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## Abstract

The field of forensic biology is an ever-evolving and constantly developing field. It utilizes knowledge of biological concepts and practical approaches that assists in a legal investigation. Forensic biology serves as a significant discipline dealing with multifarious sub-disciplines such as forensic genetics, forensic serology, forensic anthropology, forensic botany, forensic entomology, forensic microbiology, etc. For examination of different biological evidences, bodily fluids, and cellular components related to humans, animals plant or micro-organisms that are encountered at the crime scene or are relatable to the concerned crime. From the development of anthropometrical science in the 1870s by Alphonse Bertillon for personal identification to the currently emerging and developing field of DNA fingerprinting and microbial patterns, forensic biology has provided novel approaches and improved methodology for collection, preservation and analysis of compromised evidences encountered at the crime scene. This chapter deals with the basic introduction of various sub-branches of forensic biology and their utilization in the field of forensic science. The chapter also describes various bodily substances such as hairs, nails, seminal fluid, teeth and botanical evidences such as wood, leaves, pollens, etc., that are generally encountered at the crime scene along with the techniques of their identification and segregation and

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application in the forensic context. A detailed approach of wildlife forensics, forensic entomology, forensic limnology and forensic mycology towards assistance in the criminal investigation has been elucidated in this chapter.

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**Keywords**

Forensic palynology · Forensic entomology · Forensic dendrochronology · Forensic limnology · Forensic mycology · Wildlife forensics

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**17.1 Introduction**

Forensic biology deals with the application of biology, various concepts and procedures involved in biological science in the law enforcement system. It is the outspreaded discipline of forensic science involving the examination of humans, animals and plants in a legal context with diverse sub-disciplines that include forensic anthropology, forensic serology, wildlife forensic, forensic botany, forensic entomology, and forensic mycology. Examination of materials like wood, diatoms, hair, nails, teeth, leaves, seeds, pollen and other biological evidences is done to link the crime scene to the victim and the assailant as well as to establish the identity also.

Human identification is one of the major demands resolved by the application of forensic biology. Anatomical, morphological, biochemical as well as molecular examination of various ranges of biological evidences serve the purpose. Besides, utilization of botanical and zoological evidences can help in linking a suspect to a victim or scene of crime. In addition to this, botanical and zoological evidences can also assist in solving wildlife crimes such as poaching, hunting, and illegal logging of timber. Entomological as well as mycological evidences can provide significant information related to a cadaver such as time since death, cause of death as well as the location of crime.

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**17.2 Hair**

Hair is ubiquitous evidence that can be found at any crime scene in the form of shed hair or cut hair. Considered to be the defining feature for characterizing mammals, hair can be a useful evidence for developing a link between the victim, suspect and scene of crime following the Locard's principle of exchange. Hair evidences is generally encountered in cases involving vigorous combative contact between individuals, as in cases of homicide, sexual assault, etc. The basic process of analysis of hair is depended on hair growth, hair types, and care. The human hair is a vital biomaterial, that principally grows as follicles in the dermis comprises protein, particularly keratin. Hair properties vary broadly across different geographical locations and historical eras and thereby can be useful for the identification of a person's age, gender or ethnicity.

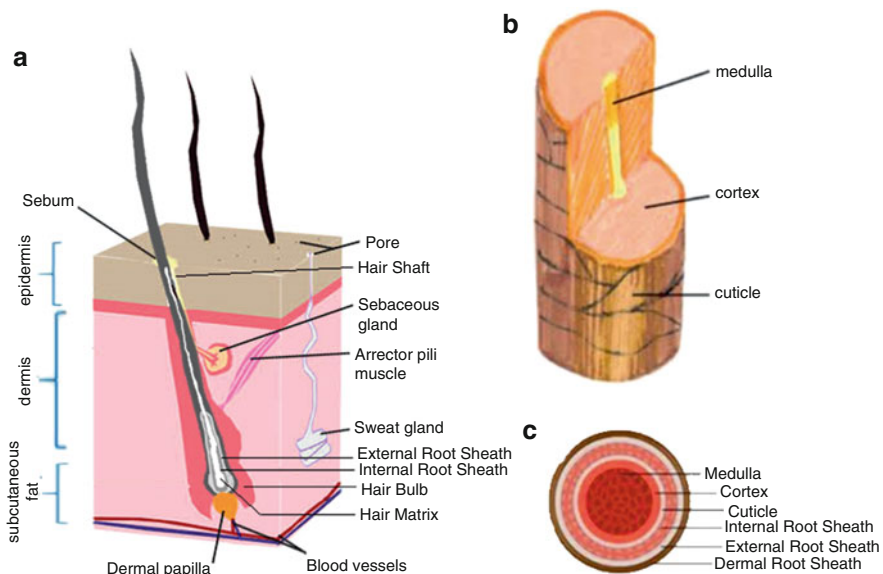
### 17.2.1 Structure of Hair Follicle

Hair follicle is a miniorgan found in mammalian skin located above the dermal layer of skin. It primarily functions in the differentiation of hair cells, growth of hair shaft and anchoring of hair shaft into the skin (Robertson 1999; Erdoğan 2017).

Development of hair follicle from the embryonic epidermis occurs in the form of an epithelial finger-like structure consisting of several cylindrical concentric cell layers (Fig. 17.1). The outermost cylindrical layer or **outer root sheath (ORS)** surrounds other cellular structure and separates them from the dermis. The middle cylindrical layer or **inner root sheath (IRS)** encases the hair shaft and directs its outward pathways. Innermost layer, the **hair shaft** has a complex arrangement containing various layers. Outermost layer is the cuticle, which comprises of layers of flat, thin overlapping one another as roof shingles followed by the middle region of cortex, which contains the keratin bundles in roughly rod like and cell structures and inner most layer of medulla that is unsystematic and exposed area in the fiber's center (Stenn and Paus 2001; Buffoli et al. 2014).

#### 17.2.1.1 Cuticle

The outer layer of the human hair shaft is called cuticle. It consists of flattened, imbricated, translucent scales, laid one over the other with the open end pointing towards the end of the hair. About fifth-sixths of each scale is covered by the attached scale (Ryder 1963). The pattern of cuticle arrangements, also known as scale patterns can be examined microscopically and are useful in species



**Fig. 17.1** Anatomy of anagen hair follicle (a) a cross-sectional anatomy of skin showing the parts of hair follicle; (b) longitudinal anatomy of hair shaft; (c) cross-sectional anatomy of hair shaft

identifications. Three categories of scale pattern include coronal, spinous and imbricate.

### **17.2.1.2 Cortex**

Cortex is a hollow cylindrical composed of fine fibers of protein material composed of spindle-shaped cells aligned parallel to the axis of hair shaft forming cell membrane complex (CMC). CMCs are responsible for tensile strength of hair and also carry pigment material. The cortical cells also contain keratin micro-fibrils embedded in the sulfur-rich proteinous matrix that provides color to the hairs. Hair cortex also contains melanocytes that produces melanin—a pigment also present in skin and is responsible for providing hair color. The dispersal pattern of melanocyte is another characterizing feature useful in species identification. In humans, the pigments are dispersed more towards the cuticle while in animals they are dispersed more towards the medulla.

