TWO-YEAR
POST GRADUATE DEGREE PROGRAMME (CBCS)
IN
BOTANY

SEMESTER - III

Course: BOTDSE T 301.1

(Forensic Botany)

Self-Learning Material

DIRECTORATE OF OPEN AND DISTANCE LEARNING
UNIVERSITY OF KALYANI
KALYANI – 741235, WEST BENGAL

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Director's Messeage

Satisfying the varied needs of distance learners, overcoming the obstacle of distance and reaching the unreached students are the threefold functions catered by Open and Distane Learning (ODL) systems. The onus lies on writers, editors, production professionals and other personal involved in the process to overcome the challenges inherent to curriculam design and production of relevant Self Learning Materials (SLMs). At the University of Kalyani a dedicated team under the able guidance of the Hon'ble Vice-Chancellor has invested its best efforts, professionally and in keeping with the demands of Post Graduate CBCS Programmes in Distance Mode to devise a self-sufficient curriculum for each course offered by the Directorate of Open and Distane Learning (DODL) University of Kalyani.

Development of printed SLMs for students admitted to the DODL within a limited time to cater to the academic requirements of the Course as per standards set by Distance Education Bureau of the University Grants Commission, New Delhi, India under Open and Distance Mode UGC-DEB Regulations, 2020 had been our endeavour. We are happy to have achieved our goal.

Utmost care and precision have been ensured in the development of SLMs, making them useful to the lesrners, besides avoiding errors as far as practicable. Further, suggestions from the stakeholders in this would be welcome.

During the production-process of the SLMs, the team continuously received positive stimulations and feedback from Professor (Dr.) Amalendu Bhunia, Hon'ble Vice-Chancellor, University of Kalyani, who kindly accorded directions, encouragements and suggestions, offered constructive criticisms to develop it within proper requirements. We gracefully, acknowledge his inspiration and guidance.

Sincere gratitude is due to the respective chairpersons as well as each and every Members of PGBOS (DODL), University of Kalyani, Heartfelt thanks is also due to the Course Writers- faculty members at the DODL, subject-experts serving at University Post Graduate departments and also to the authors and academicians whose academic contributions have enriched the SLMs. We humbly acknowledge their valuable academic contributions. I would especially like to convey gratitude to all other University dignitaries and personnel involved either at the conceptual or operational level of the DODL of University of Kalyani.

Their persistent and co-ordinated efforts have resulted in the compilation of comprehensive, learners friendly, flexible text that meets curriculum requirements of the Post Graduate Programme through distance mode.

All the Self Learning Materials are self-writing and collected from e-Book, journals and websites.

Director
Directorate of Open & Distance Learning
University of Kalyani

SYLLABUS
COURSE – BOTDSE T 301.1
Forensic Botany
(Full Marks – 50)

Course	Group	Details Contents Structure		Study hour
BOTDSE T 301.1	**Forensic Botany**	**Unit 1. Introduction**	1. Introduction: Introduction to forensic botany.	1
		Unit 2. Use of Botanical Evidence in Criminal Investigation:	2. Use of Botanical Evidence in Criminal Investigation: Botanical evidence and crime scene; source, transfer, evidence recognition, collection, preservation and documentation of botanical evidences in criminal investigation.	1
		Unit 3. Branches of Botany in Forensic Study	3. Branches of Botany in Forensic Study: Palynology, Limnology, Plant Anatomy, Plant ecology, Plant Molecular Biology.	1
		Unit 4. Analyses of Samples – I	4. Analyses of Samples: Pollen grains; Anatomical structures; Diatoms.	1
		Unit 5. Analyses of Samples – II	5. Analyses of Samples: DNA.	1
		Unit 6. Drug Enforcement	6. Drug Enforcement: Botanical contributions to drug enforcement.	1
		Unit 7. Classic Forensic Botany Cases	7. Classic Forensic Botany Cases: Famous case histories by using different botanical evidences.	1

Content

COURSE – BOTDSE T 301.1

Forensic Botany

Soft Core Theory Paper **Credits = 2**

Content Structure

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1. Introduction
2. Course Objective
3. Introduction: Introduction to forensic botany.
4. Use of Botanical Evidence in Criminal Investigation: Botanical evidence and crime scene; source, transfer, evidence recognition, collection, preservation and documentation of botanical evidences in criminal investigation.
5. Branches of Botany in Forensic Study: Palynology, Limnology, Plant Anatomy, Plant ecology, Plant Molecular Biology.
6. Analyses of Samples: Pollen grains; Anatomical structures; Diatoms, DNA.
7. Drug Enforcement: Botanical contributions to drug enforcement.
8. Classic Forensic Botany Cases: Famous case histories by using different botanical evidences.
9. Let's sum up
10. Suggested Readings
11. Assignments

1. Introduction

Forensic science is the application of sciences such as physics, chemistry, biology, computer science and engineering to matters of law. Forensic science plays a vital role in the criminal justice system by providing scientifically based information through the analysis of physical evidence. During an investigation, evidence is collected at a crime scene or from a person, analyzed in a crime laboratory and then the results presented in court. Each crime scene is unique, and each case presents its own challenges. Over the course of a Forensic Science degree you'll study a wide range of aspects of the discipline.

2. Course Objectives

You should gather knowledge after studying the course:

- Understand, from a legal standpoint, the importance of properly securing a crime scene
- Classify the various types of biological evidence that may be obtained at a crime scene Use technology to perform descriptive and inferential data analysis for one or two variables.
- Gather information about outdoor crime scence.
- Analyze the biological samples in legal proceedings
- Describe DNA typing and Drug enforcement.
- Understand different case histories by using Plant anatomy and systematics, Palynology, Plantecology, Limnology, Molecular biology and DNA.

3. Introduction: Introduction to forensic botany.

"Forensic" comes from the Latin word "forensis" meaning forum. During the time of the Romans, a criminal charge meant presenting the case before the public. Both the person accused of the crime & the accuser would give speeches based on their side of the story.

Forensic botany is a marriage of many disciplines and results ultimately in their application to matters of law. The botanical aspects of forensic botany include plant anatomy, plant growth and behavior, plant reproductive cycles and population dynamics, and plant classification schemes (morphological and genetic) for species identification. The forensic aspects require an understanding of what is necessary for botanical evidence to be accepted as evidence in our judicial system. Forensics requires recognition of pertinent evidence at a crime scene, appropriate collection and preservation of evidentiary material, maintenance of a chain of custody, an understanding of scientific testing methods, validation of new forensic techniques, and admissibility criteria for court.

- ✓ Application of botany in law enforcement i.e. scientific use of plant materials to solve crimes
- ✓ Examples of plant life or plant remains that can be used as evidences are wood, seed, fruit, leaf, twig, trichrome, pollen, spore, algal cell etc.
- ✓ Plant allow forensic botanists to identify things such as what season the crime took place or geographical location, whether or not a body has been moved following a murder, and how long a body has been buried if it was buried
- ✓ These forms of physical evidences can be sometimes traced to an individual suspect
- ✓ The scientific methods utilized in forensic botany, and these methods range from simple techniques (e.g., light microscopy) to more technical molecular biology techniques (e.g., DNA sequencing).
- ✓ Plants have been used as evidence in criminal cases for kidnapping, child abuse, hit-and- run motor vehicle accidents, drug enforcement, homicide,

sexual and physical assault, the establishment of time of death, and verification of an alibi. In addition, new applications are under development to use plant material in forensics as "tracers" to aid in the identification of missing persons, to track drug distribution patterns, and to link bodies to primary crime scene locations after they have been dumped at secondary sites.

✓

Matters of Law" include:

- **Crimes**

  Homicide, sexual assault, burglary, etc.

- **Disputes among individuals**

  Wrongful death, patents, etc.

- **Establishing rights**

  Parentage; immigration, land disputes

- **Investigation of disasters**

  Natural and man-made

CIVIL LAW	**CRIMINAL LAW**

• **case filed by a private party**
  ○ **a corporation**
  ○ **an individual**
• **Penalty:**
A guilty defendant pays the plaintiff *(a person who brings a case against another in a court of law)* for losses caused by their actions.
  ○ no incarceration
	• **case filed by the government**
	• **Penalty:**
	A guilty defendant ispunished by
	  ○ incarceration (in jail/prison)
	  ○ Fine paid tothe Govt.
	  ○ execution (death penalty)

Unique roles of forensic scientists:

The individual with the best argument would determine the outcome of the case.

- Assist in recognition and collection of physical evidence
- Document and maintain chain-of-custody
- Analyze and evaluate the evidence using a variety of scientific approaches
- Interact with the legal system
- Assist attorneys (and often law enforcement personnel)
- Testify in Court

What botanical evidences can do?

- Determine the circumstances and cause of death
- Estimate time frames in relations to the death
- Establish where the death could have taken place
- Determine if there were multiple crime scenes
- Prove or disprove an alibi
- Solve crimes by matching crime scene evidence to suspect
- Identify illegal products from endangered species

Advantages of using plant sample:

- ✓ Plant evidence is long lasting, which means that plant parts to remain identifiable for a very long periods of time
- ✓ Plant cell wall is made of some chemical compounds which are nearly indestructible and do not decay quickly
- ✓ Pollen grains and spores also have walls that are made of decay resistant material- sporopollenin
- ✓ Ecological and molecular restraints of various plant species allow a forensic botanist to narrow down the possibilities of where a crime was committed, when it was committed and who committed the crime.

Plant in our society:

To understand the widespread application and potential utility of plants in forensics, we

discuss a few brief examples of plant usage in human society. As these examples are presented, consider the numberof plant-based items that may be found on your person, among your private possessions, and in your home and workplace — and consider, one day, that they may be useful as critical trace evidence.

1. **Food**

Apples are generally considered to be a wholesome, healthful addition to the daily diet as a good source of vitamins and fiber. In actuality, this concept was promoted by the apple industry in responseto the renouncement of apples by Carry Nation as part of the Prohibition Act. The Women's Christian Temperance Union was opposed to apples because they were, in part, responsible for alcohol use on the frontier. In the early 1900s, the apple industry began promoting the healthful benefits of apples, and today, we have many apple cultivars to choose from at our markets. Interestingly, burglars often sample fruit and other foods in the homes they are invading while pilfering goods.

2. **Fiber**

One prevalent clothing fiber in our society is cotton. Cotton comes from the elongated epidermal hairs on the seeds of the Gossypium hirsutum plant. Prior to mechanized harvesting of cotton fibers, flax (Linum usitatissimum) was the most common fiber plant for the textile industry. Flax fibers, unlike cotton, are sclerenchyma fibers from the stem of the flax plant. Flax fibers are commonly woven into linen, and certain cultivars are used to produce cigarette papers and linseed oil. As trace evidence, the source of clothing, carpet fibers, rope, twine, and threads can be useful for associating a victim to a suspect or individuals back to a primary crime scene for an investigative lead.

3. **Medicine**

Herbal remedies and folklore investigations to identify active chemical components have long been part of human culture and have played an important role in the discovery of useful medicinal compounds (e.g., aspirin). In addition, well-preserved stomach contents from ancient human remains have yielded insight into rituals involving herbs and food. For example, in 1984, a peat cutter near Manchester, England, discovered a well-preserved human leg and called in the police to investigate. The body of Lindow Man was recovered, and radiocarbon dated to approximately A.D. 50–100,

and he was determined to be a member of the Celtic tribes.

4. Beauty

Plants are key components in many herbal shampoos, soaps, cosmetics, and perfumes. Not only are botanicals used in human cosmetics and have appealing scents, but they beautify our environment as well. The Dahlia flower, for example, has an unusual history. Originating from Mexico and called Cocoxochitl by the Aztec Indians, it was discovered and seeds were sent to a French priest studying botany in Madrid, Spain.

5. Recreation

The American obsession with green lawns can be visualized in the numerous golf courses, city parks, and extensive front and backyards that are ingrained parts of suburban life. Prior to the Civil War, few Americans had lawns. However, in the 1950s, the term "lawn" was used in reference to a portion of land kept closely mown in front or around a house. In the 1950s, turfgrass breeding programs gave rise to several new grass varieties that offered improved heat, drought, and disease resistance. In the 1970s and '80s, lawn specialists began recommending blends of grasses (e.g., fescue, bluegrass, perennial ryegrass) rather than a monoculture of a single grass species. Today, American homeownersnspend enormous capital and energy on achieving a perfectly groomed, green lawn as a setting for their homes. In fact, an entire lawn- care industry has developed around this particular aspect of suburban homes. Grass samples may be one of the most abundant types of botanical evidence found at crime scenes simply due to the American obsession with the lawn.

6. Law Enforcement

Plants may be present as biological evidence in many ways:

- Seeds caught and carried in a pant cuff
- Grass stains on a dress after a sexual assault
- Plant leaves and stems snagged and carried in a vehicle's undercarriage, grill, wheel wells,hood, or trunk
- Stomach contents with vegetable matter to aid in verification of an alibi
- Use of pollen to date the burial of skeletal remains in a mass grave

All of these examples and more can assist the forensic community in associating a

person to an object, a person to a crime scene, or a suspect to a victim. “Every criminal leaves a ‘trace’ (evidence)”is a phrase with some accuracy. That “trace” may very well be biological plant material.

4. Use of Botanical Evidence in Criminal Investigation: Botanical evidence and crime scene; source, transfer, evidence recognition, collection, preservation and documentation of botanical evidences in criminal investigation.

Botanical Samples:

Botanical samples in forensic investigation include not only the study of whole plants, but their seeds, leaves, flowers, spores, pollen grains, wood, fruits, cells, glandular & other hairs, & many more parts may be considered as evidences. Botanical evidences consisting of microscopic features like spores and pollen, make this field of study more challenging.

The botanical aspect majorly consists of anatomy, growth, development, taxonomy, classification of plants that help in the identification of the particular species of the plant, whereas the forensic aspect deals with the recognition of appropriate evidence at the crime scene, collection, and packaging of evidence, maintaining the chain of custody, conducting scientific tests of the collected samples & admissibility of the evidence in the court of law.

Considerations for the Use of Forensic Botanical Evidence

When considering the use of forensic botanical evidence found at a crime scene, one must eventually factor in the integration of –

- crime scene collection
- forensic laboratory testing
- and finally, the presentation of the evidence in court

At the Crime Scene

A review of the entire crime scene should be made to determine –

- If any botanical evidence might have been overlooked for collection.
- In particular, small plant fragments, seeds, pollen, and flowering plants should be noted.

