensive number of victims unidentified in different civil wars of the 20th century.

Another problem present in many armed conflicts is the movement of individuals, both during and after the conflict. In the case of the deceased, displacement may have occurred during the war, either as a means of escape or as part of the conflict. In these cases, families completely lose track of the family member, not knowing where to start the search. If in that specific territory, there is no national database with data from victims and possibly family members, the identification of human remains may be impossible. If the conflict has spread to more than one country, it is practically impossible to carry out the identification of the human remains, since there is no way to attribute them to a specific family. A clear example of this is the two world wars.

The identification process could be very extensive, involving a large number of professionals from the criminalistic field but also professionals related to documentary research, topographers, photographers, historians, and archaeologists (Fernández-Álvarez et al. 2016; Vullo 2019; Etxeberria et al. 2021). On the other hand, scarce funding leads to some parts of the work, such as the osteological study and archival research, being carried out by volunteers, who are usually professionals who are not dedicated full-time to this investigation. On the other hand, the reports generated can be dissimilar in terms of the quantity and quality of the data obtained, since they are created by independent teams, and are not organized under a centralized system. All this has given rise to unreliable files and enormous complexity in maintaining a complete chain of custody of the remains, from their appearance to their return to relatives, passing through distinct laboratories. It also creates the problem of who will be in charge of contact with the victim's family in case of identification (Vullo 2019). For example, in the case of the Spanish Civil War, Spanish Law 20/2022 of October 19, on Democratic Memory, proposes in its article 23 the creation of a "State Bank of DNA of Victims of War and Dictatorship", guaranteeing collaboration among different associations of victims, autonomous communities, and with other government research institutes. So far, genetic research is usually carried out at the community level, that is, relatives often search for their missing relatives in that same community. However, many human remains located in a specific part of the territory could be from another region, and this is why implementation of this initiative is so relevant, as well as the efforts carried

out by the National Commission for Forensic Use of DNA (CNUFADN) to implement this measure equally at the state level.

In this way, it is common for the funding granted to end, exhausted before an identification case is completed, or for the results to be communicated to the relatives up to three years after the request, sometimes after the death of the relatives who have requested the identification (Ríos 2012; Palomo-Díez et al. 2019).

In the case of the European civil wars of the 20th century, one of the difficulties is that, with time, there are fewer and fewer relatives of the first degree to carry out the identifications. For this reason, it is very important to analyze all possible markers, including lineage markers, allowing a possible future identification.

DNA Degradation

As a consequence of the degradation process, two biological samples, even if they are of the same type (two teeth, two blood or saliva samples, etc.) and/or from the same individual, must be analyzed according to their state of conservation. The factors that most affect DNA are pH, temperature, humidity, and certain components in the soil (C. Gomes et al. 2017; I. Gomes et al. 2020). Humidity favors hydrolytic and oxidative degradation reactions. In such environments, the proliferation of fungi and bacteria is favored. Microbial contamination brings two problems: nucleases that degrade the genetic material of interest and the exogenous DNA of the microorganisms (Pääbo et al. 2004). High temperatures, although, provide dryness and the absence of microorganisms, usually lead to the denaturation of DNA chains, and can promote their degradation. Slightly basic pH helps to preserve DNA, but acidic pH causes the degradation of hydroxyapatite from bone samples (Figüero et al. 2007). A very acidic or basic pH, very different from the physiological one, causes changes in the nitrogenous bases of the nucleotides and decreases and weakens the hydrogen bonds between complementary bases. On the other hand, samples deposited on the soil usually have another problem that prevents an effective genetic analysis, the presence of inhibitors (Baeta 2012; C. Gomes et al. 2017). Many are compounds present in the soil such as humic acids and porphyrin residues. However, other compounds can also work as an inhibitor, such as the heme group of hemoglobin present in blood samples. Postmortem molecular modifications are usually based on oxidation modifications in nitrogenous bases, due to certain free radicals, mainly hydrogen peroxide and hydroxyl groups. In general, the forensic routine is prepared for these types of cases, and some protocols facilitate the analysis of samples with presumptive inhibitors.

For these reasons, it is essential to carry out methodologies that allow a complete obtainment of the genetic material. Specifically, to carry out an efficient extraction, adapted to the nature of the sample (for example, bones, teeth, fetal tissue, skin, and hair, among others) and adapted to the possible concentration of genetic material, is normally very low. Other fundamental steps are the purification of the extract and the quantification of the genetic material obtained, since these will allow adapting the genetic amplification to the real concentration of the genetic material. One of the fields that can most contribute to the identification of victims of armed conflicts, normally in an advanced state of degradation, are investigations that apply laboratory protocols within the field of “ancient DNA”, since in most cases these succeed with extremely low genetic concentrations.

Another relevant problem is the selection of samples in the case of victims. In the specific case of buried victims, the commonly used samples are bone or dental samples. Although bones such as the femur or teeth are traditionally used, in the latter case with the advantage of protecting the enamel, the utility of the petrous portion of the temporal bone (Pilli et al. 2018; González et al. 2020; Pajnič et al. 2021) is increasingly being demonstrated, not only because of its consistency but also because of its location, which is why it is protected from certain wear and tear produced, for example, by certain professions. One of the major difficulties is the fact that in certain cases this sample is not available when the skull is separated from the rest of the body, and the disadvantage of having to partially destroy the individual’s skull to analyze this sample.

However, the great difficulty remains in the analysis of biological samples with a low concentration of genetic material (C. Gomes et al. 2019a), and, over time, the increasing absence of direct relatives of the victim. For this reason, acting as quickly as possible is crucial, since the longer the biological sample remains in the soil, or is exposed to weather conditions, the more difficult it will be to perform an identification (C. Gomes et al. 2019b).

7. Conclusions

Owing to the efforts carried out, to a large extent, by family members and/or associations for the recovery of victims of armed conflicts, identifications are increasingly a reality. It is usually seen that the accomplishment of these identifications depends not only on scientific–technical mechanisms, such as identification by fingerprints, dentistry, or genetic analysis but also on the budget and predisposition of each government to carry out this work rigorously. It is also crucial that the entire identification process is carried out by independent scientific teams, allowing intercountry collaboration, since many victims of armed conflicts are from other territories. These international collaborations can be decisive for successful identification. As time goes by, it is more complex to find close biological relatives to carry out the identification of victims. A possible answer to this problem would be the existence of national databases that contain the genetic information for each person and/or family who is searching for a specific relative. Additionally, it would be important to include information about each victim, not only historical records but possible genetic profiles based on the analysis of antemortem data, in cases where it is possible.

The identification of victims of armed conflicts should be a priority of every government, not only for the repair of the identity and dignity of these citizens but also for the example to the citizenry of the importance of each individual in that society.

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