### **17.2.1.3 Medulla**

Medulla is the innermost pith of hair shaft that originates lesser or more near the root. Its proteinous matrix differs from cortex and cuticle as it contains granules filled with an amino acid—citrulline. Cellular components of medulla are dispersed in a manner that it appears as a narrow canal with spaces filled with air. The pattern of cellular distribution in medulla are characterizing features in certain species, occupying more than half of hair's diameter, thereby are indicative of species origin (Saferstein 2007). The ratio of diameter of medulla to the diameter of hair shaft is termed as medullary index. In many animals, the medulla is very broad, occupying more than one-third of the shaft's diameter. In human medullary index is generally less than one-third (Zafarina and Panneerchelvam 2009). On the basis of level of modulation, human hair can be classified as no medulla or if present, continuous, interrupted and fragmented.

Arrector pili muscles are the bands of smooth muscle that connects the shaft with the dermis and aids in thermal regulation. Besides, one or more sebaceous glands are also associated with the hair shaft and secrete an oily substance called sebum that helps in lubrication of hair and skin.

## **17.2.2 Morphology of Hairs**

Hair can be differentiated on the basis of color, length, structure, bodily source of origin. Dyed or bleached hair may delude the examination, therefore proper cleaning of hair evidence is a major prerequisite prior to the examination. Also, the root portion of hair must be examined in such cases as roots retain the natural color of hair. Morphological characteristics of hairs such as medullary index, scale pattern, pigment distribution pattern can be employed in the examination. Characteristics features of hair that can be used for identification are tabulated below (Table 17.1):

**Table 17.1** Various characteristic features of hairs

S no	Characteristics	Types of hairs
1	Color	White, blonde, light brown, gray brown, dark brown, grey, black, auburn, red.
2	Length	Fragment, 1", 1–3", 3–5", 5–8", 8–12", 12–18", 18–30", segment.
3	Spatial configuration	Undulating, kinky, curly, wavy, curved, straight, sinuous.
4	Site of location	Scalp, pubic, vulvar, anal, chest, beard, axillary, eyebrow, eyelash, limb, ear, nose.
5	Tip	Singed, uncut, tapered, rounded, sharp cut, cut at angle, frayed, split, crushed, broken.
6	Root	Stretched, absent, bulbous, sheathed, atrophied, follicular, wrenched.
7	Pigment	Absent, non-granular, granular, multicolor, chain, massive (clumped), dense, streaked, opaque.
8	Medulla	Absent, sparse, scanty, fractional, broken, globular continuous, irregular, double, cellular.
9	Cosmetic treatment	Bleached, rinsed, natural, dyed, damaged.
10	Cuticle	Ragged, serrated, looped, narrow, layered, wide, cracked, absent, clear, dyed.
11	Scales	Flattened, smooth, level, arched, prominent, and serrated.

### 17.2.3 Human Hairs vs. Non-human Hairs

Differentiation of human hair and non-human hair is significant in forensic caseworks. Variations in characters of the above-mentioned anatomical regions of hairs such as scale patterns and medullary index are useful in distinguishing human and non-human hairs. Macroscopic as well as microscopic examination of fine details of hair structure is essential for accurate comparison (Zafarina and Panneerchelvam 2009). Hair-based difference between humans and animals are described in Table 17.2.

Non-human hairs have primary function of thermal regulation. Three types of non-human hairs can be observed namely—vibrissae, bristle and wool. Vibrissae are the long, coarse hairs present in the muzzle area of animals and helps in sensation. Bristles are the short coarse hairs that act as guard hairs. Color of bristle hair is an important criterion for species identification. Wools are the fine hairs that aids in insulation.

Human hairs grow throughout the body except mucus membranes and glabrous skin such as lips palms, feet, labia minora and penis. The human hair is of four types—primordial, lanugo, vellus and terminal. Primordial hairs are formed in the beginning of 3 months after conception. Lanugo hairs are the fetal hairs generally fine and soft in nature and are shed before birth. Vellus hairs are short and fine hair covering entire body except the palms and soles of feet. Terminal hairs are longer and thicker, sex-limited hairs that replaces the vellus hairs. These are produced by hair follicles with sebaceous glands (Robertson 1999).

**Table 17.2** Microscopic difference between human and animal hair

Characteristic	Human hair	Animal hair
Hair length	Longer	Generally shorter
Hair color distribution	Consistent throughout the shaft	Shows banding, a radical color change at shorter distance
Cuticle	Scale is small, flattened or imbricate and surrounds the shaft uniformly	Scales are large, polyhedral, wavy and their structure varies throughout the shaft
Medulla	Narrow, may be absent, fragmented, amorphous or discontinuous	Broad, always present and continuous with defined structure
Cortex	Thick, 4–10 times broad as medulla	Thin, rarely more than twice the breadth of medulla
Medullary index	Less than 0.3	More than 0.5
Pigment	More towards the periphery of cortex	Uniform, peripheral or central

Hair shape is one of the characterizing features that helps in determining bodily origin as well as aids in racial identification. Human hairs generally lack medulla, and if present, they are of fragmented types. Mongoloids race exceptionally have scalp hairs with continuous medulla. Mongoloids have auburn hairs with thick cuticle and round cross-section. Caucasoids have hairs with oval cross-section, evenly distributed pigmentation and moderate cuticle. Negroids hairs are dense and clumped with flat cross-section and extremely thin or no cuticle.

Hairs can also be distinguished on the basis of their body origin. Scalp hairs are generally long and soft textured with thin medulla as compared to hairs from other body parts. Medulla may range from being continuous to absent. Pubic hairs are coarser hairs with stiff wiry or buckled texture. The medulla if present is broad and continuous. Axillary hairs have appearance similar to pubic hairs with less extent of buckling. Limb hairs appear in arc-like shape. They have fine diameter with soft texture and have wide medulla with granular appearance. Beard or moustache hairs have triangular cross-section with wide and continuous medulla.

Besides species identification, medullary index can be employed for gender differentiation. Medullary index varies somewhat in male and female hairs and in the hairs from different parts of the body. Ordinarily, the medullary index is greater in woman when hairs from the corresponding parts of the body are compared. The male beard hairs have greater medullary index than hairs from other parts (Srettabunjong et al. 2016).