If plant matter seems to have a significant value at a crime scene-

- The scene must be reviewed to determine and document the location of any similar plant species (e.g., is there more than one plant representing the species at the crime scene)?
- Reference samples need to be collected from all plants for later examination.
- A schematic diagram for indoor and outdoor aspects of the scene should be made
- All plants of significance should be noted.

During Collection

For bodies:

- all of the seams (a line where two pieces of fabrics are sewn together), pockets, cuffs, shoes, etc. of clothing should be checked for adherent plant matter
- all of the body orifices (opening of body like nostrils, anus etc.) must be examined for plant matter (e.g., algae, stems, seeds)

For vehicles-

- the undercarriage, windshield wipers, vents, floor mats, air filters, and so forth should be checked for seeds or leaf and flower fragments

Sometimes knowledge of plant ecology (knowledge of plant growth patterns and geography) estimates the time for establishing the death.

For example, if a plant is growing through skeletal remains, the age of the plant can be approximately determined and perhaps a season (or other time estimate) assigned for when the body may have been laid at that location.

Are any of the plant samples unusual in appearance (i.e., not a common species)?

- The less common a plant is, the higher the probative value for the case.

Others

- ❖ Flowering plants that may be pollen reference samples should be noted
- ❖ Samples should be collected for later species identification
- ❖ Leaf and flower samples should be pressed flat and stored in paper envelopes to maintain original shape.

After Collection

- ➢ If evidence has been identified, all collected samples should be packaged and sealed appropriately (e.g., in porous, non-porous packets).
- ➢ If any unusual forms (pollen, fecal matter, etc.) are present, proper care should be taken regarding packaging and storage temperature. This step may be critical if DNA identification and individualization steps are to be performed later at the laboratory.
- ➢ Appropriate chain-of-custody records should be maintained.

Is there other forensic evidence in the case?

Often, botanical evidence is not considered until the other forms of forensic evidence are deemed nonprobative. Consider in advance the fact that bloodstains may seem informative at the scene; however, if DNA testing later shows that they are all originating from the victim, then the additional evidence may become more significant to the case.

At the Forensic Testing Laboratory

Check the chain of custody and storage conditions prior to the evidence's submission to the laboratory, especially if DNA testing may be performed later.

- ✓ Make a visual examination of the evidence for gross physical characteristics that may be useful for botanical identification to the species level:
- ✓ Leaf morphology — shape, size, texture, edges, colour.
- ✓ Vein patterns in the leaf — where are the major veins and how do they connect to the minor veins?
- ✓ Flower morphology — shape, size, texture, edges, colour, fragrance.
- ✓ Arrangement of flower on a stem or in relation to each other if multiple flowers are present.
- ✓ Seed morphology — shape, number, size, patterns or etching on the surface, colour.
- ✓ Roots — texture (fine versus coarse), fibrous versus tap, size, colour

Perform a microscopic examination of the evidence for minute characteristics that may be useful for botanical identification to the species level:

- ❖ Leaf morphology — surface features (e.g., trichomes, stomatal patterns); in a cross section, what is the arrangement of cell types (e.g., parenchyma cells versus epidermal cells)? Note presence or absence of inclusion bodies (e.g., crystals and their shapes/sizes).
- ❖ Flower morphology — petal features, presence of fluorescence under ultraviolet light; in a cross section, is there any special arrangement of cell types? What is the shape and orientation of pigment containing cells?
- ❖ Arrangement of tissue types and cells - in a cross section for stems and roots can significantly aid in the identification of certain plant fragments.
- ❖ Seed morphology — surface patterns that may be helpful in plant identification to the genus or species level.

If plant species identification is in question after examining physical traits, DNA extraction may be necessary for the molecular identification of a plant species.

- Is the plant sample free of any visible mold or fungi or adherent pollen?
- Have all seeds been removed from the vegetative tissue?
- Is the plant sample of sufficient size to yield enough DNA for testing?
- Once DNA has been extracted, is the quality and quantity sufficient for further analyses?
- If species identification is to be performed using DNA, what types of comparisons can be made and are any known reference samples available?
- If the individualization of a plant species is to be performed using DNA, what will be the possible outcomes of the testing: a match, a no match, or inconclusive?If a match has been made between an evidentiary sample and a known reference sample, what is the probative value?
- For plant species that are "unusual" or rare, a match may be very significant unto itself.
- However, if a plant species is more common, a comparative reference database may have to be constructed to establish a statistical value for the match.
- Have the report conclusions been clearly stated and explained to the submitting agency?

In The Court

A review of the chain of custody and documentation should be made.

- A review of the report and the report conclusions should be made.
- Has plant evidence ever been introduced into the courts in your state?
- What are the qualifications of the examiners and/or those performing testimony?
- What are the qualifications of the laboratory performing the testing?
- If the examination and testing were performed at an academic institution, are there records of validation for the use of the techniques and the experience of the examiner with court testimony?

If not, a careful review may be necessary to avoid having the evidence not introduced into court.

- Was a blind test performed or possible when attempting plant species identification?

  This step may not be necessary with sufficient examiner experience and if adequate reference samples are available for comparison.

- What is the predicted level of discrimination power for the form of plant DNA testing used in the case?

  This may vary depending on the test method or the propagation method for the plant species in question.

For example,

- in the case of clonally propagated marijuana, linkages may be made between users, dealers, and growers based on the commonality of plant DNA profiles. The establishment of one hundred plants all with identical DNA in a localized geographic region strongly supports clonal propagation for sale and distribution.
- Alternatively, seed-propagated plant samples that match are especially probative because it is not anticipated that populations of identical plants are present in a limited geographic region.

Are all of the participants in the case apprised (informed/told) of the benefits and limitations of the botanical evidence to avoid any discrepancies in testimony?

- If these questions are asked prior to the use of botanical evidence in testing and in court, the evidence can be assured of the appropriate level of representation in criminal cases.
- Botanical evidence is inherently variable due to the great number of plant species present in land and water.

A comprehensive understanding of the plant species in question, the test methods, and a cooperative effort between crime scene personnel, forensic scientists, academic

practitioners, and attorneys can lend clarity to a judge and jury when forensic botany is presented in court.

Relevant Terminologies Related To Collection and preservation of botanical evidences are –

Biohazards:

Materials that contain blood or other potentially infectious contents. These materials include many of those found in biological evidence, including semen, vaginal secretions, or any bodily fluid that is visibly contaminated with blood, and all bodily fluids in situations in which it is difficult or impossible to differentiate between bodily fluids as well as any unfixed tissue or organ from a human (living or dead) that can be collected at a crime scene and stored (OSHA 2012).

Biological Evidence:

Biological evidence refers to samples of biological materials or evidence items containing biological material. Biological material recovered from crime scenes commonly appears in the form of hair, tissue, bones, teeth, blood, semen, other bodily fluids. Botanical evidences generally remain unnoticed.

Bloodborne Pathogens:

Microorganisms that are present in human blood and can cause disease in humans. These pathogens include, but are not limited to, hepatitis B virus and human immunodeficiency virus (OSHA 2012).

Chain of Custody:

Identification of the person or agency having custody of evidence and the place where that evidence is kept, in chronological order from the time evidence is collected to its destruction. A formal, written process that records the persons having custody of evidence from initial point of receipt or custody by a representative of a law enforcement agency to its final disposition by the agency. The record also reflects the

dates and reasons if evidence is transferred from one location or person to another. A chain-of-custody record could also be included in a court transcript.

Exceptionally Cleared:

A case status where an offender is not arrested and formally charged due to some element beyond law enforcement control. Examples of exceptional clearances include, but are not limited to, the death of the offender (e.g., suicide or justifiably killed by police or citizen); the victim's refusal to cooperate with the prosecution after the offender has been identified; or the denial of extradition because the offender committed a crime in another jurisdiction and is being prosecuted for that offense (Federal Bureau of Investigation, 2013).

Contamination:

The unwanted transfer of material from another source to a piece of physical evidence (National Institute of Justice "Crime Scene Investigation: A Guide for Law Enforcement" 2000).

Crime Laboratory:

A facility (Government or private) that analyses physical evidence.

Crime Scene:

A location at where (or a person upon who) a crime may have occurred.

Degradation:

The transition from a higher to a lower level of quality.

Desiccant:

A substance used as a drying agent.

DNA:

The genetic material; a double helix composed of two complementary chains of paired bases (nucleotides) (National Institute of Justice "The Future of Forensic DNA Testing: Predictions of the Research and Development Working Group" 2000); deoxyribonucleic acid (DNA), often referred to as the “blueprint of life,” it is the genetic material present in the nuclei of cells that is inherited, half from each biological parent. DNA is a chemical substance contained in cells that determines each person’s individual characteristics. An individual’s DNA is unique, except in cases of identical twins.

Dried Down:

Evidence that has been fully dried so that no liquid (e.g., blood, semen) can drip from the object.

Evidence:

Property that may be related to a crime and/or that may implicate a person in or clear a person of a crime.

Evidence Collector:

The person who initially took ownership of an item for evidentiary purposes.

Evidence Custodian:

The person who is responsible for **evidence processing in a given location** (e.g., property and evidence room, hospital, court, crime laboratory). This person can be an evidence collector or handler as well.

Evidence Handler:

Any person who **has/had evidence in his or her possession at any given time**. A record of this handler must be kept in the chain-of-custody record.

Evidence Packaging:

The manner in which items with potential evidentiary value are wrapped, bagged, or boxed to be preserved, documented, and labelled (Latta and Bowers 2011).

Extracted DNA:

Genomic DNA extracted from biological evidence; DNA in its raw form.

First Responder:

The initial responding law enforcement officer(s) and/or other public safety official(s) or service provider(s) arriving at the scene before the arrival of the investigator(s) in charge (National Institute of Justice "Crime Scene Investigation: A Guide for Law Enforcement" 2000).

Frozen:

A storage condition in which the temperature is maintained thermostatically at or below –10°C (14°F).

Hepatitis B:

A viral disease that causes inflammation of the liver and is primarily spread through exposure to infectious blood or bodily fluids, such as semen and vaginal secretion.

Hepatitis C:

A viral disease that causes inflammation of the liver and is primarily spread through blood-to-blood contact.

High-Efficiency Particulate Air (HEPA) Filter:

A filter that removes 99.97% of all particles greater than 0.3 micrometer from the air that passes through.

Human Immunodeficiency Virus (HIV):

A virus that causes a condition in humans that leads to the progressive failure of the immune system and can be spread by the transfer of blood, semen, vaginal fluid, pre-ejaculate, or breast milk.

Integrated Software Systems:

A collection of computer programs designed to work together to handle an application, either by passing data from one to another or as components of a single system. Integrated systems may include Computer Aided Dispatch, Records Management System, Laboratory Information Management System, and Property Evidence Module.

Law Enforcement Agency:

Any agency that enforces the law. This may be local or state police or National agencies, such as the National Investigation Agency (NIA) or the Drug Enforcement Administration like Narcotics Control Bureau.

Long-Term Storage:

A location that is designated to secure evidence or property items in the custody of an agency until the items are diverted, sold, released, or destroyed.

Nonporous Container:

Packaging through which liquids or vapors cannot pass (e.g., glass jars, metal cans, and plastic bags) (National Institute of Justice "Crime Scene Investigation: A Guide for Law Enforcement" 2000).

Packaging:

Container used to house individual items of evidence.

Parent/Child Tracking:

A tracking system capability that maintains information about an **original evidence sample** (or **parent**) and the **resulting samples** (or **children**) that have been devised or extracted to obtain testing results.

Personal Protective Equipment (PPE):

Items used to prevent an individual's direct contact with bloodborne pathogens. PPE includes disposable gloves, disposable overalls, disposable shoe covers, laboratory coats, masks, and eye protection.

Porous Container:
Packaging through which liquids or vapors may pass (e.g., paper bags and cloth bags) (National Institute of Justice "Crime Scene Investigation: A Guide for Law Enforcement" 2000).

Property Officer:A worker responsible for the intake, submission, and/or retrieval of evidence in a property room.

Property Room:
A location dedicated to housing evidence for criminal investigations. This location can be in a law enforcement office, a crime laboratory, a hospital, or a court.

Property Room Manager/Supervisor:
A worker responsible for managing the property and/or the personnel who handles the intake, submission, and/or retrieval of evidence in a property room.

Refrigerated:
A storage condition in which the temperature is maintained thermostatically between 2oC and 8oC (36oF and 46oF) with less than 25% humidity.

Refrigerator:
Equipment used to keep an item or group of items cooler than room temperature.

Room Temperature:
A storage condition in which the temperature is equal to the ambient temperature of its surroundings; storage area may lack temperature and humidity control methods.

Sexual Assault Kit:

A collection of items used by medical personnel to collect and preserve physical sexual assault evidence that can be used in a criminal investigation.

Stabilizing Solution:

A compound that is added to biological material designed to enable the storage and transportation of DNA samples without freezing (Swinfield et al. 2009).

Standard Operating Procedure (SOP):

A set of guidelines that can also be equated to general orders, policies and procedures, and rules and regulations.

Temperature Controlled:

A storage condition in which temperature is maintained thermostatically between 15.5°C and 24°C (60°F - 75°F) with less than 60% humidity. Technical Working Group on Biological Evidence Preservation

Temporary Storage/Short-Term Storage:

Storage of evidence from the time collected to reception by property room personnel.

Tickler File:

A file that serves as a reminder and is arranged to bring matters to timely attention; can be manual (e.g., folders into which copies of property records are placed when an item is temporarily signed out to the laboratory, court, investigation, etc.), or can be automated as part of a computer application that sets a reminder date that triggers a notification that an action is overdue (e.g., an item has not been returned from court).

Touch DNA (Trace DNA or Low Copy Number [LCN] DNA):

"Touch DNA" is DNA obtained from biological material transferred from a donor to an object or a person during physical contact. This DNA contained in shed skin cells that

transfer to surfaces that humans touch (Daly, Murphy, and McDermott 2012). This particular kind of evidence could play an essential role in forensic laboratory work and is considered an important tool for investigators. It only requires very small samples, for example from the skin cells left on an object after it has been touched or casually handled, or from footprints. The analysis of Touch DNA requires only seven or eight cells from the outermost layer of human skin and only a sterile cotton swab is needed for the collection of samples.