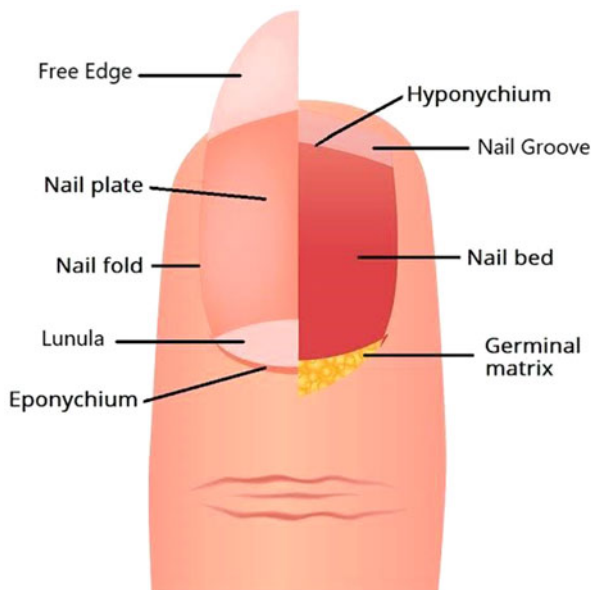
Biological examination of hair includes techniques such as scale casting, cross-sectioning and micrometrical analysis such as determination of scale count, scale count index, medullary index, hair index etc. Besides these, molecular examination of hair can be useful in sex-determination and individualization of the hair. Hairs in the anagen phase that have sheath cells are most preferable material for molecular examination. Mitochondrial DNA analysis is the most approachable type of DNA

typing technique. Elemental analysis of hair using techniques such as emission spectroscopy (ES), X-ray fluorescence (XRF), energy dispersive X-ray microanalysis (EDX), inductively coupled plasma arc mass spectrometry (ICP-MS), and the nuclear-based analyses of neutron activation analysis (NAA) are informative regarding presence of atypical elements or abnormal concentration of any common element in cases of deliberate poisoning or intake from polluted environment.

### 17.3 Nails

Nail as physical evidence is easily encountered in homicide and sexual assault cases and can aid in linking the criminal with victim and the crime scene. In cases of assaults, if the victim scratches the perpetrator, cells (skin) or hairs can be trapped under fingernails. Then during examination, the accused can be linked to crime. The study about fingernails and toenails is known as ‘Onychology’ (Onuks-nails and Logia-study). A nail is envelope like tough keratinized covering that protects the fingertips and the adjacent tissues from injuries. The epithelial matrix beneath the nail plate cells aids in the formation of a nail by pushing older nail plate cells forward. The only living part of the nail lies at the proximal end below the epidermis. White crescent-shaped lanula can be seen clearly in the thumb while it may not be visible in the little finger. A healthy nail generally gives slight pinkish hard but flexible smooth, shiny appearance and unspotted without any ridges, pits or splits. The average growth rate of nails is 3 mm in a month and is more in children than adults (Devi and Banu 2015; Kumar et al. 2017) (Fig. 17.2).

**Fig. 17.2** Structural representation of human nail



The major advantage of this evidence is that it is unaffected of most of the external conditions (environmental conditions) and unlike other biological evidence, does not break down easily and can remain at the crime scene for longer time even at a decomposed environment. In addition it is relatively unnoticeable to the untrained eye; therefore a criminal is not likely to make special efforts to destroy the nail evidence, as it is not known to many people that this small nail clipping can also play important role in linking the criminal to either to crime scene or a victim. Its successful evaluation can help in providing useful information in individualization and personal identification. Thus taking this point as a major advantage of using nails for identification purpose in contrast to other body tissues is that the sample size does not matter and sample processing is generally non-destructive and non-invasive and yet each nail retains a discrete record of detailed information on genetic inheritance and individualization. Also, in contrast to other tissues like bones, nails can be easily decontaminated from effect of external environment. The important thing to be kept in mind is that they must be collected carefully in a container (clear micro-centrifuge tube or similar) where dislodged exogenous material would not be lost and medium size clippings should be used. The evidence should be dry in order to prevent degradation of DNA (Hebda et al. 2014). The common methods to collect fingernail evidence are clipping the nail, swabbing beneath the nail using a small, moistened swab, or scraping beneath the nail, generally using a wooden applicator and collecting the debris. While collecting it from the body, hyponychium, the area below the free edge of the fingernail should be thoroughly checked for accumulation of biological and non-biological foreign materials (Bozzo et al. 2015; Devi and Banu 2015). Samples can be collected on a plain sheet of paper or in sterile micro-centrifuge tubes (for DNA analysis). No nail cosmetic or nail treatment should have been done and hands should be thoroughly wash with soap and warm water and then allowed to dry. Conventional and sterilized metallic nail clippers should be used. The samples can easily be stored at room temperature (Grover and Bansal 2017).

Once the nail evidence reaches the laboratory, its examination can be done using scanning electron microscopy and atomic force microscopy for obtaining discrete information (Devi and Banu 2015). Microscopic examination looking for any external material (blood, skin, hairs etc.) can be done (Foran et al. 2015). Nail surface can also be examined using compound microscope in order to determine any possible difference in color. FT-IR (ATR) spectroscopy technique can be used for non-destructive identification of molecular species. The “fingerprint region” of the keratin fiber (FTIR spectral region between 1750 and 750  $\text{cm}^{-1}$ ) can be analyzed as it mainly consists of the major amide bands, CH deformations cysteine oxides. In cases where control samples are not readily available or in cases where the unknown samples cannot be matched to either nail from the particular animal or suspect animal, it is then possible, through FT-IR microscopy to establish the characteristics of a nail (Italiya et al. 2009). The nail clippings can be compared using comparison microscope for individual nail striations (Loveleen 2017).

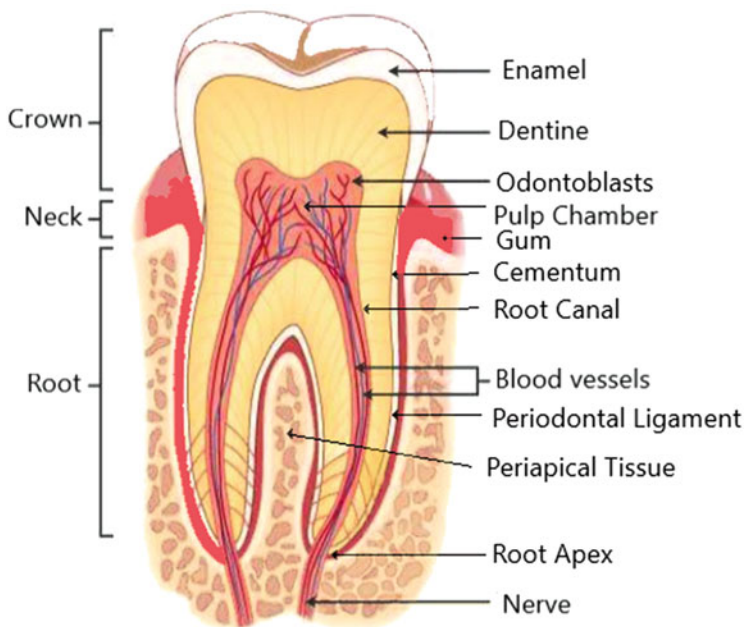
Nails can be an excellent source of germline DNA for genetic analyses in almost all forensic and clinical aspects. Although, the special structure of nails (DNA in



keratinized cells) makes DNA extraction more complex but it can yield a high amount of DNA if standard and well-defined protocols and reagents are used for lysing keratin, without compromising the quality of DNA.

## 17.4 Teeth

Teeth as a forensic evidences are the most commonly traced and sometimes, the only evidences left for forensic human identification in cases of mass disasters, mutilated, decomposed and burnt body remains (Shah et al. 2019). The persistence and longevity of teeth in extremely harsh condition of extreme heat and pressure is attributable to their structural composition comprising of calcified minerals (Yukseloglu et al. 2019). Biologically, a tooth is composed of four dental tissues, comprising of three calcified hard tissue namely enamel, dentine and cementum and a non-calcified soft tissue—pulp. Enamel of a tooth made up of calcium phosphate is considered as the hardest part of human body that protects the internal components of tooth. Dentin is a hard tissue similar to bone but is sensitive to touch and thermal variation. Cementum helps in cushioning the tooth firmly to the jaw bone. Pulp is the central part of tooth that holds the nerves and blood vessels. An adult human dentition comprises of 32 teeth categorized into 4 types of teeth namely incisors, canines, pre-molar and molar (Arola et al. 2017) (Fig. 17.3).



**Fig. 17.3** Structure of human tooth

Forensic dentistry aids in human identification by determining age, gender and race based on the variation in dental pattern that includes inter-space between teeth, tooth's individual unique characteristics, dental anomalies etc. Also, forensic dentistry plays an important role in cases of sexual abuses, criminal deaths etc. involving bite mark patterns left in the form of defensive wounds. The field of forensic dentistry encompasses examination of bite-mark patterns, tooth prints, radiographic and photographic examination, dental DNA analysis as well as rugoscopy, and cheiloscopy (Adler et al. 2011; Krishan et al. 2015; Divakar 2017).

### **17.4.1 Age Estimation**

Assessment of dental age is one of the accurate methods for estimation of chronological age as it is least affected by nutritional and endocrinal activities. Development of tooth is a continual process that initiates at embryonic stage, progressing till early adult life and is reliable on various factors such as inter-spacing, and other local as well as systemic factors (Divakar 2017; Shah et al. 2019). Chronological sequence of mineralization and eruption sequences of both deciduous and permanent set of teeth has been described in the Table 17.3.

Various methods for estimation of dental age have been proposed under the following categories.

#### **17.4.1.1 Visual Examination**

Assessment of eruption condition of tooth, dental anomalies and degenerative changes in teeth such as subsequent wear and tear, attrition etc. are some of the reliable criteria that provide information related with individual's chronological age.

#### **17.4.1.2 Histological Examination**

Histological examination is evidential for determining the extent of mineralization especially in neonatal cases where the teeth are not sufficiently radiopaque for radiographic visualization. Assessment of neonatal lines is a reliable method for determining age of babies in days. Also, the increment lines in the cementum, known as cementum annulations, cross striation in enamel due to gradual deposition of enamel are also informative for estimation of age.

#### **17.4.1.3 Morphological Examination**

Conditions of completion of dental development stages, i.e. in case of elderly adults, require alternate method for age determination. Morphological examinations of teeth for age determination fits well in such cases and rely on the subsequent variation in the dental structures throughout one's life. Generally, morphological assessment is applicable to teeth from body remains of dead adults and involves assessment of mineralization extent of adult teeth.

**Table 17.3** Chronology of mineralization and eruption sequences of Human Dentition Set

Jaw	Tooth	Eruption sequence	Initiation of calcification	Completion of crown	Completion of root
<i>Permanent set of dentition</i>					
Maxillary	Central incisor	4	3–4 months	4–5 years	10 years
	Lateral incisor	6	10–12 months	4–5 years	10–11 years
	Canine	12	4–5 months	6–7 years	14–15 years
	First premolar	8	1.5–1.75 years	6–7 years	12–14 years
	Second premolar	10	2–2.5 years	7–8 years	13–14 years
	First molar	2	At birth	4–5 years	9–10 years
	Second molar	14	2.5–3 years	7–8 years	15–16 years
	Third molar	16	7–9 years	12–16 years	18–25 years
Mandible	Central incisor	3	3–4 months	3–4 years	9 years
	Lateral incisor	5	3–4 months	4–5 years	9–10 years
	Canine	7	4–5 months	5–6 years	12–13 years
	First premolar	9	1–2 years	6–7 years	12–14 years
	Second premolar	11	2–3 years	7 years	14–15 years
	First molar	1	At birth	3–4 years	9–10 years
	Second molar	13	2–3 years	7–8 years	14–15 years
	Third molar	15	8–10 years	12–16 years	18–25 years
<i>Deciduous set of dentition</i>					
Maxillary	Central incisor	2	3–4 months in utero	2 months	2.5 years
	Lateral incisor	3	4 months in utero	2–3 months	2.5 years
	Canine	7	4–5 months in utero	9 months	3.5 years
	First molar	5	4 months in utero	6 months	3 years
	Second molar	10	5 months in utero	11 months	4 years
Mandible	Central incisor	1	3–4 months in utero	2–3 months	2.5 years
	Lateral incisor	4	4 months in utero	3 months	2.5 years

(continued)

**Table 17.3** (continued)

Jaw	Tooth	Eruption sequence	Initiation of calcification	Completion of crown	Completion of root
	Canine	8	4–5 months in utero	9 months	3.5 years
	First molar	6	4 months in utero	6 months	3 years
	Second molar	9	5 months in utero	10 months	3.5 years

#### 17.4.1.4 Radiological Examination

Radiological examination provides graphical visualization of teeth structure and helps in determining dental age in two approaches, i.e. by tooth eruption pattern and dental maturity based on their extent of mineralization. Advanced imaging technologies offer visualization of radiopaque spots on teeth that extends from the period prior to teeth calcification till the closure of teeth apex. In simpler words, radiographic examination of teeth provide information regarding dental age for age group of children to adolescents. Atlas method and scoring method are the commonly used radiographic methods for assessment of chronological age.

#### 17.4.1.5 Bio-chemical Examination

Bio-chemical examinations are beneficial in cases of pre-natal ages ranging up to 6 months, in which the radiographic examinations are unable to provide accurate age due to radiolucent conditions of teeth. Bio-chemical examinations in such conditions involve correlation of dry weights and heights of tooth's crown with chronological age. Racemization of amino acids is another bio-chemical marker of age determination in cases of adults.

### 17.4.2 Gender Determination

Gender of the unknown deceased is another criterion for narrowing down the range of persons to be identified for positive identification of such deceased. Like skeletons, teeth possess the characteristics of sexual dimorphism, i.e. different morphological features depending upon the gender of an individual. Morphological as well as molecular examination of teeth assists in determining gender of an individual.

#### 17.4.2.1 Morphological Examination

Morphological measurements of dental indices such as mandibular inter-canine arch widths, distances of molar cusps, canine dimorphism, root length and crown diameter are the common grounds for gender determination. However, ratio of enamel, dentin and pulp tissue play a major role in describing the sexual dimorphism in crown morphology and size of the permanent dentition (Khangura et al. 2011; Krishan et al. 2015).

#### **17.4.2.1.1 Root Length and Crown Diameter**

Mesiodistal crown diameter of incisors and canines, bucco-lingual crown diameter along with the length of root region are useful morphological criteria for distinction of gender.