5. Branches of Botany in Forensic Study: Palynology, Limnology, Plant Anatomy, Plant ecology, Plant Molecular Biology.

Forensic botany is defined as the use of plant evidence in court. It is subdivided into several botanical subspecialties, including palynology (the study of pollen), limnology (the study of freshwater ecology), plant anatomy (the study of cellular features) and plant ecology (plant succession patterns). In the past decade, molecular biology and the use of DNA methods have been important tools to further the research of these disciplines.

Palynology:

Forensic palynology refers to the use of pollen in criminal investigations. The major plant groups identified as pollen sources include flowering plants, conifers, and ferns. Ferns technically produce spores instead of pollen but are included in pollen types. Pollen is microscopic and not visually obvious trace evidence during crime scene collection, but is retained on clothing, embedded in carpets, and pervasive in soil. Pollen grain morphology can be used to identify a plant genus and often the species. Crime scenes that are restricted to a few square meters, such as a rape scene or the

entry point of a burglary, are good choices for pollen evidence.

Localized areas have a specific pollen distribution pattern representing the combination of plant species found in the surrounding vegetation. Common pollen types from plants that use wind for distribution (e.g., grass, bracken spores) will be less useful than pollen from uncommon, poorly distributed species (e.g., flax, willow). Insect-distributed pollen is typically deposited within a few feet of the source plant. Pollen analysis consists of species identification and an estimation of the percentage that each plant species represents in an evidentiary sample. A similar pollen composition from shoeprints and from the shoes that made the prints indicates a strong match correlation. Pollen evidence collected from a burglary entrance and a suspect's shoes, for example, could provide a linkage in a case.

A case that exemplifies the use of pollen in criminal casework is described by Horrocks et al. In Auckland, New Zealand, a prostitute alleged that the defendant had raped her in an alleyway approximately seven meters from his car after failing to pay her in advance for her services. The defendant claimed that he had never been more than one meter away from the car and had not entered the alleyway. Furthermore, he claimed that he had not had sex with the victim and the soil on his clothing was from the driveway area. An examination of the crime scene and the evidence showed no footprints and no seminal fluid stains. A soil sample was collected from the defendant's clothing, the disturbed area of ground in the alleyway, and from the driveway area near the defendant's car. All the soil samples were prepared for pollen analysis by deflocculation with potassium hydroxide, acetylation to remove cellulose and organic matter, and a silicate removal step using hydrofluoric acid. Samples were bleached to remove additional organic matter and analyzed under a microscope for pollen identification and counting. The types of pollens were similar between the two locations, but the amounts of each type were different in each sample. The alleyway contained 76% *Coprosma* (an evergreen shrub) pollen, but the driveway sample contained only 8%.The defendant's clothing contained approximately 80% *Coprosma* and only small amounts of other pollen species. These results support the victim's account of the sexual assault taking place in the alleyway. Pollen analysis has also been utilized to establish time of death.

In Magdeburg, Germany, a mass grave containing 32 male skeletons was discovered in February of 1994. The identities of both the victims and the murderers was unknown. Two hypotheses were proposed: (1) the victims were killed in the spring of 1945 by the Gestapo at the end of World War II, or (2) the victims were Soviet soldiers killed by the secret police after the German Democratic Republic revolt in June of 1953. The ability to differentiate between the spring and summer was critical to solving the case. Pollen analysis was performed on 21 skulls. Seven of the skull nasal cavities contained high amounts of pollen from plantain, lime tree, and rye. All of these plant species release pollen during the months of June and July. Pollen analysis supported the hypothesis that the remains were of Soviet soldiers killed by the Soviet secret police after the June 1953 revolt.

Limnology:

Limnology is the study of freshwater ecology and can be applied to a subset of forensic cases. In particular, aquatic plants (e.g., algae, diatoms) have been useful to link suspects to a crime scene or to establish that drowning occurred in freshwater

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Properties for which diatom is used as clue:

a: Diatom populations vary seasonally in lakes, rivers, and ponds.

- In early spring, diatom populations expand in freshwater.
- Following this expansion, the live diatoms decline but a large number of dead diatoms remain in summer water.
- In the fall, a second diatom expansion occurs and then progressively declines throughthe winter months.

b: Each species has a characteristic shape and refractive pattern from the silica

in thecell wall which can be used for identification.

c: Diatoms do not occur naturally in the body.

When a person drowns in freshwater, diatoms are taken in along with water into the lungs.The diatoms are dispersed to the internal organs of the body.

In a study of 771 cases, the diatom test was positive for 28% of presumed freshwater drowning cases but was rarely positive for domestic water drowning. The low rate of diatoms observed in domestic drowning could be traced back to cleaning agents containing crushed diatoms for abrasives.

In 1991, two young boys were brutally attacked by teenage assailants while fishing at a suburban pond in Connecticut. The boys were held at knifepoint, bound with duct tape, and savagely beaten anddragged into the pond to drown. One boy managed to get free, save himself, and rescue his friend. After many hours of criminal investigation, three suspects were apprehended. To link the suspects to the crime scene, investigators seized the sedimentencrusted sneakers of both the victims and the assailants and analyzed them for algal and diatom species. microscopic analysis of samples from each pair of sneakers plus reference samples from the pond showed the same species and distribution pattern of each species. These results supported the position that the samples all originated from a common freshwater location.

Plant Anatomy:

Plant Anatomy is a broad discipline that includes the study of plant species and taxonomy (the identification of plant species). Species identification is a typical firststep in analyzing botanical evidence for casework.

Plant anatomy uses features such as leaf morphology and tree growth ring patterns to aid in species identification and in performing physical matches of evidence, respectively.

The kidnapping and death of Charles Lindbergh's young son in 1932 was the first modern-era case to use such botanical evidence in court. A wooden ladder was usedto gain access to the second-story nursery to kidnap Lindbergh's son.

Arthur Koehler, a wood identification expert for the Forest Products Laboratory of the

U.S. Forest Service in Wisconsin, was able to provide critical evidence against Bruno Richard Hauptman, who was later convicted of the crime.

Koehler had an excellent academic record and had provided evidence in several cases prior to the famous Lindbergh trial. His testimony is noteworthy since the use of scientific experts in the mid- 1930s was generally limited to fingerprints, handwriting, bullet comparisons, and analyses of stomach contents. Koehler first identified the four tree species used to construct the ladder as yellow pine, ponderosa pine, Douglas fir, and birch, via microscopic analysis of wood-grain patterns. Next, Koehler analyzed the tool marks left on the wood from both the commercial planing mill and the hand plane used by Hauptman during the construction of the ladder. Koehler used oblique light in a darkened room to observe the plane patterns left on the wood. Amazingly, he was able to trace the wood by the mill plane marks to a shipment of yellow pine delivered to the National Lumber and Millwork Company in Bronx, New York. The hand-plane marks on the ladder exactly matched those made by a hand plane found in Hauptman's possession. Finally, Koehler compared the annual growth rings and knot patterns on rail 16 of the ladder to a section of wood in Hauptman's attic. The pattern of knots and growth rings on rail 16 exactly matched the exposed end of wood.

Plant Ecology:

Plant ecology involves studying the **growth patterns of vegetation in areas that have been disturbed**. These patterns and the vegetative (non-flowering) portion of plants can be useful in estimating time of death.

- For example, when a body is discovered lying on top of a weed plant with broken top, useful information can be obtained to define time windows for when the death occurred. A certain amount of shading will eventually kill a plant, so if the weed plant is lacking chlorophyll, a minimum amount of time must have already elapsed.
- If new shoots are present at the base of the plant, this may establish a second time window. Agricultural research on many plant species has defined the time for new shoot initiation after the top of a plant has been removed.

- The length of the new shoot can sometimes establish a third time window.

In one case, the brain cavity of a skull was filled with plant roots. The anatomy and developmental stage of the roots indicated that the plant was approximately one year old, and the plant was putatively identified as *Ranunculus ficaria* L (buttercup family). The predictable stages of plant development were useful in estimating the time that the skeletal remains had been in their present location. The investigators were able to determine that the skeleton had been there for at least one year; however, a maximum time could not be established. The plant could have developed secondarily sometime after the body had lain in its present location, so a maximum time estimate was not possible.

Plant Molecular Biology:

In the age of DNA analysis, forensic botany is using molecular biology to aid in criminal and civil investigations. The first criminal case to gain legal acceptance using plant DNA typing was a homicide that occurred in 1992 in Arizona's Maricopa County. A woman's body was found under a paloverde tree in the Arizona desert. Near the body was a beeper eventually traced to a suspect, Mark Bogan. A few seed pods from a paloverde tree were found in the back of Bogan's truck. Officials wanted to know if DNA could match those seed pods to the tree where the body was discovered. Dr. Timothy Helentjaris from the University of Arizona used a technique called randomly amplified polymorphic DNA (RAPD) analysis to generate a band pattern from the evidence in question. He also surveyed a small population of other paloverde trees to determine if the band patterns were unique to each individual. His convincing testimony on plant evidence helped convict Mark Bogan of murder. RAPD marker analysis has also been utilized in civil court cases to identify patent infringements. In Italy, RAPD analysis of a patented strawberry variety "Marmolada" helped settle a lawsuit involving the unauthorized commercialization of the plant.

Molecular methods can be used to identify a plant species from minute leaf fragments and pollen grains. Forensic botanists have utilized DNA technology because often botanical trace evidence does not contain the necessary morphological or histological

features that would allow one to identify a plant at the genus or species level. This is particularly true for fragmented and deteriorated plant material. The Bode Technology Group Inc. (Dr. Robert Bever; Springfield, VA) is developing and utilizing molecular methods to analyze botanical trace evidence. This type of analysis is a valuable tool for potentially linking an individual to a crime scene or physical evidence to a geographic location. One useful application for the molecular analysis of botanical trace evidence is the identification of a geographic region where a kidnapped individual may be located. Based on flowering times and the plant species represented in the trace pollen evidence found with a ransom note, a geographic region may be identified and would provide the police with an investigative lead. Plant systematists have characterized many loci that are useful for the identification of plants, including several nuclear (18S, ITS1, ITS2) and chloroplast (rbcL, atpB, ndhF) genes.

Bode Technology Group has identified a DNA extraction, cloning, and sequencing procedure to identify plants using some of those genes. Using these methods, they have identified numerous species of plants from physical evidence. These include species of algae, evergreens, and many flowering herbs, shrubs, and trees. Many plants have a limited geographic distribution or grow in specific habitats. Some of these locations will be general areas, such as roadsides or areas of new construction. Other locations will be more specific, like the Mohave Desert or southern Florida, for plant species that have a severely restricted geographic range. Linking botanical trace evidence to a geographic region could provide law enforcement and investigators with valuable information.

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6. Analyses of Samples: Pollen grains; Anatomical structures; Diatoms, DNA.

Pollen Grain Analysis:

- Pollen and spores are chemically extracted from samples
- To identify pollen and spores, specialists can use a compound light

microscope, a scanning electronic microscope, reference collections that may consist of photos and illustrations or perhaps even actual dried specimens arranged systematically (herbariums).

- ❖ Pollen and spore evidence that has been collected, analyzed, and interpreted can be presented in court.
- ❖ These "fingerprints" can be used to confirm certain aspects of a crime.

How to collect pollen and spore?

- During an investigation, control samples must be collected as well as evidence samples.
- Samples must be collected wearing gloves and with clean tools (such as brushes and cellophane tape) and placed in sterile containers, which then must be sealed and labeled with care.
- Sampling instruments must be cleaned after each use, or new ones must be used.
- Collected evidence must be secured, and the chain of custody must be maintained.

Analyzing Pollen and Spore Samples:

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- To identify pollen and spores, specialists can use a compound light microscope, a scanning electronic microscope, reference collections that may consist of photos and illustrations or perhaps even actual dried specimens arranged systematically (herbariums).
- Pollen and spore evidence that has been collected, analyzed, and interpreted can be presented in court.
- These "fingerprints" can be used to confirm certain aspects of a crime.

Pollen Fingerprint

Pollen fingerprint is the number and type of pollen grains found in a geographic area at a particular time of year.

Four (4) essential parts-

(1) number of pollen grains
(2) type of pollen grains
(3) found in a certain area
(4) at a particular time

What it does?

Pollen fingerprint can link a piece of evidence to a particular place and time.

Advantages of pollen analysis in forensic botany:

Since both pollen and spores have resistant structures, they at times can help to determine such thingsas

- ✓ Whether a victim/suspect was moved or not
- ✓ Where is the crime's location, whether it occurred in a city or in the village In which season itmay have occurred
- ✓ How do you think pollen collected here differs from pollen in Darjeeling?
- ✓ How would pollen collected in the summer differ from pollen collected in the winter? Howshould you analyze the pollen?
- ✓ What instruments or techniques should you use?

Analysis of Anatomical samples:

Cell shape and orientation of certain structures within a cell can be helpful in classification of a species. In order to learn about plant anatomy and specific plant structures within the plant body plan, it is important to take a practical approach.

Plant Anatomy

The study of the internal structure of plants is called plant anatomy. When a stem, root, or leaf is dissected, the cells can be arranged in specific patterns that may be useful for classification and identification. For example, the internal arrangement of cells in the root structure of a dicotyledonous (the first true leaves of a seedling occur in pairs) versus a monocotyledous (the first true leaf is a single leaf) plant is characteristic.

In the following sections we describe examples of in expensive laboratory exercises that can be performed as training for forensic botanists.

Plant Anatomical Features used in Forensic Botany

Since 1930s: became increasingly more common in forensic applications (Lane et al., 1990). Cell wall: particularly important for two reasons:

- it is not easily digested by most organisms and therefore persists when other plant features aredestroyed (Bock and Norris, 1997)
- the size, shape, and pattern of cell walls is often taxon-specific (Lane et al., 1990).

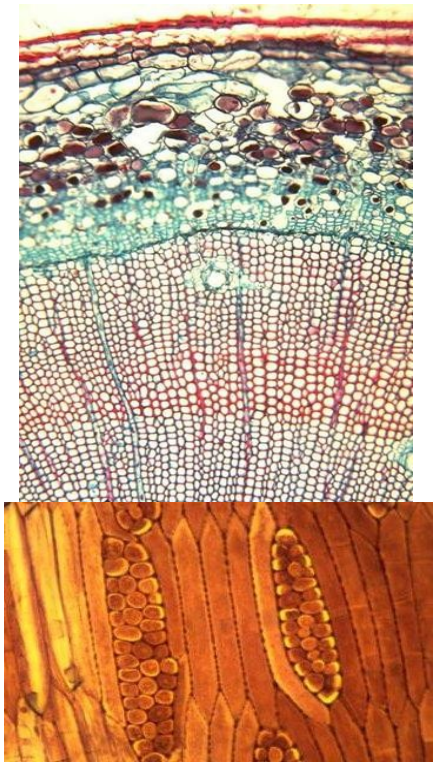
Unique cell types: sclereids, trichomes- are useful inidentifying botanical material (Lane et al., 1990).