#### **17.4.2.1.2 Canine Dimorphism**

Variation in measurements of canine parameters such as mesiodistal arch width, buccolingual width, canine size, height etc. between males and females can be employed for gender determination of any evidence.

Besides other canine dimorphic features, inter canine arch width can also be an useful parameter for differentiating males and females. Inter canine arch width is the distance between the cusp tips of both the canines and are found to be higher in males than in females.

#### **17.4.2.2 Molecular Examination**

Molecular analysis of teeth for sex determination involves examination of barr bodies, F-bodies, Sex determining regions of Y (SRY) gene, enamel protein-amelogenin gene.

##### **17.4.2.2.1 Barr Bodies**

Sex chromatins found in the nuclei of cells are found to be highly stained by nuclear dyes in females and are often termed as Barr bodies. These are indicative of the inactive X chromosome present in the somatic cells of females, thereby can be useful in gender determination.

##### **17.4.2.2.2 F-Bodies**

Y-chromosomes (found in males) possess a binding fluorescent dye, quinacrine, creating a bright fluorescent spot (F body) visible in ultraviolet light.

##### **17.4.2.2.3 Sex Determining Regions of Y (SRY) Gene**

The sex-determining region Y (SRY) gene is responsible for generation of HMG box protein that assists in testis formation by means of specific DNA-binding activity. SRY gene is located on the short arm of the Y-chromosome at p11-31. Detection of SRY gene in odontological evidence may point to a male (but not female) genotype.

##### **17.4.2.2.4 Amelogenin Gene**

Amelogenin (AMEL) is a componential protein essential for development of tooth enamel. The AMEL gene is located on both X and Y chromosome. AMEL-X allele is 2872 base pair long and located at the Xp22.1–Xp22.3 area of X-chromosome, while the human AMEL Y-allele is a 3272 base pair component situated at Yp11.2 section of Y-chromosome.

## 17.5 Botanical Evidences and Their Forensic Examinations

Evidences originated from plants and plant materials that are informative in forensic cases are categorized under botanical evidences. These botanical materials can be unique and specific to a location and thereby can be useful in linking a perpetrator, victim and crime scene. Plants can provide information such as seasonal duration, geographical location of crime as well as identification and location of primary and secondary crime scene. The botanical aspects deals with the study of anatomy, morphology, development, taxonomy of plant and the forensic aspects deals with the recognition, collection, preservation and admissibility of botanical evidence in court of law. These evidences can be found on the dead-bodies, on cloths, footwear, nails or hairs of victim and/or accused. Forensic botany is classified into sub-categories such as dendrochronology, palynology, limnology, ecology etc. These sub-disciplines are engrossed with the analysis of plant materials and their relation with the environment in which they are found. Some of the important botanical evidences and their forensic examination techniques are discussed hereunder.

### 17.5.1 Forensic Examination of Woods

Woods can be related to a crime in various forms ranging from large logs or sticks as a weapon of crime, or tiny twigs as a trace evidence that can aid in linking victim-perpetrator-crime scene. Besides, illicit trade of wood and timber is another condition that requires forensic identification of wood (Wiedenhoeft 2006). The contemporary techniques of identification are much useful in identification of wood. Physical examination of wood involves assessment of color, texture, hardness, weight, lustre (Barbour 2004).

#### 17.5.1.1 Color

Color distinction between the light-colored outer sapwood and dark-colored inner heartwood is well defined in many woods and thereby helps in distinct identification of wood. Sapwood is involved in conduction of minerals from roots to stem. Heartwood is deposition of tannins, resins, oils, gums and helps in mechanical support to the stem. Size of sapwood differs largely across a variety of species from the wide sapwood as seen in black cherry to the narrow sapwood as in locust. The color darkness and the odor of heartwood in tree species such as red cedar, black walnut is distinct and individualized feature of these species.

#### 17.5.1.2 Florescence

Florescence is a distinct character in woods such as Staghorn sumac (*Rhus typhina*), Bijasal (*Pterocarpus marsupium*), Black Locust (*Robinia pseudoacacia*), and Padauk (*Pterocarpus dalbergioides*). Black Locust (*Robinia pseudoacacia*) and Mulberry (*Morus* spp.) are identical woods in terms of appearance and weight. They can be differentiated on the basis of florescence. Black locust emits a strong yellow

green light while Mulberry shows no florescence when exposed to a black light or UV-light (Davies 2017).

### **17.5.1.3 Odor**

Odor is a subtle but sometimes very useful characteristic in examination of woods. Woods such as teak, deodar, chir, rosewood and ton can be identified by means of their characteristic odors.

### **17.5.1.4 Hardness**

Hardness of wood can be related to its density, strength and weight. It is the characteristics of wood to resist the penetration by any foreign body. Soft woods are readily indented by fingernails while moderately harder woods can be dented with sharp knife. Hard woods are difficult to be penetrated by means of sharp objects.

Visual analysis of anatomical characteristics of woods at both macroscopic and microscopic level is beneficial for wood identification. Heartwood and sapwood can also be classified on the basis of vessel elements or pores that are present only in heartwoods. Classification of woods into porous and non-porous, pore size, pore number and pore arrangement in case of porous woods (heartwoods), presence of parenchyma, rays and fibers are some of the common anatomical grounds essential for identification of woods (Miller 1991).

### **17.5.1.5 Pore Size and Pore Arrangements**

Pore size can vary from large (observable through naked eye) to small (not visible to naked eye). Pore or vessel arrangement can be categorized on the basis of their position relative to each other into the following.

#### **17.5.1.5.1 Solitary Pores**

Solitary pores are the single pores that do not possess any contact with the nearby pores.

#### **17.5.1.5.2 Pore Multiples**

Pore multiples is the arrangement of pores that involves clumping of two to five pores.

#### **17.5.1.5.3 Pore Chains**

Pore chains are the radial arrangement of multiple pores.

#### **17.5.1.5.4 Nested Pores**

Nested pores includes multiple pores clustered together in radial as well as tangential directions.

#### **17.5.1.5.5 Wavy Band**

Wavy band is the arrangement of pores in the manner of irregular concentric bands.

### 17.5.1.6 Parenchyma

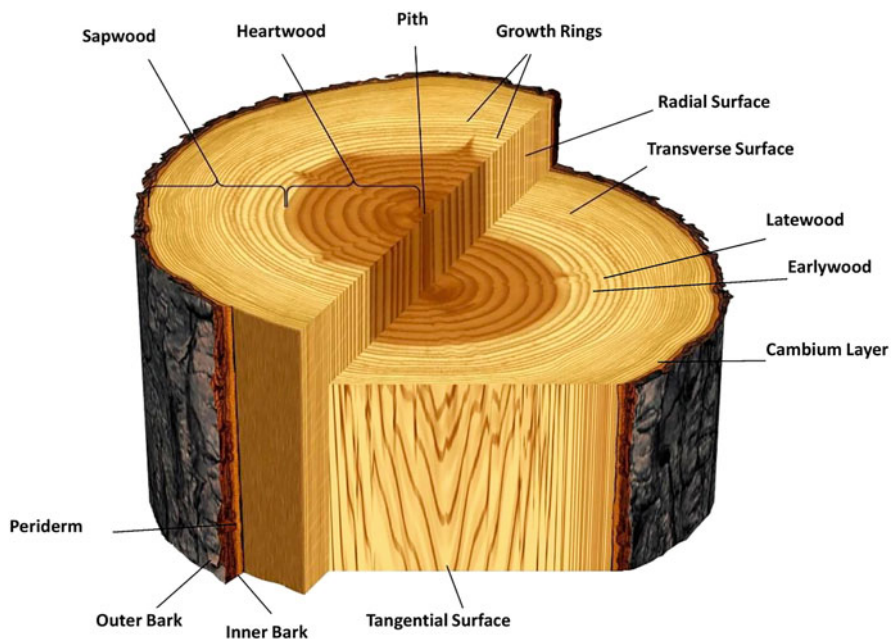
Wood or xylem parenchyma cells are vertically and axially arranged elements of a complex tissue and are concerned with the storage of food in form of carbohydrates, fats and conduction of water. Parenchyma cells are characteristic elements of hardwoods and are scarcely found in softwoods. Based upon the location of parenchyma with respect to the pores, it can be categorized into two broad types—paratracheal and apitracheal and therefore, are counted upon as one of the useful features in wood identification. Paratracheal parenchyma is the one that is located in close proximity of the pores whereas apitracheal parenchyma are separated from pores by means of rays and fibers.

### 17.5.1.7 Wood Rays

Wood rays or medullary rays are narrow strips or ribbon-like structure running perpendicular to the growth rings running from inner to outward directions. They are involved in transportation of food and water across the wood's diameter. Woods of different species can be distinguished on the basis of size and distribution of rays across the specimen.

### 17.5.1.8 Tyloses

Tyloses are the balloon-like ingrowths of parenchyma protruding into the lumen of adjacent vessels obstructing the opening of vessel elements. Presence or absence and distribution of tyloses (if present) is a ground useful for distinguishing wood species (Fig. 17.4).



**Fig. 17.4** Cross-sectional diagram of wood



### 17.5.1.9 Dendrochronology

It is a well characterized discipline that is useful for wood identification. It involves the determining age of wood by examining the tree growth increments. Growth rings are concentric annular rings layer seen in secondary xylem in a cross-section of wood formed during one growth season. Spring wood or early wood is formed during spring season in which cambium is in highly active state and produces ample number of xylem substances. On the contrary, autumn wood or late wood is produced during winter season in which the activity of cambium is suspended thereby producing few number of xylem substances. Counting the number of annual tree growth rings can provide details regarding the minimum age of wood. Also, the comparison of pattern of the rings on a wood to that of a parent tree can help in individualization of the wood and its identification (Dormontt et al. 2015). But, application of dendrochronology is confined to a limited group of timbers from tropical region that produces growth rings.

Radio-carbon dating is more promising technique for determining the age of wood. It involves estimating the ratio of  $C_{14}$ - $C_{12}$  isotope of carbon and comparing with the standard value that provides the radio-carbon age of that particular wood. Similarly, ratio of stable isotopes of several chemicals synthesized from the trees such as Sulphur, Strontium can provides information regarding the geological area of origin of plant and its climatic conditions (Allen and Huebert 2014). Near Infra-red spectroscopy (NIRS) technique that involves exposure of sample to near infrared light can reveal information regarding physical and chemical components of the wood and helps in distinguishing different species of same genus as well as same species found in different location (Ma et al. 2019). Phytochemicals present within the heartwood of timber in form of exudates and extractives can be traced by means of mass spectrometry (MS) technique that involves ionization of chemical substances into charged molecules and measurement of the mass-charge ratio (Altemimi et al. 2017).

Anatomical evaluation of woods is well-to-do for identification of woods, however, the accuracy of identification decreases with decreasing size of wood. Molecular examination of wood can successfully aid in identification of trace samples of wood.

At molecular level, the technique of DNA barcoding is a promising technique for identification of plant species. The range of samples for DNA barcoding extends to all stages of a plant's life, i.e. from flowering, seedling condition to mature plants as well as decomposing plant specimens under environment condition and in the gut and fecal remnants of animals (Kress—plant DNA barcodes) applications today and in the future. Frequently used barcoding region of plants include *rbcl* gene, *trnH-psbA* gene, *matK* gene and internal transcribed spacer (ITS) region of ribosomal cistrons (Kang et al. 2017).

## 17.5.2 Leaves

Leaves are one of the easiest parts of a plant that aids in identifying the specific plant, its growth conditions, soil type and thereby can help in identifying the location of primary and secondary scene of crime. Such evidences can easily be located clutched in victim's hands or stuck in hairs; trapped in wheels of vehicles used in crime or at the bottom soles of shoes of victim/suspect. A leaf is defined as an expanded lamina attached to the plant's stem by means of stalk or petiole.

### 17.5.2.1 Structure of a Leaf

Leaf is a bilateral, flattened part on the stem developing from the bud and comprises three basic structures—leaf-base, petiole and lamina. Leaf base helps in attachment of leaf to the stem. Petiole is a stalk-like structure that extends from the leaf lamina to leaf base and helps in holding the leaf as per desired exposure to sunlight. A leaf with no petiole is known as sessile leaf and the one with petiole is known as petiolate leaf. Lamina is a broad, flattened and expanded structure of leaf consisting of meshy network of midrib, veins and veinlets. These veins help in providing rigidity to the lamina and act as network of water and nutrients for the leaf (Fig. 17.5).

Microscopic examination of morphological and anatomical structure of non-degraded and dried leaves helps in distinguishing them and identifying their origin plants (Thyagarajan and Raji 2019). The shape, margin and surface of lamina vary in different leaves and therefore can be used for identification of a leaf. Presence or absence of incision on leaf lamina is another useful criterion for distinguishing leaves. If the incisions do not touch the midrib, the leaves can be classified as **simple** leaves. In case of incisions reaching the midrib and dividing the lamina into

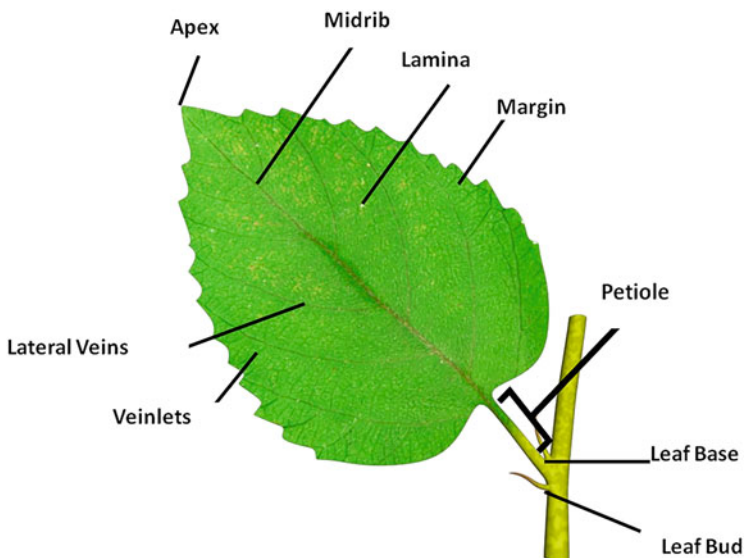


Fig. 17.5 Anatomy of a leaf

numerous leaflets, the leaves are said to be **compound** leaves. Compound leaves with common axis are said to be **pinnately compounded** leaves while the leaflets attached at the tip of petiole, at a common point, the leaves are said to be **palmately compounded** leaves (Oguchi et al. 2018). Various other grounds such as vein counts, stomata index, palisade ratio, types of trichomes, nature of epidermal cells and stomata, that can be utilized for morphologically distinguishing the leaves are discussed below (Bhatia et al. 1973).