Tissue organization of different plant parts (Willey andHeilman, 1987).

Wood characteristics (secondary tissue organization)

The Vascular Cambium

- Lateral meristematic region
- Division of cells here produces secondary xylemand secondary phloem tissues
- Diameter expansion forces tangential elongationof phloem cells
- Epidermis and primary phloem layers eventuallyfall off
- Dead phloem cells compose the outer bark



Two Cell Types

- **Fusiform Initials** - divide to produce new xylem or phloem cells that have longitudinally

elongatedshapes

- **Ray initials** - short, rounded cells that divide to produce new xylem or phloem ray cells

Cambium: the growing (generative) layer between the xylem and phloem.

Xylem: principle strengthening and water conducting tissue of the stem, roots, and branches.

Phloem: inner bark, principal function to distributemanufactured foodstuffs.

Bark: dead, outer tissue that protects the cambium from the external environment and exposure topathogens and physical injury.

Vessel: the composite, tube-like structure found in hardwoods from the fusion of cells in alongitudinal column.

Fibre: an elongated cell with pointed ends and a thick or infrequently thin wall.

Rays: ribbon-shaped tissue extending in a radial direction across the grain of the wood.

Sapwood and Heartwood:

In mature trees, the xylem has both living and dead cells.

Sapwood contains the only living cells in the xylem (not all sapwood cellsare alive either) and has a conductive function.

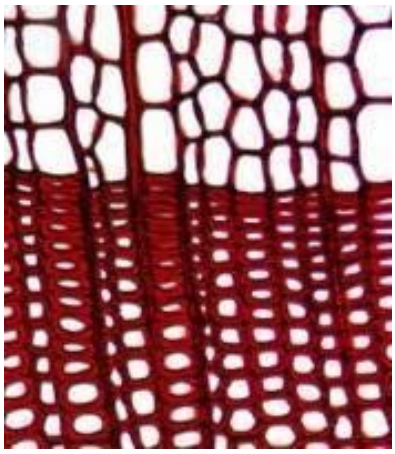
Heartwood is composed of dead cells and lends mechanical support only.

Growth rings:

- Mark annual growth boundaries in trees grown in temperate climates Often composed of 2 distinct segments
- Early wood (spring wood) Late wood(summer wood)

Early wood and latewood cells have differentcharacteristics

Cell Differences within Growth Rings



Earlywood

- Large Radial diameter cells
- Lower density than latewood

Latewood

- Smaller radial diameter cells
- Thicker cell walls

Irregularities in Annual Ring Formation

False rings

- Growth interrupted by environment (e.g. defoliation)
- Slow growth may cause formation of latewood type cells

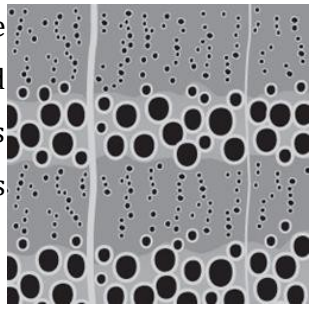
Discontinuous rings

- Cambium was dormant in one region
- One-sided crowns, suppressed, or over mature trees

Trees grown in tropical environments

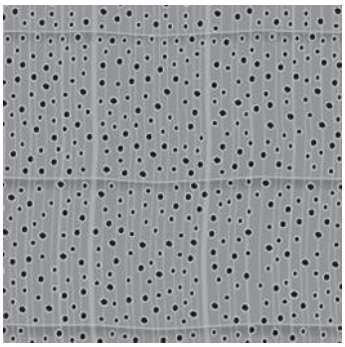
- ✓ Almost continual growth can limit occurrence of rings
- ✓ In some climates, stopping and restarting of growth can give more than one growth increment in a year

Ring-porous: The largest pores are in the early wood while those in the late wood are more evenly distributed and uniform in size. These woods typically have distinct figures and patterns, and the uneven uptake of stain (the large pores soak up more color).



Semi- ring/diffuse porous- Pores are large in the early wood and smaller toward the latewood, but without the distinct zoning, as seen in ring-porous woods.

Diffuse porous- Pores are distributed fairly evenly across the early wood and latewood. Most domestic diffuse-porous woods have relatively small-diameter pores, but some tropical woods of this type (e.g. mahogany) have rather large pores.

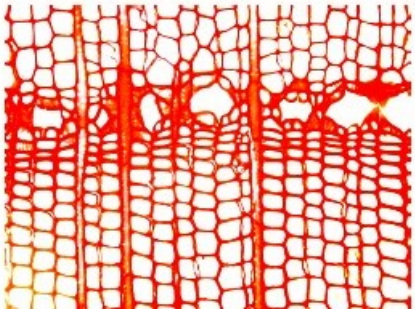


Non porous- Softwoods (gymnospermous wood) don't have vessel cells. However, different softwoods have different growth-ring characteristics. In white pine, the rings are non-distinct, and stain uptake is fairly even, as in diffuse porous woods. In yellow pine, where the rings are clearly visible, stain uptake in early wood is more pronounced than in latewood, as in ring-porouswoods.

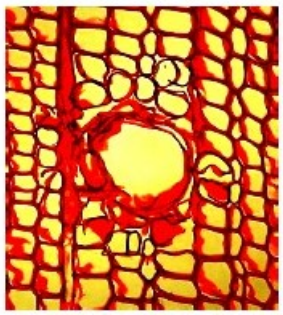
Criteria for identification

- Structure of tracheid walls and xylem rays
- Resin canals
- Pitting in rays
- Crystals

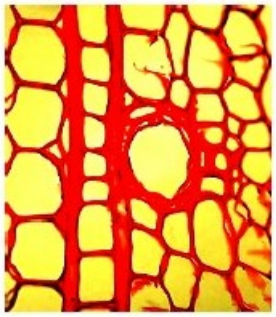
Resin canals:



Traumatic resin canals (produced by injury)



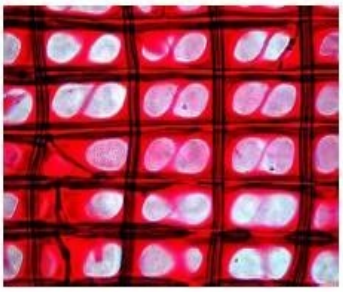
Resin canals with thin-walled epithelial cells



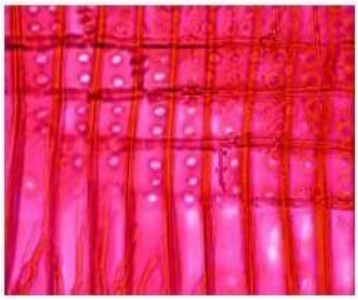
Resin canals with thick-walled epithelial cells

The wood formed immediately after wounding will contain traumatic resin canals. Wounding may be caused by freezing, fires, or mechanical damage. Traumatic resin canals do not have as regular a shape as the normal resin canals, and are formed by some species that normally do not have resin canals.

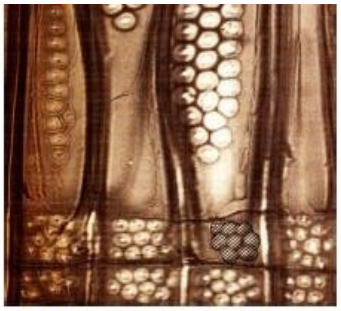
Pits:



Large window-like pits (large pinoid pits-occupying practically)



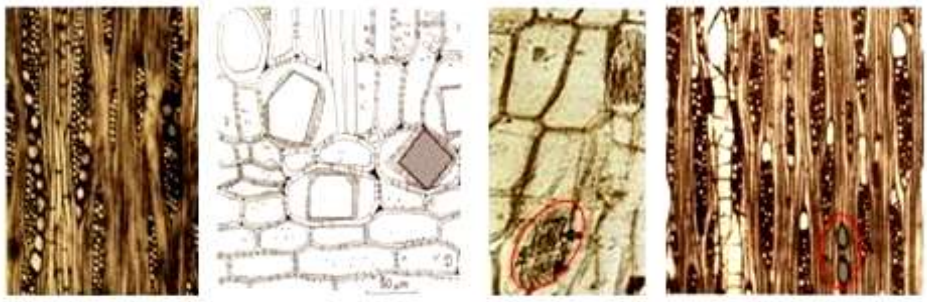
Cupressoid pits (The pit aperture is approx. the same size as the pit border)



Araucaroid (pits 2 or more rows alternating pits side by side)

Crystals:

- In ordinary cells
- In chambered cells
- In idioblasts
- Raphides/ druses
- Oil/ mucilage cells



In chambered cells (*Citrus maxima*)

In idioblasts (*Zelkova acuminata*)

Raphides (*Morinda lucida*)

Oil cells (*Litsea populifolia*)

Roots: Frequently found with exposed or shallowly buried bodies

Plant roots, like their above-ground counterparts, exhibit annual growth rings that can be useful in pinning down the post-mortem interval, or at least the time since the body came to be at the location where it was found.

Three ways in which the roots can be used to date the remains or otherwise characterize the burial site:

(1) Examine root development after it has been damaged. When a grave is dug or the ground otherwise disturbed, roots can be damaged but still continue growing. If the meristematic zone is damaged, no secondary xylem cells can be produced, leaving a permanent lesion. The number of growth rings laid down after the lesion indicates the number of years since the damage occurred.

(2) Examine roots in direct contact with the remains. Roots in contact with the bones or personal effects of the deceased can be cross-sectioned at the point of contact and the annual rings counted, establishing a minimum time frame for time since death. The contact must be penetrative, i.e. the roots must be growing through clothing or bones, in order for the interpretation to be valid.

(3) Examine branch growth. Annual longitudinal growth of the roots, in addition to radial growth, can be estimated, and a time frame determined from the length of a root from its point of contact with the remains to its distal end.

Stomach contents:

Characteristic cell types from food plants can be used to identify a victim's last meal (Bock and Norris, 1997). knowledge about which can be useful in determining the victim's whereabouts or actions prior to death.

Some of these cell types include (Dickison, 2000):

- Sclereids (pears)
- Starch grains (potatoes and other tubers)
- Raphide crystals (pineapple)
- Druse crystals (citrus, beets, spinach)

- Silica bodies (cereal grasses and bamboos)

Time since death can be approximated by the state of digestion of the stomach contents

It normally takes at least a couple of hours for food to pass from the stomach to the small intestine.

A meal still largely in the stomach implies death shortly after eating, while an empty or nearly- emptystomach suggests a longer time period between eating and death (Batten, 1995).

However, there are numerous mitigating factors to take into account:

- the extent to which the food had been chewed
- the amount of fat and protein present
- physical activity undertaken by the victim prior to death
- mood of the victim
- physiological variation from person to person.

All these factors affect the rate at which food passes through the digestive tract.

A Case Study:

In a case where a young woman had been stabbed to death, witnesses reported that she had eaten herlast meal at a particular fast food restaurant.

Provenance

Her stomach contents did not match the limited menu of the restaurant; leading investigators concluded that she had eaten at some point after being seen in the restaurant.

The investigation led to the apprehension of a man whom the victim knew, and with whom she hadshared her actual final meal (Dickison, 2000).

Diatoms:

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DNA analysis:

DNA is composed of four chemical constituents (labelled A, T, C and G), known as bases, attached toa sugar backbone which can form a strand millions of bases long. There are two such strands in DNA,which run in opposite directions. The bases pair up to form a twisted ladder. Each base pair exclusively with one other base on the opposite strand: A to T and G to C.

Forensic DNA analysis focuses on examining specific sections of DNA that are known to be particularly variable between individuals in order to create a DNA profile. The part of the DNA that isexamined is called a locus (plural loci), which is a unique site along DNA of a chromosome characterised by a specific sequence of bases. Currently, an individual's entire genome is not analysedto create his or her DNA profile. This means that part or all of the same DNA profile could be shared by more than one person. The statistical analysis of forensic DNA data therefore focuses on establishing the weight of evidence that should be attached to the similarity etween the DNA profile of a person of interest and DNA taken from a crime scene.

DNA profiling/ typing/ fingerprinting:

DNA profiling (also called DNA fingerprinting, DNA tying) is the process of determining an individual's DNA characteristics, which are as unique as fingerprints. DNA analysis intended to identify a species, rather than an individual, is called DNA barcoding.

DNA profiling is a forensic technique in criminal investigations, comparing criminal suspects' profilesto DNA evidence so as to assess the likelihood of their involvement in the crime. It is also used in parentage testing, to establish immigration eligibility, and in genealogical and medical research. DNA profiling has also been used in the study of animal and plant populations in the fields of zoology, botany, and agriculture.

Who invented it?

The process of DNA fingerprinting was invented by Alec Jeffries at the University of Leicester in England in 1984.