### 17.5.2.2 Vein Counts and Venation

The arrangement of veins and veinlets on the lamina of leaves is known as venation. The hierarchy of veins forms a complex reticulate mesh. The first-order veins or major veins are one or more in number, ribbed with vascular tissue and sclerenchyma that stretches from the petiole to leaf apex. Second-order veins split off from major veins at certain intervals and the third-order veins branches between the second-order veins, connecting them. Angiosperms have wide diversity in venation extending upto four orders of veins that can be distinguished on the basis of time of formation, size and branching (Sack and Scoffoni 2013).

### 17.5.2.3 Stomatal Index

Stomatal index can be defined as the ratio of number of stomata to the total number of stomata and epidermal cells present in a given area of leaf. It can be formulated by the equation:  $\text{Stomatal index} = S * 100 / (E + S)$ , where,  $S$  is the number of stomatal cells and  $E$  is the number of epidermal cells in a specified area of leaf (Rowson 1946). Stomatal density can be described as number of stomata cells present in unit area of leaf. Stomata cells are concerned with exchange of gases in plants and therefore, the pattern of their distribution can be useful differential marker for identifying a plant species (Khan et al. 2014).

### 17.5.2.4 Palisade Ratio

Palisade ratio can be described as the average number of palisade cells that contains maximum number of chloroplast and are prime site of photosynthesis, present under each upper epidermal cell. Palisade ratio has paramouncy over other features as it remains constant despite of the varying environment and location of plant. However, it is less used in monocot leaves as mesophylls cannot be differentiated into palisade and spongy cells in such leaves.

### 17.5.2.5 Types of Trichomes

Trichomes are outgrowth appendages of epidermal layer present in the form of epidermal hairs that does the function of preventing water loss during transpiration, protection from UV rays, stress-resistance. Trichomes may be unicellular or multicellular; glandular or non-glandular; branched or unbranched. They are found with diverse structure as per function in the form of hairs, thorns, scales etc.

Several climatic factors such as temperature, pressure, humidity, amount of available sunlight, etc. influence the structure of leaves (Sharma et al. 2020b). Morphological and physiological variations among the leaves depending upon the climatic factor have been described in Table 17.4. In case of leaves with initial

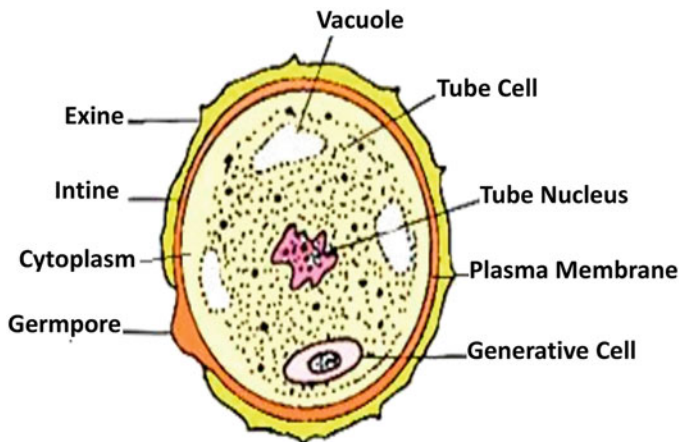
**Table 17.4** Effect of different climates on the structure of leaves

Climate	Effect on leaves
Tropical cold climate	Leaves are large and typically hunter-green and have the capacity to absorb sunlight because the sun is visible for a very short period of time. These kinds of leaves are present mainly towards the pole.
Tropical dry climate	Leaves are much more variable including many compound-leaved legumes and more species with thorns.
Subtropical humid climate	The leaves present in this region are mostly wax coated because of the regular rainfall in the area and the plant and leaves are well nourished because of all the nutrients available.
Temperate climate	All size of leaves is present in this kind of climate because in this type of climate, winters are mild and rainfall is moderate.

degradation stage, molecular examination of leaves can help in tracing the plant source.

### 17.5.3 Pollen Grains

Forensic palynology was used as a crime-solving tool (murder case) for the first time in 1959 in Sweden. Forensic palynology is the utilization and analysis of pollens and spores in criminal and civil cases (Alotaibi et al. 2020). Due to its morphology and microscopic size, protection from mechanical and chemical destruction and its endurance in the intestine for 21 days, pollen analysis has become an advancing scope for establishing a link between victim(s), suspect(s) and crime-scene(s) and is considered among the best application of Locard's principle of exchange (Bennett and Willis 2002; Arguelles et al. 2015; Boi 2018; Alotaibi et al. 2020). Pollens are an ideal material for investigation as they are microscopic, highly variable and can be found on things which are exposed to or interact with the air (Alotaibi et al. 2020). Pollens, often considered as trace evidences are one of the least destroyable evidence that easily gets attached to any surfaces, skins, and cloths and can remain at a location for a long interval of time. Also, the bi-layered walls of pollens are tough and resistant to the adverse environmental conditions due to the presence of sporopollenin in the outer exine layer, thereby enhancing the retrieval rate of such trace evidences even after a long span of time. Any geographical region can be distinguished from the other in terms of pollen prints of that location. Pollen prints are clusters of pollens from different plants of a location that gets shed due to air current and settle down on the ground in a form of a thin layer. Soil, dirt, dust, ropes and twines, clothing and fabrics, drugs, air filters, plant material, and animal and human material, such as fur, hair and stomach contents are common elements at almost every crime scene (Hirapure et al. 2014; Alotaibi et al. 2020). They are held firmly by their surface due to static charges and are not effectively shed, making them highly valuable as evidence. The structure and outer surface of pollens and spores is highly resistance to environmental factors like heat, cold, washing, smudging and degradation etc. and may remain preserved for many years. Pollen is the male gametophyte of gymnosperms and angiosperms. Its size ranges from 15 to 200  $\mu$ m; its shape, when dry, is generally oval or spherical. Pollination is the



**Fig. 17.6** Structural anatomy of pollen grain (microspore)

transport of pollen from its site of production to the female landing site. Analysis of pollen helps in the identification of plants which can determine the geographical origin of a specimen. This creates a link between crime scene and individuals and can determine possession of prohibited or endangered species (Alotaibi et al. 2020) (Fig. 17.6).