Methods of DNA typing:

Sample collection –

A sample of DNA is taken from

- Blood
- Hair follicles
- Saliva
- Semen
- Body tissue cells, such as cheek epithelial cells

Cells are then broken down to release their DNA

"Touch" DNA

Humans shed tens of thousands of skin cells each day, and these cells may be transferred to surfaces we touch. Touch DNA has been successfully sampled (by swabbing) items such as

- Door knobs
- Steering wheels
- Gun grips
- Eating utensils

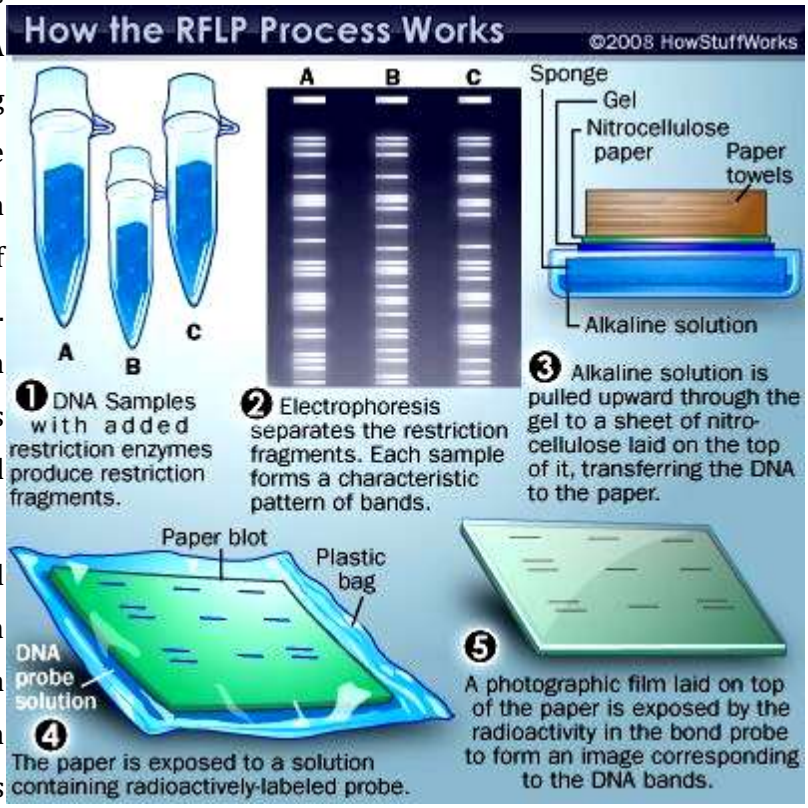
DNA extraction

When a sample such as blood or saliva is obtained, the DNA is only a small part of what is present in the sample. Before the DNA can be analyzed, it must be extracted from the cells and purified. There are many ways this can be accomplished, but all methods follow the same basic procedure. The cell and nuclear membranes need to be broken up to allow the DNA to be free in solution. Once the DNA is free, it can be separated from all other cellular components. After the DNA has been separated in solution, the remaining cellular debris can then be removed from the solution and discarded, leaving only DNA. The most common methods of DNA extraction include organic extraction (also called phenol chloroform extraction), Chelex extraction, and solid phase extraction. Differential extraction is a modified version of extraction in which DNA from two different types of cells can be separated from each other before being purified from the solution. Each method of extraction works well in the laboratory, but analysts typically selects their preferred method based on factors such as the cost, the time involved, the quantity of

DNA yielded, and the quality of DNA yielded. After the DNA is extracted from the sample, it can be analyzed, whether it is by RFLP analysis or quantification and PCR analysis.

RFLP analysis

The first methods for finding out genetics used for DNA profiling involved RFLP analysis. DNA is collected from cells and cut into small pieces using a restriction enzyme (a restriction digest). This generates DNA fragments of differing sizes as a consequence of variations between DNA sequences of different individuals. The fragments are then separated on the basis of size using gel electrophoresis.



The separated fragments are then transferred to a nitrocellulose or nylon filter; this procedure is called a Southern blot. The DNAfragments within the blot are permanently fixed to the filter,and the DNA strands are denatured. Radiolabeled probemolecules are then added that are complementary to sequences in the genome that contain repeat sequences. These repeatsequences tend to vary in length among different individuals and are called variable number tandem repeat sequences or VNTRs. The probe molecules hybridize to DNA fragments containing the repeat sequences and excess probe molecules are washed away. The blot is then exposed to an X-ray film.Fragments of DNA that have bound to the probe molecules appear as fluoresent bands on the film.

The Southern blot technique requires large amounts of non-degraded sample DNA. Also,

Karl Brown's original technique looked at many minisatellite loci at the same time, increasing the observed variability, but making it hard to discern individual alleles (and thereby precluding paternity testing). These early techniques have been supplanted by PCR-based assays.

Polymerase chain reaction (PCR) analysis:

Developed by Kary Mullis in 1983, a process was reported by which specific portions of the sample DNA can be amplified almost indefinitely (Saiki et al. 1985, 1985) The process, polymerase chain reaction (PCR), mimics the biological process of DNA replication, but confines it to specific DNA sequences of interest. With the invention of the PCR technique, DNA profiling took huge strides forward in both discriminating power and the ability to recover information from very small (or degraded) starting samples.

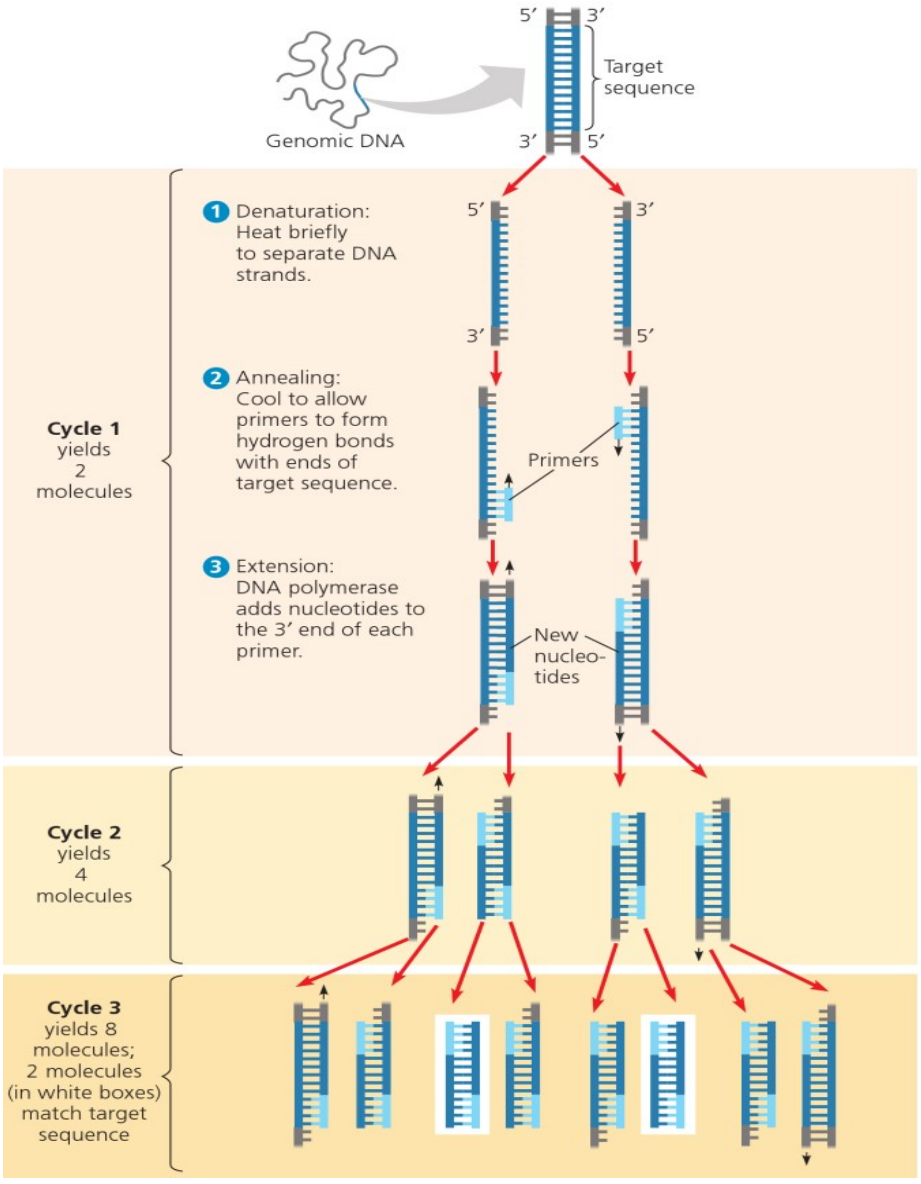
PCR greatly amplifies the amounts of a specific region of DNA. In the PCR process, the DNA sample is denatured into the separate individual polynucleotide strands through heating. Two oligonucleotide DNA primers are used to hybridize to two corresponding nearby sites on opposite DNA strands in such a fashion that the normal enzymatic extension of the active terminal of each primer (that is, the 3' end) leads toward the other primer. PCRuses replication enzymes that aretolerant of high temperatures, such asthe thermostable Taq polymerase. In thisfashion, two new copies of the sequence of interest are generated. Repeated denaturation, hybridization, and extension in this fashion produce an exponentially growing number of copies of the DNA of interest. Instruments that perform thermal cycling are readily available from commercial sources. This process can produce a million-fold or greater amplification of the desired region in 2 hours or less.

Early assays such as the HLA-DQ alpha reverse dot blot strips grew to be very popular due to their ease of use, and the speed with which a result could be obtained. However, they were not as discriminating as RFLP analysis. It was also difficult to determine a DNA profile for mixed samples, such as a vaginal swab from a sexual assault victim.

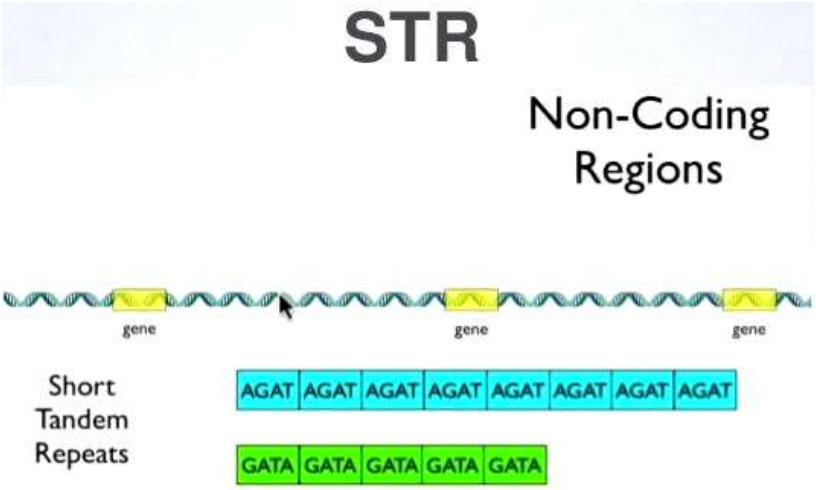
However, the PCR method was readily adaptable for analyzing VNTR, in particular STR loci. In recent years, research in human DNA quantitation has focused on new "real-

time" quantitative PCR (qPCR) techniques. Quantitative PCR methods enable automated, precise, and high-throughput measurements. Inter-laboratory studies have demonstrated the importance of human DNA quantitation on achieving reliable interpretation of STR typing and obtaining consistent results across laboratories.

STR analysis:



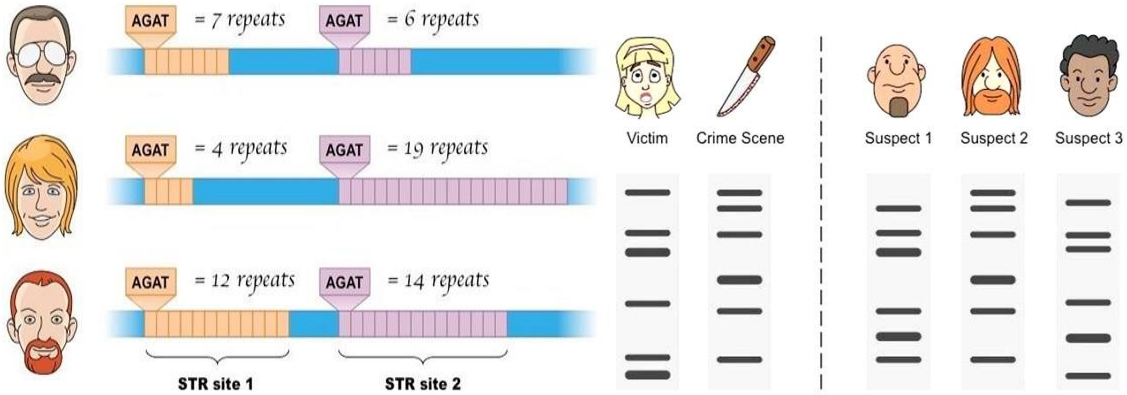
The system of DNA profiling used today is based on polymerase chain reaction (PCR) and uses simple sequences or short tandem repeats (STR). This method uses highly polymorphic regions thathave short repeated sequences of DNA (the most common is 4 bases repeated, but there are other lengths in use, including 3 and 5 bases). Because unrelated people almost certainly have different numbers of



repeat units, STRs can be used to discriminate between unrelated individuals. These STR loci (locations on a chromosome) are targeted with sequence-specific primers and amplified using PCR. The DNA fragments that result are then separated and detected using electrophoresis. There are two common methods of separation and detection, capillary electrophoresis (CE) and gel electrophoresis.

Each STR is polymorphic, but the number of alleles is very small. Typically each STR allele will be shared by around 5 - 20% of individuals. The power of STR analysis comes from looking at multiple STR loci simultaneously. The pattern of alleles can identify an individual quite accurately. Thus STR analysis provides an excellent identification tool. The more STR regions that are tested in an individual the more discriminating the test becomes.

From country to country, different STR-based DNA-profiling systems are in use. In North America, systems that amplify the CODIS 20 core loci are almost universal, whereas in the United Kingdom the DNA-17 17 loci system (which is compatible with The National DNA Database) is in use, and Australia uses 18 core markers. Whichever system is used, many of



the STR regions used are the same. These DNA-profiling systems are based on multiplex reactions, whereby many STR regions will be tested at the same time.

The true power of STR analysis is in its statistical power of discrimination. Because the 20 loci that are currently used for discrimination in CODIS are independently assorted (having a certain number of repeats at one locus does not change the likelihood of having any number of repeats at any other locus), the product rule for probabilities can be applied. This means that, if someone has the DNA type of ABC, where the three loci were independent, we can say that the probability of having that DNA type is the probability of having type A times the probability of having type B times the probability of having type C. This has resulted in the ability to generate match probabilities of 1 in a quintillion (1x1018) or more. However, DNA database searches showed much more frequent than expected false DNA profile matches. Moreover, since there are about 12 million monozygotic twins on Earth, the theoretical probability is not accurate.

In practice, the risk of contaminated-matching is much greater than matching a distant relative, such as contamination of a sample from nearby objects, or from left-over cells transferred from a prior test. The risk is greater for matching the most common person

in the samples: Everything collected from, or in contact with, a victim is a major source of contamination for any other samples brought into a lab. For that reason, multiple control-samples are typically tested in order to ensure that they stayed clean, when prepared during the same period as the actual test samples. Unexpected matches (or variations) in several control-samples indicates a high probability of contamination for the actual test samples. In a relationship test, the full DNA profiles should differ (except for twins), to prove that a person was not actually matched as being related to their own DNA in another sample.

AFLP:

Another technique, AFLP, or amplified fragment length polymorphism was also put into practice during the early 1990s. This technique was also faster than RFLP analysis and used PCR to amplify DNA samples. It relied on variable number tandem repeat (VNTR) polymorphisms to distinguish various alleles, which were separated on a polyacrylamide gel using an allelic ladder (as opposed to a molecular weight ladder). Bands could be visualized by silver staining the gel. One popular focus for fingerprinting was the D1S80 locus. As with all PCR based methods, highly degraded DNA or very small amounts of DNA may cause allelic dropout (causing a mistake in thinking a heterozygote is a homozygote) or other stochastic effects. In addition, because the analysis is done on a gel, very high number repeats may bunch together at the top of the gel, making it difficult to resolve. AmpFLP analysis can be highly automated, and allows for easy creation of phylogenetic trees based on comparing individual samples of DNA. Due to its relatively low cost and ease of set-up and operation,AmpFLP remains popular in lower income countries.

Y-chromosome analysis:

Recent innovations have included the creation of primers targeting polymorphic regions on the Y- chromosome (Y-STR), which allows resolution of a mixed DNA sample from a male and female or cases in which a differential extraction is not possible. Y-chromosomes are paternally inherited, so Y- STR analysis can help in the identification of paternally related males. Y-STR analysis was performed in the Sally Hemings

controversy to determine if Thomas Jefferson had sired a son with one of his slaves. The analysis of the Y-chromosome yields weaker results than autosomal chromosome analysis. The Y male sex-determining chromosome, as it is inherited only by males from their fathers, is almost identical along the patrilineal line. This leads to a less precise analysis than if autosomal chromosomes were testing, because of the random matching that occurs between pairs of chromosomes as zygotes are being made.

Mitochondrial analysis:

For highly degraded samples, it is sometimes impossible to get a complete profile of the 13 CODIS STRs. In these situations, mitochondrial DNA (mtDNA) is sometimes typed due to there being many copies of mtDNA in a cell, while there may only be 1-2 copies of the nuclear DNA. Forensic scientists amplify the HV1 and HV2 regions of the mtDNA, and then sequence each region and compare single-nucleotide differences to a reference. Because mtDNA is maternally inherited, directly linked maternal relatives can be used as match references, such as one's maternal grandmother's daughter's son. In general, a difference of two or more nucleotides is considered to be an exclusion. Heteroplasmy and poly-C differences may throw off straight sequence comparisons, so some expertise on the part of the analyst is required. mtDNA is useful in determining clear identities, such as those of missing people when a maternally linked relative can be found. mtDNA testing was used in determining that Anna Anderson was not the Russian princess she had claimed to be, Anastasia Romanov. mtDNA can be obtained from such material as hair shafts and old bones/teeth. Control mechanism based on interaction point with data. This can be determined by tooled placement in sample.

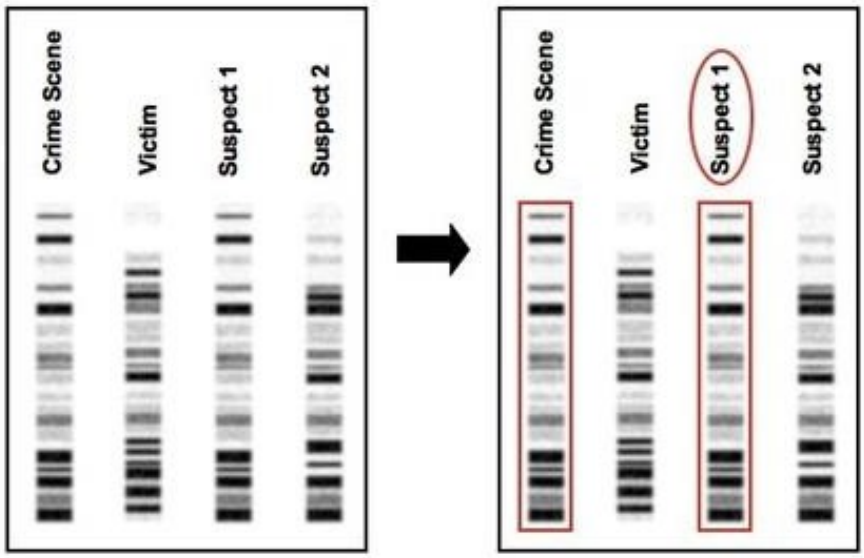
Applications of DNA Fingerprinting:

Since its discovery, DNA fingerprints have been used as evidences in the identification of criminals inmurder case, rapists in rape case, parents in case of doubt•ful parentage etc. This method has also been used in a wide range of other applications, including immigration cases, disputes invol•ving purebred dogs, and in animal conserva•tion studies.

In Forensic Analysis:

If a DNA profile from tissue found at a crime scene matches with that of a suspect, it does not prove that the tissue belongs to the suspect; instead, it excludes all those who have a different DNA profile. Therefore, to generate five to more DNA profiles from the same sample, different probes have to be used.

The more profiles that match, between the sample and the suspect, the more unlikely it is that the sample at the crime scene came from someone other than the suspect. Sometimes DNA fin-gerprinting in criminal case may not involve the suspect's own DNA, but rather the DNA of plantsor animals that are closely present at the crime scene.



In Paternity case:

If a mother of a new•born accuses a particular man of being the father of her child, and the man denies it, the DNA typing or fingerprinting can at least reach conclusive decisions. In this case, DNA samples are taken from blood samples of mother, baby and the suspected father.

DNAs are cut with restriction enzyme for the marker to be analysed and the resulting fragments are separated by electrophoresis, transferred to a membrane filter by Southern blotting and probed with alabeled mono-locus STR or VNTR probe.

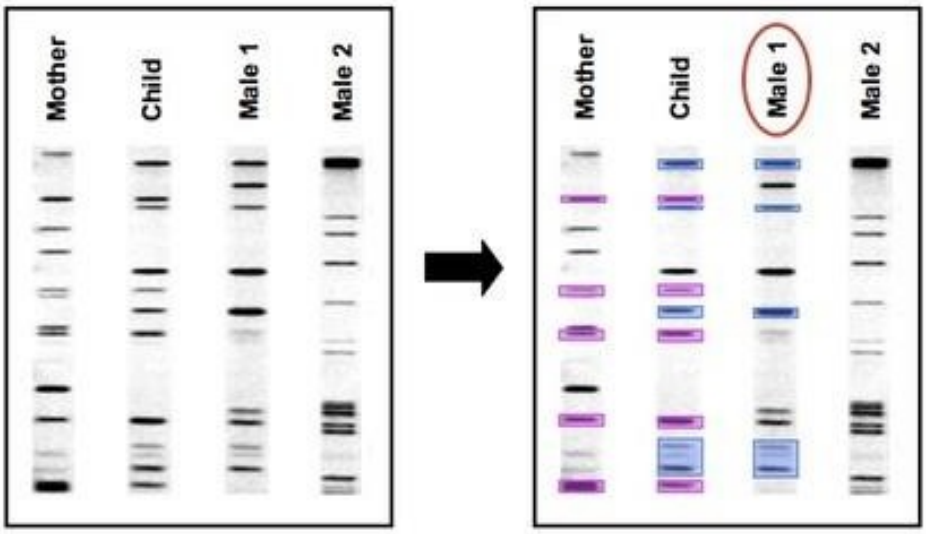
After autoradiography or chemiluminescence detection, the DNA banding pattern – the DNA fingerprint or DNA profile is analyzed to compare the samples. The data shown can be interpreted as follows. In second lane two DNA fragments are shown from mother's sample.

Therefore, the mother is heterozygous for one particular pair of alleles at the STR or VNTR locus under study. Similarly, two DNA fragments are also detected in the third lane for the baby.

It indicates that the baby is also heterozygous. It is also shown that one fragment for the baby matches the larger fragment of the mother's DNA. The other fragment of the baby's DNA is much larger, indicating presence of more repeats in that allele. In the autoradiogram it is also shown that the lar-ger fragment of the baby's DNA matches with one DNA fragment of the alleged father.

We know that the baby receives one allele from its mother and one from its father. The present data indicate that the man shares an allele with the baby, but he denied being its father. If the man had no alleles in common with the baby, then the data would have proved that he is not the father. This is called exclusion result. The inclusion result that indicates positive identity is difficult to establish through DNA fingerprinting.

It depends on frequencies of STR or VNTR alleles identified by the probe in the ethnic population from which the accused person comes. However, for better' confirmation, it is suggested to probe the DNA fragment with different mono-locus STRs or VNTRs and if the data for each probe indicated that the same person contributed a particular allele to the child, then the conclusion regarding the paternity will be more confirmed.



Other Applications of DNA Fingerprinting:

(i) Detection of genetically modified organisms (GMOs):

Genetically modified crops typically contain certain genes that were introduced for the development of new crops. In general, these genes express a particular promoter and a particular transcription terminator, enabling PCR primers to be designed on the basis of these sequences. Such primers can be used to test their presence.

A positive result will indicate that the plant is genetically modified or that the food contains one or many GMOs. On the other hand a negative result does not rule out the presence of a GMO, because the plant may have been genetically modified using genes with a different promoter or terminator.

(ii) Phylogenetic relationship:

Analysis and comparative study of DNA extracted from very ancient organisms, such as 40 million year old fossil leaf or 20 million year old insect in amber, or 40,000 year old mammoth with present day related organisms may be helpful to determine the actual phylogenetic relationship among present-day descendant.

(iii) Determination of variability in population or ethnic groups may be done by DNA typing.

(iv) In horses, dogs etc. pedigree status can be determined for certain breeds to prepare their breedregistration.

(v) Conservation biology may also use DNA typing to determine genetic variability amongendangered species.

(vi) Presence of pathogenic strains of bacteria like E. coli in food can be tested by DNA typing.

Plant DNA Typing Considerations:

For DNA analysis of botanical samples, many of the same considerations for human identity testing still apply. The four factors (quantity, quality, purity, and mixture ratios) may all play a role in determining whether further testing should be performed or whether an interpretable DNA profile can be obtained from a plant sample. Two factors

need to be considered for obtaining a sufficient quantity of plant DNA for profiling: size of the plant fragment and ability of the analyst to mechanically break the plant cell wall for the sufficient release of nuclear DNA contents. The size of a plant fragment can seldom be enhanced other than to initially collect as much sample as possible from the crime scene. However, new technologies are aiding in the DNA extraction process for improving DNA yield from plant samples. Traditionally, plant cells have been disrupted by the mechanical pressure of grinding by hand in a mortar and pestle with the addition of liquid nitrogen to increase the fragility of the cell wall. Some useful equipment for breaking plant cell walls may be a mechanical homogenizer (consisting of a rotating blade with a serrated tip) or the addition of metallic beads prior to high-speed oscillation of samples. In addition, several companies manufacture commercial plant DNA extraction kits that minimize the number of centrifugation steps and maximize DNA yields. The use of some of these commercial kits also seems to improve the final purity of the plant DNA so that the PCR amplification process is not inhibited by the presence of secondary plant metabolites (e.g., tannins, resins, phenolic compounds). Finally, care in collection and preservation of botanical evidence as well as the development of plant species-specific probes and PCR primer sets in the future will address mixture interpretation issues.

Preparation of Genomic DNA from Plant Tissue

Although many commercially available plant DNA extraction kits have recently become available, there are some very reliable traditional chemical extractions for plant DNA purification. The following protocol is one example of such a procedure.

Materials:

Cold, sterile water Liquid nitrogen

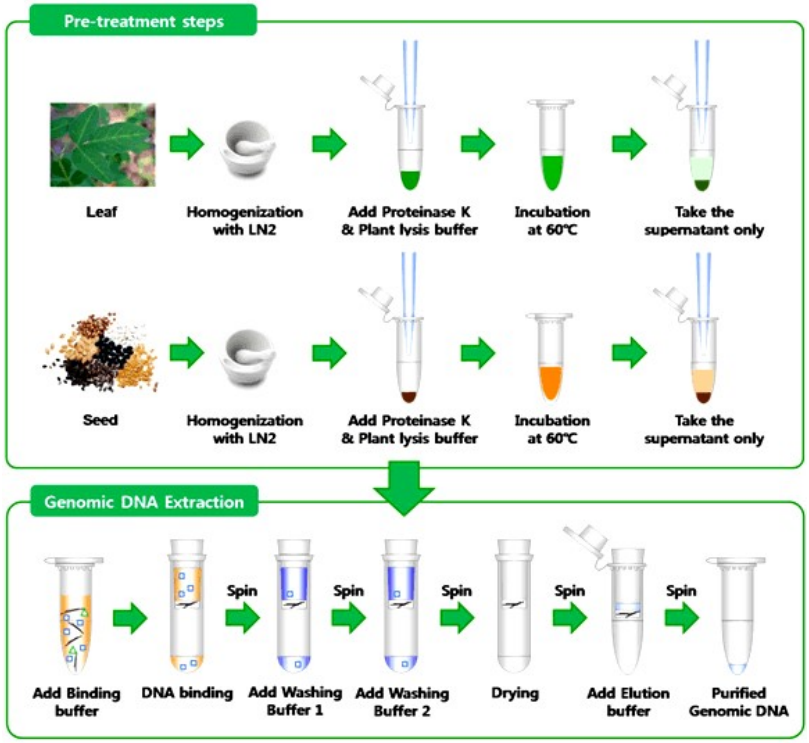
Extraction buffer (100 mM Tris-Cl, pH 8; 100 mM EDTA; 250 mM NaCl; 100 µg/mL proteinase K)10% (wt/vol) N-lauroylsarcosine Isopropanol

TE buffer (10 mM Tris-Cl, pH 8; 1 mM EDTA) Cesium chloride (CsCl)10 mg/mL ethidium bromide CsCL-saturated isopropanol Ethanol

3M sodium acetate, pH 5.2

Beckman JA-14, JA-20, JA-21, and Vti80 rotors

- Harvest 10–50 g of fresh plant tissue.
- Rinse with cold, sterile water; dry with tissues; and freeze with liquid nitrogen. Mechanically grind with a mortar and pestle.
- Transfer frozen powder to a 250-mL centrifuge bottle and immediately add 5 mL of extraction buffer per gram of starting fresh plant tissue; gentle mix. Add 10% N lauroylsarcosine to a final concentration of 1%; incubate 2 h at 55°C.
- Centrifuge for 10 min in a JA-14 rotor at 6000 rpm chilled to 4°C. Save the supernatant and repeat this step if debris is still present.
- Add 0.6 volumes of isopropanol and mix. If no visible precipitate forms, place at –20°C for 30 min. Centrifuge for 15 min in a JA-14 rotor at 8000 rpm at 4°C. Discard the supernatant.
- Resuspend the pellet in 9 mL TE buffer, add 9.7 g of solid CsCl, mix, and incubate 30 min onice. Centrifuge for 10 min in a JA-20 rotor at 8000 rpm at 4°C and save the supernatant.



- Add 0.5 mL of 10 mg/mL ethidium bromide and incubate 30 min on ice.
- Centrifuge 10 min in a JA-20 rotor at 8000 rpm at 4°C. Transfer supernatant to two 5-mL ultracentrifuge tubes and seal.
- Centrifuge in a Vti80 rotor at 20°C for 4 h at 80,000 rpm. Collect the band of DNA using a 15-G needle and syringe.
- Remove any residual ethidium bromide by repeated extraction of the DNA band with CsCl- saturated isopropanol.
- Add 2 volumes of water and 6 volumes of 100% ethanol, mix, and incubate for 1 h at – 20°C. Centrifuge for 10 min in a JA-20 or JA-21 rotor at 8000 rpm at 4°C.
- Resuspend the pellet in TE buffer and precipitate again by adding 1/10 volume of 3M sodium acetate and 2 volumes of 100% ethanol. Repeat the centrifugation step. Air-dry the pellet by inverting the tube over a paper towel for 15 min, then resuspend the pellet in the TE buffer. The expected plant DNA yield should be 104 μg of 50 Kb high molecular- weight DNA per gram of starting plant tissue.

Applications of DNA fingerprinting in forensic botany:

In theory, DNA fingerprints obtained from plant fragments should be able to provide important evidence in crime investigations but success has been limited so far, probably due to problems with isolating DNA of sufficient quality from poorly preserved plant material. SSR markers are often chosen for forensic work since they work comparably well also with heavily degraded DNA. One famous early case, however, involved RAPD analysis of seed pods of the Palo Verde tree, *Cercidium* sp., recovered both from the crime site and from the pick-up truck of a suspect, while another case made use of SSR and RAPD analysis to compare fragments from clonally reproducing bryophytes (mosses) collected both on the crime site and on the suspect himself. In subsequent experiments, a high likelihood of picking up fragments of bryophytes by walking outdoors wearing rubber boots was shown, as well as the ability to isolate DNA of sufficient quality after several months of storing bryophyte material under adverse

conditions. These facts together with the high level of clonality in many bryophyte species make them an ideal target for forensic analysis. In yet another criminal case, seedlings of the inbreeding herbaceous knotweed Polygonum aviculare obtained from germinating seeds found in the wheelhouse of a suspect's car tire, and from a large number of soil samples taken at the crime site and various reference localities, were analyzed with AFLP.

Detection of adulterations of food, drink and medicinal products is another area for forensic botany. Licensing arrangements sometimes require that a specified clone, cultivar or landrace is utilized in the manufacturing of food and beverages. Thus, well-defined grapevine clones must be used to receive "appellation d'origine controllée" labelling in France. In one study, musts (that is, freshly pressed grape juice destined for wine-making) from two different grape cultivars could be identified using two SSR markers. In another study, musts containing different proportions of two grape cultivars were analyzed with densitometry measurements of the SSR amplification products after separation and staining on polyacrylamide gels. In Greece, Nemea wines are marketed with protected denomination of origin (PDO). Instead of using only the prescribed cultivar 'Agiorgitiko', the more productive 'Cabernet Sauvignon' is sometimes added. DNA samples from fresh and fermented products, containing various mixtures of these two cultivars, were therefore subjected to a CAPS assay. Presence of the adulterant could be detected down to 10% throughout the fermentation process.

Olive oil is also often marketed with PDO labelling. RAPD, ISSR and SSR analysis of Portuguese olive oils allowed the determination of geographic origin of the cultivars on which they had been based. Similarly all 10 olive cultivars involved in samples of Italian oil samples could be identified with only one AFLP primer pair. For rice, the adulteration of the expensive Basmati rice is an important issue, not only for European and US customs but also for consumers. Basmati cultivars have often been mixed with crossbred Basmati varieties and long-grain non-Basmati varieties. Several DNA-based markers have been proposed, and some were commercialized for adulteration tests, such as the multiplexed SSR markers developed by Archak and colleagues. DNA analyses of various plant-based food products have similarly been used for authentication. The

presence of the apple 'Annurca' could thus be verified by SSR analysis in highly processed nectar and puree products. Using relativelyshort SSR target sequences (<160 bp), it was also possible to amplify genomic DNA from canned pear fruit and fruit juice while markers with longer target sequences failed.

Medicinal drugs constitute another important product area where adulterants cause major problems. Based on nine SNP sites, all populations except two could be distinguished in DNA isolated from the dried stems of the orchid Dendrobium officinale, which is a valuable source of 'Fengdou' drugs usedin traditional Chinese medicine. The latter two populations could instead be distinguished using a more complex procedure known as suppression subtraction hybridization which involves PCR amplification, differential DNA fragment cloning and sequencing. Using these protocols, origination of the plant material could be determined for 50 drug samples obtained at a commercial market. For more information on DNA marker use in medicinal plants, see the reviews by Nybom and Weising and Sarwat and colleagues.

A variety of DNA marker methods have been used to demonstrate infringement of Plant Breeder's Rights, either in court or, in our experience much more common, leading to a settlement outside of court. A related field concerns the identification of plants, the possession of which is considered illegal. Thus several studies have been published on the identification of Cannabis sativa specimens as part of drug enforcement. In one approach, 15 SSR loci were combined into a single multiplex to enable fast and user-friendly discrimination between Cannabis genotypes. One of the detected genotypes, however, proved to be very common in police seizure-derived evidence material, suggesting that many illicit growers had access to the same clone. This clonal propagation of course makes it difficult to determine the origination of a particular batch. A related DNA marker application concerns violation of trade restrictions. A special situation is encountered when products from protected trees are involved since woody tissue usually yields heavily degraded DNA. Nevertheless, a set of SNP markers derived from cpDNA intergenic spacers have proven useful for identification of tropical tree species using wood-derived DNA samples.

7. Drug Enforcement: Botanical contributions to drug enforcement.

Often in drug seizures, identification of the seized substance is a problem, especially if the plant material is fragmented and dried. A variety of methods are currently employed to identify *Cannabis sativa* L (marijuana).

- Marijuana can be identified by classical botanical characterization, especially if the type of cystolith found in an individual's vehicle back to a plant from a growing area near the suspect's home, for example.
- The Connecticut State Forensic Science Laboratory is developing a molecular strategy for creating unique band patterns from marijuana samples, which uses a technique called amplified fragment length polymorphism (AFLP) analysis.

AFLP analysis is based on the selective PCR amplification of restriction fragments from a total digest of plant DNA to generate a fluorescent band pattern. Validation of the AFLP technique on marijuana samples and the construction of a marijuana AFLP database for comparative purposes were developed at the Connecticut State Forensic Science Laboratory.

Cannabis has special trichomes. The resin within these trichomes has encouraged our ancestors to work with this plant for millennia, spreading the seeds world wide. *Cannabis* resin contains strong smelling medicinal compounds that reflect in the sun to call extra attention. Trichomes, the resin gland heads, protect the cannabis plant from insects and predation by being sticky and intoxicating. Trichomes also protect growing seeds from the sun and wind by reflecting solar radiation and creatinga physical barrier.

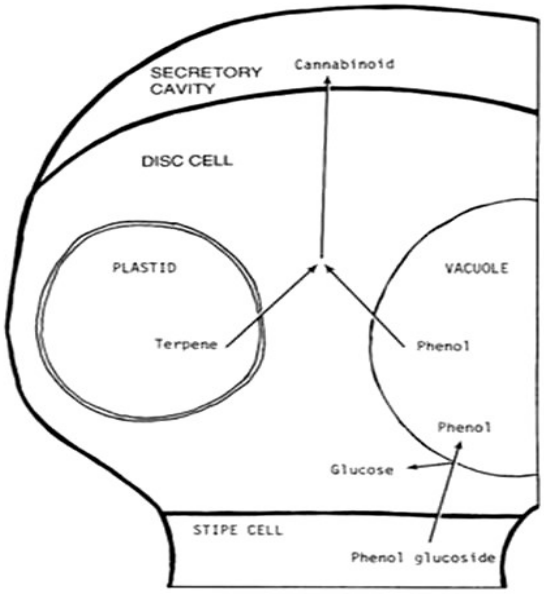
The Cannabinoids and Terpenoids in Resin:

Cannabis resin includes: cannabinoids (THC, CBD, CBN, THC-V, over 90 discovered so far!), terpenoids (smell and "high" modulation), and plant waxes/oils. One group of cannabinoids, the THC group, is primarily responsible for the psychoactive properties of cannabis.

Forensic botany uses plant DNA to trace crimesDate: February 29, 2016

Source: Sam Houston State University Summary: The field of forensic botany is being advanced with the publication of two recent studies that use marijuana DNA to link drug supplies and pollen DNA to aid in forensic investigations.

Publication in International Journal ofLegal Medicine



A test was developed to individualize samplesof marijuana that could be used to link drugs across cases. The study examined 11 cases containing a total of 199 samples from U.S. Customs and Border Protection, which included four matching genotypes indicating drugs came from the same source.

"The use of a DNA-based method for identification will allow federal law enforcement agencies (e.g., U.S. Customs and Border Protection (CBP) and Drug Enforcement Administration (DEA)) to form links between cases involving the cross-border trafficking of *Cannabis*," said Dr. David Gangitano, one of the authors on the study.

Publication in Science and Justice

Researchers found that pine pollen could provide a viable source of DNA for criminal investigations. Pine pollen remains viable for DNA testing for at least two weeks on cotton clothing and can help linka suspect or victim to a location. The study examined a new collection device, a high-throughput method for DNA extraction and amplification, and a newly-developed system for genotyping.

This study has shown that pollen can be a stable source of forensic DNA evidence, as a proof-of- principle, and that may persist on cotton clothing for at least 14 days of wear. This method can be applied in forensic cases where pollen grains larger than 10 µm

(e.g., from herbs or trees) may be transferred to clothing (worn by suspect or victim) by primary contact.

8. Classic Forensic Botany Cases: Famous case histories by using different botanical evidences.

1. A Case Study (*Using Plant Anatomy and biosystematics*):

The kidnapping and death of Charles Lindbergh's young son in 1932 was the first modern-era case to use such botanical evidence in court. A wooden ladder was used to gain access to the second-story nursery to kidnap Lindbergh's son.

Arthur Koehler, a wood identification expert for the Forest Products Laboratory of the U.S. Forest Service in Wisconsin, was able to provide critical evidence against Bruno Richard Hauptman, who was later convicted of the crime.

Koehler had an excellent academic record and had provided evidence in several cases prior to the famous Lindbergh trial. His testimony is noteworthy since the use of scientific experts in the mid- 1930s was generally limited to fingerprints, handwriting, bullet comparisons, and analyses of stomach contents. Koehler first identified the four tree species used to construct the ladder as yellow pine, ponderosa pine, Douglas fir, and birch, via microscopic analysis of wood-grain patterns. Next, Koehler analyzed the tool marks left on the wood from both the commercial planing mill and the hand plane used by Hauptman during the construction of the ladder. Koehler used oblique light in a darkened room to observe the plane patterns left on the wood. Amazingly, he was able to trace the wood by the mill plane marks to a shipment of yellow pine delivered to the National Lumber and Millwork Company in Bronx, New York. The hand-plane marks on the ladder exactly matched those made by a hand plane found in Hauptman's possession. Finally, Koehler compared the annual growth rings and knot patterns on

rail 16 of the ladder to a section of wood in Hauptman's attic. The patternof knots and growth rings on rail 16 exactly matched the exposed end of wood.
All these exactly matched and thus identified the original criminal.

- **Wood features in determining Art Fraud:**

Tree ring analysis (Dendrochronology) is a common technique for dating masterworks by Europeanpainters, many of which were painted directly on wood.
Given that the samples are in good condition, analysts can pinpoint the exact year when the tree, fromwhich the wood for the painting was taken, was cut down.

2. A Case Study (Using Dendrochronology techniques):

A Peter Paul Reubens painting originally dated 1616 was shown to be at least 10 years younger, and a painted wall panel recovered from a house in Switzerland in the 1970s was determined to have been painted on spruce harvested in 1497 (Schweingruber, 1988).

Dendrochronology techniques are useful in determining the provenance of wooden art objects and musical instruments

In one case, two violins forming part of an inheritance were claimed to have been made by AntonioStradivari.
The sounding-boards of the instruments were x-rayed and compared to standard curves for sprucefrom the Alpine region of northern Italy, where Stradivarius is known to have worked.
The oldest rings from the samples dated to 1902 and 1894 respectively for the two violins. Furthermore, these oldest rings were not the outermost rings of the wood from which the violins wereconstructed.
Allowing for a period of seasoning before the wood could be used to make the instruments, analysesshowed that the violins could not have been made before 1910.

Given that Stradivari did his best work at the turn of the 17th century, the instruments weredeemed to be fakes (Schweingruber, 1988).

The Kidnapping of Charles Lindbergh, Jr. (March 01, 1932)

A critical piece of evidence in the case was a crude homemade woodenladder left at the

scene. Xylotomist Arthur Koehler of the United States Forest Service undertook a meticulous examination of the ladder and when the case finally came to trial four years later, offered the first botanical testimony ever to be heard and accepted in American courts.

The ladder had been constructed in three sections, presumably for ease of transport Koehler identified each side rail and rung with a number and identified each piece to species. Through careful examination of the characteristic milling marks left on each piece and comparisons with local mills, he was able to trace all components of the ladder back totheir respective retail sources.

He also noted distinctive marks left on the wood by a dull, nicked hand plane. Of particular interest was rail #16, a piece of low-grade pine which had four distinctive square nail holes. It was also relatively unweathered.

The low grade of the wood, the nail holes, and its unweathered condition suggested that particular piece of wood had been removed from some interior construction.

Without a suspect however, progress on the case was slow. In September of 1934, some of the notes used to pay the ransom were used at a gas station by Bruno

Hauptmann, a carpenter who lived in the Bronx, New York City. He was arrested when $14 600 of the ransom money was found in his garage. Upon searching the attic for more ransom money, police noticed that one of the floorboards was eightfeet shorter than the others. The square nail holes in rail 16 lined up exactly with holes in one of the attic floor beams, and the annual ring pattern of rail 16 matched that of the short floorboard. A hand plane recovered from Hauptmann's garage was indeed dull and damaged, and made marks identical to those on the ladder and on a homemade shelf in the Hauptmann garage.

Hauptmann was convicted of kidnapping and murder and was executed on April 3rd, 1936.

1. A Case Study (*Using Limnology*):

In a study of 771 cases, the diatom test was positive for 28% of presumed freshwater drowning cases but was rarely positive for domestic water drowning. The low rate of

diatoms observed in domestic drowning could be traced back to cleaning agents containing crushed diatoms for abrasives.

In 1991, two young boys were brutally attacked by teenage assailants while fishing at a suburban pond in Connecticut. The boys were held at knifepoint, bound with duct tape, and savagely beaten anddragged into the pond to drown. One boy managed to get free, save himself, and rescue his friend. After many hours of criminal investigation, three suspects were apprehended. To link the suspects to the crime scene, investigators seized the sedimentencrusted sneakers of both the victims and the assailants and analyzed them for algal and diatom species. microscopic analysis of samples from each pair of sneakers plus reference samples from the pond showed the same species and distribution pattern of each species. These results supported the position that the samples all originated from a common freshwater location.

1. A Case Study (*Using Palynology*):

Pollen analysis consists of species identification and an estimation of the percentage that each plant species represents in an evidentiary sample. A similar pollen composition from shoeprints and from the shoes that made the prints indicates a strong match correlation.

Pollen evidence collected from a burglary entrance and a suspect's shoes, for example, could provide a linkage in a case. A case that exemplifies the use of pollen in criminal casework is described by Horrocks et al. In Auckland, New Zealand, a prostitute alleged that the defendant had raped her in an alleyway approximately seven meters from his car after failing to pay her in advance for her services. The defendant claimed that he had never been more than one meter away from the car and had not entered the alleyway. Furthermore, he claimed that he had not had sex with the victim and the soil on his clothing was from the driveway area. An examination of the crime scene and the evidence showed no footprints and no seminal fluid stains. A soil sample was collected from the defendant's clothing, the disturbed area of ground in the alleyway, and from the driveway area near the defendant's car. All the soil samples were prepared for pollen analysis by deflocculation with potassium hydroxide, acetylation to remove cellulose and organic matter, and a silicate removal step using hydrofluoric acid.

Samples were bleached to remove additional organic matter and analyzed under a microscope for pollen identification and counting. The types of pollens were similar between the two locations,but the amounts of each type were different in each sample. The alleyway contained 76% *Coprosma* (an evergreen shrub) pollen, but the driveway sample contained only 8%.The defendant's clothing contained approximately 80% *Coprosma* and only small amounts of other pollen species. These results support the victim's account of the sexual assault taking place in the alleyway. Pollen analysis has also been utilized to establish time of death.

In Magdeburg, Germany, a mass grave containing 32 male skeletons was discovered in February of 1994. The identities of both the victims and the murderers was unknown. Two hypotheses were proposed: (1) the victims were killed in the spring of 1945 by the Gestapo at the end of World War II, or (2) the victims were Soviet soldiers killed by the secret police after the German Democratic Republic revolt in June of 1953. The ability to differentiate between the spring and summer was critical to solving the case. Pollen analysis was performed on 21 skulls. Seven of the skull nasal cavities contained high amounts of pollen from plantain, lime tree, and rye. All of these plant species release pollen during the months of June and July. Pollen analysis supported the hypothesis that the remains were of Soviet soldiers killed by the Soviet secret police after the June 1953 revolt.

2. A Case Study (*Using Palynology*):

Srebrenica massacre or Srebrenica genocide (part of the Bosnian War)

Some of the more than 6,100 gravestones at the Srebrenica-Potočari Memorial and Cemetery for the Victims of the 1995 massacre	
Location	**Srebrenica, Bosnia and Herzegovina**
Date	**11–22 July 1995**
Target	**Bosniak men and boys**
Attack type	**Military assault, mass murder, ethnic cleansing, genocide**
Deaths	**8,373**
Perpetrators	**Army of Republika Srpska Scorpions paramilitary group**

In July 1995 a massacre of civilians followed the burial in seven mass graves. Three months later thebodies were exhumed and transported to a number of new burial sites in an attempt to conceal evidence of the massacre and to deflect blame.

Could palynology help in relating the secondary burial sites with the original primary burial sites and thereby more closely link the massacre to known or suspected perpetrators?

Re-exhumation was commenced by the United Nations International Criminal Tribunal for theformer Yugoslavia (ICTY) in 1997.

The objective of the palynological and associated soil analyses was to determine the environmentalprofile of the original burial sites and to try and find a connection with

the secondary sites wheredifferent environmental profiles existed. These analyses were done independently of all other forensic investigations being undertaken at the same time to ensure credibility. Five of the original sites and 19 secondary sites were investigated in detail. Analyses indicated that the original mass graves each had a different geological and botanical profile which easily separated each site. Samples from all sites were taken from the fill of the graves close to and from varying distances away from bodies or bodyparts and from sediment surrounding the mass graves. Over 240 comparator samples were collected from various sources to determine the background pollen profile of each site, the local vegetation wasrecorded and abundance of major species determined. Results showed that sediments and associatedspores and pollen from the original mass graves had indeed been transferred along with the bodies tothe numerous secondary burial sites and that even some botanical evidence at the primary burial sites. pointed to the original execution site or sites. Pollen found at the original burial sites consisted of cultivated grasses (cereals including wheat and maize), wild grasses (Poaceae), pines (Pinus), spruces (Picea), sedges (Cyperaceae), beeches (Fagus) and walnut (Juglans). Various combinations of thesepollen types, plus many others, were subsequently found in exotic material sampled from within the graves at the secondary burial sites, proving a link between the original and subsequent burial sites. The accuracy of the evidence provided by the pollen was confirmed by other types of forensic evidence and presented in court. The investigation showed the importance of being able to differentiate between imported and local fill used at grave scenes. This was probably the first time that environmental profiling was used systematically in a war crimes investigation.

3. A Case Study (*Using Palynology*):

"Murder on the Danube" case

The first time police used pollen to solve a crime was in Austria in 1959. A forensic scientist studyingthe mud on a murder suspect's boot found what turned out to be a 20-million-year-old pollen grain from a hickory tree. That species no longer grew in Austria then. But investigators were able to locate a Miocene sediment outcrop on the Danube

River, from which such a pollen grain could have becomerecycled into the environment. "We know you killed him," they told the murder suspect, in the best police procedural fashion, "andwe know where." Then they took him to the outcrop. The suspect was so unnerved that he led them straight to the victim's grave.

4. A Case Study (*Using Palynology*):

Here some examples are given in assessing an alibi by the use of forensic palynology.

A man was found shot in the back on Mount Holdsworth in the Tararua Ranges north of Wellington, the capital city of New Zealand. Police investigations pinpointed one individual who had been seen in the area, knew, and had the means and motive to kill the victim. His alibi was that an eyewitness was mistaken as he never had been in the area and the jacket he was reported to have been wearing had been purchased in The Netherlands and brought to Wellington, where it never had left the city. Furthermore the distinctive board shorts that he was reported to have been wearing had been purchased in a small coastal New Zealand town after the victim had been murdered. Pollen of *Nothofagus menziesii*, a mountain plant, on the clothing suggested that the alibi was untrue and that the clothing had been in mountains in the vicinity of Mount Holdsworth or a similar mountain scene where *Nothofagus menziesii* was growing.

Example:

Part I: Two male intruders entered a house in which the sole female occupant slept having left the back door unlocked for the return of her husband.

She awoke and saw strangers in her bedroom.

The intruders ran off, one leaving a jacket behind on the kitchen floor.

One of the intruders subsequently returned to recover his jacket, but in his rush to leave the house he brushed against a flowering *Hypericum* bush growing just outside the back door.

A suspect was arrested later that day and charged with indecent assault on a female and burglary, but denied any involvement and refused to name any associate.

Part II: A day following the offence the suspect's clothes were taken for forensic examination.

Pollen analysis of selected parts of his clothing showed that his track pants contained 14% *Hypericum* pollen, denim jacket 24%, and polo shirt 27.5%.

Traces of *Hypericum* pollen occurred on other items. Most of these pollen grains still had their cell contents preserved and were on the clothing in clumps consistent with having recently been collected by the clothing and not having been aerially dispersed.

The pollen from the *Hypericum* bush was identical in colour, shape, development, and size range tothe pollen from the clothing.

The clothes had so much *Hypericum* pollen on them that they had to have been in direct and intimate contact with a flowering bush.

Part III: The suspect may have been in contact with *Hypericum* elsewhere, but detailed investigationsindicated that this was unlikely.

This is but one way in which forensic palynology can assist law enforcement agencies to determine the history behind a criminal action, and demonstrates that forensic palynology should be considered as an integral part of any criminal investigation.

Pollen evidence is by its nature circumstantial and often cannot be used on its own to convict, ormore strictly to determine the truth.

1. A Case Study (*Using Plant Molecular Biology & DNA*):

In the age of DNA analysis, forensic botany is using molecular biology to aid in criminal and civil investigations.

The first criminal case to gain legal acceptance using plant DNA typing was a homicide that occurred in 1992 in Arizona's Maricopa County. A woman's body was found under a paloverde tree in the Arizona desert. Near the body was a beeper eventually traced to a suspect, Mark Bogan. A few seed pods from a paloverde tree were found in the back of Bogan's truck. Officials wanted to know if DNA could match those seed pods to the tree where the body was discovered. Dr. Timothy Helentjaris from the University of Arizona used a technique called randomly amplified polymorphic DNA (RAPD) analysis to generate a band pattern from the evidence in question. He also surveyed a small

population of other paloverde trees to determine if the band patterns were unique to each individual. His convincing testimony on plant evidence helped convict Mark Bogan of murder. RAPD marker analysis has also been utilized in civil court cases to identify patent infringements.

9. Let's sum up

- Forensic botany is a marriage of many disciplines and results ultimately in their application to matters of law. The botanical aspects of forensic botany include plant anatomy, plant growth and behavior, plant reproductive cycles and population dynamics, and plant classification schemes for species identification.
- Forensics requires recognition of pertinent evidence at a crime scene, appropriate collection and preservation of evidentiary material, maintenance of a chain of custody, an understandingof scientific testing methods, validation of new forensic techniques, and admissibility criteria for court.
- Plant evidence is long lasting, which means that plant parts to remain identifiable for very long periods of time. Plant cell wall is made of some chemical compounds which are nearly indestructible and do not decay quickly. Pollen grains and spores also have walls that are made of decay resistant material-sporopollenin.
- The collection of plant fragments, seeds, flowers, and fruits should all be performed by hand. Whole plants and any fragments that may potentially be useful for a physical match should be collected as well as any pieces associated with a body. Botanical fragments in and on motor vehicles should be collected; in particular, the wheel wells, in and under floor mats, the undercarriage, pedals, windshield wipers, vents, trunk, and engine compartments should be fully

examined.

- Outdoor crime scenes also warrant a special note. Investigator and technicians are often working under greater time pressure because, given certain weather conditions, some of the physical evidence can be altered or destroyed.
- All trace evidence, including blood and body fluids, prints, soil, hairs and fibers, pollen, plant fragments, and stains, should be photographed in place prior to collection and packaging. Body orifices should be examined for semen and other body fluids, hairs and fibers, and other trace materials. It is important to collect the evidence when possible prior to moving the body.
- Cell shape and orientation of certain structures within a cell can be helpful in classification of a species. In order to learn about plant anatomy and specific plant structures within the plant body plan, it is important to take a practical approach.
- Plant roots, like their above-ground counterparts, exhibit annual growth rings that can be useful in pinning down the post-mortem interval, or at least the time since the body came to be at the location where it was found.
- Characteristic cell types from food plants can be used to identify a victim's last meal. Knowledge about which can be useful in determining the victim's whereabouts or actions prior to death. Some of these cell types include: sclereids, starch grains, raphide crystals, ruse crystals and silica bodies.
- Pollen fingerprint is the number and type of pollen grains found in a geographic area at a particular time of year. Four (4) essential parts-number of pollen grains, type of pollen grains, found in a certain area and at a particular time.
- Many different types of biological evidence are commonly submitted to forensic science laboratories for examination. Initially, evidence that was suitable for DNA analysis was limited to human biological substances that contain nucleated cells. This limitation has been overcome in the last 5 years with the implementation of mitochondrial DNA sequencing, and plant and animal DNA testing in the forensic arena.
- Plant ecology involves studying the growth patterns of vegetation in areas that

have been disturbed. These patterns and the vegetative (non-flowering) portion of plants can be useful inestimating time of death.

- Limnology is the study of freshwater ecology and can be applied to a subset of forensic cases.In particular, aquatic plants (e.g., algae, diatoms) have been useful to link suspects to a crime scene or to establish that drowning occurred in freshwater.

10. Suggested Readings

1. Coyle. H. M. Forensic_Botany-Principles and Applications to Criminal Casework CRCPRESS Boca Raton London New York Washington, D.C.
2. https://en.wikipedia.org/
3. http://www.notesonzoology.com/dna/dna-fingerprinting/

11. Assignments

1. What is 'touch' DNA?
2. What do you meant by pollen fingerprinting?
3. Describe the application anatomical samples in foresic botany.
4. Write a short note on outdoor crime scene.
5. Differentiate between civil and criminal law.
6. Write one famous case study solved through use of plant anatomical evidences.

7. How Drug enforcement help to solve forensic cases?
8. What is typing? Give an overview of DNA typing.
9. Mention the role of pollen in case study. What are the advantages?
10. How palynological evidences helps to solve criminal cases?
11. What is Limnology?
12. Name two DNA markers and mention its role in forensic botany.
13. How Diatoms help to solve forensic cases?
14. Distinguish ring porous wood and diffuse porous wood.
15. Briefly describe different steps of DNA fingerprinting with suitable diagram
16. What botanical evidence can do? How botanical samples are collected?

All the materials are self writing and collected from ebook, journals and websites.