Production and dispersion of pollens and spores are important considerations in forensic analysis. If one can guess the expected pattern of production and dispersion of spores and pollens, one can easily know the type of pollen fingerprint of the area (Kumari et al. 2017). Sample collection and recovering pollen from the samples is the crucial step for the identification of pollens (Mildenhall 2006). Soil, dirt, and dust are the most common elements one can find at every crime scene. They should be collected thoroughly and carefully because these elements contain abundant pollen and spores (Kumari et al. 2017). Methods such as tape-lifting for the collection of the microscopic samples, chemical treatments for removal of debris, minerals and other organic substrates, sieving for obtaining the desired size ratio, grain preparation by acetolysis for constant sizing, staining for enhanced visualization are the chronological steps to be performed prior to visual analysis under a microscope. The mounted slide containing the pollen specimen is analyzed using a transmitting light microscope that basically involves the transmission of light through the sample and visualization of the sample through a lens. Quantitative Evaluations of Minerals using a Scanning Electron Microscope (QEMSCAN) is an advanced microscopic technique that involves simultaneous assessment of minerals and other substances as it gives greater pictures and diminutions for pollen grains and has been used in routine analysis since the 1970s. The imaging of pollen grains can also be done due to its increased accuracy, time-saving method, clarity of pictures and decreased human effort. There are three methods for obtaining useful pollen grains images, including transmitted-light microscopy (TLM), the wide field fluorescent method and the structured illumination (Apotome) method. The highest recall is shown by

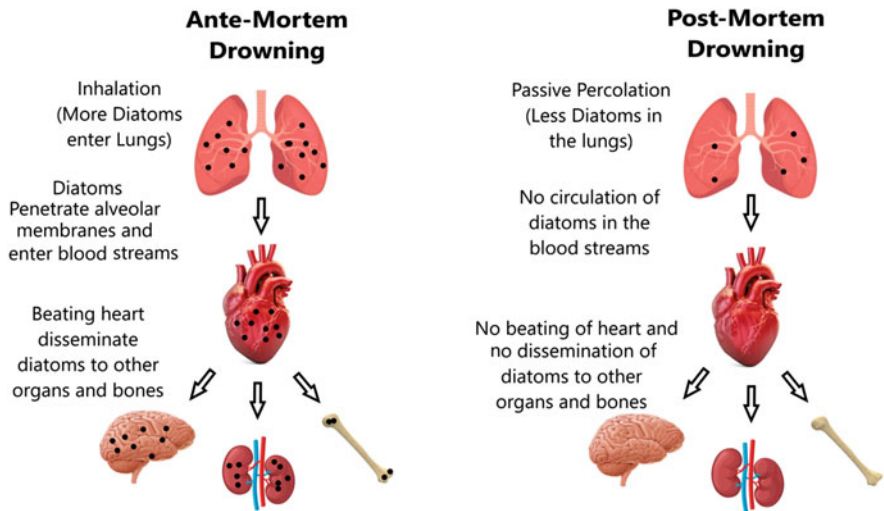
transmitted-light microscopy (TLM) for all types of imaging. All these analyses depend on the role of dispersal (Alotaibi et al. 2020). Recently, using DNA barcoding, it has recently been demonstrated that DNA analysis can be done from even a single pollen grain. Multiple taxonomies groups can be identified using this method along with the identification of parts of the organism that do not appear in the morphology (Bell et al. 2016). One of the fastest ways to differentiate between pollens is by using DNA barcoding (Alotaibi et al. 2020).

Pollen and spores have various advantages over many other biological sources of evidence. Due to their smaller size (avg. 20–60  $\mu\text{m}$ ), criminals cannot clean the crime scene as they cannot be seen by the naked eyes. They can adhere to any surface and may become firmly attached even after washing in domestic detergent. Another advantage is that pollens don't decompose. Due to their multi-layered cell wall (composed of cellulose and sporopollenin), they can be preserved for hundreds of millions years as sporopollenin is one of the most chemically resistant organic molecules (Mildenhall 2008). Palynology can be helpful in various aspects such as: relate materials at the disclosure scene or crime scene with a suspect, reduce potential suspects list, determine the movement of things and their origin including their geographic location, helps to decide the perimortem destiny of an unfortunate casualty and finally, help to determine the age of human remains. It can be a profoundly significant precise and powerful method of forensic analysis in cases of rape, murder and other types of crimes, particularly in open areas, but even if they occur in closed premises (Mildenhall 2006; Alotaibi et al. 2020).

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## 17.6 Diatoms

Diatoms are considered to be the most effective and reliable evidence in forensic cases of drowning deaths due to their large diversity of species, expressed morphologically as the difference in size and shape of frustules (Bogusz and Bogusz 2018; Calder 1984). Forensic application of diatoms is studied under Forensic limnology. Diatoms (Bacillariophyta) are microscopic, photosynthetic, uniflagellate alga with a siliceous cell wall found in all varieties of water bodies including fresh water and salt water (springs, rivers, ponds, lakes, ditches) that have been recorded and classified for over 200 years (Horton et al. 2006; Levkov et al. 2017; Vinayak and Gautam 2019). It consists of two overlapping valves: the top called the lid (epitheca) and the bottom valve, called the bottom (hypotheca). The hard cell wall of diatoms impregnated with silica (over 60% of dry weight of diatom), called as frustules which makes it resistant to most chemicals, including strong inorganic acids, hydrogen peroxide and high temperature, making it a specific protective cover for the protoplast (Bogusz and Bogusz 2018; Cameron 2004). The basic principle behind employing diatom analysis in drowning deaths is the potential of diatoms to exist in the organs of the drowned person in case of ante-mortem aspiration of diatom-rich water into the lungs. The smaller size of diatoms ranging between 20 and 200  $\mu\text{m}$  and their morphological structure allow them to seep through the alveolo-capillary barrier and subsequently enter into the blood flow and thereby entering other vital organs such as the spleen, brain, liver, kidneys and bone marrow



**Fig. 17.7** Principle of utilizing diatoms for differentiation of ante-mortem and post-mortem drowning

prior to the death of the person. This forms a major ground to distinguish ante-mortem and post-mortem drowning of a person. Also, the category of diatoms (such as freshwater diatoms, and marine diatoms) detected in a body during autopsy can give an idea about the location of death (Bogusz and Bogusz 2018; Anand and Unmesh 2016; Kaushik et al. 2017). Identifying the provenance of individuals, clothing or materials using diatom analysis can be of further use in forensic science (Horton et al. 2018) (Fig. 17.7).

As per Bogusz and Bogusz (2018), the process of drowning consists of several stages-periods:

1. Fast breathing period (few to several seconds).
2. Resistance period (half a minute to several minutes)—submersion under water.
3. Significant respiratory movement's period (one to two and a half minute)—water entering the respiratory tract.
4. Mortification period (one to one and a half minute)—loss of sensation and unconsciousness.
5. Final breathing period (around 1 min)—tonic convulsions and body strain occur.

Prior to diatoms analysis, the samples to be processed must be thoroughly cleaned from substances such as pigments, cells, sand, mud etc. that might interfere with the visual examination of diatoms. A small section of the sample (~10 g), preferably femoral or sterna bone marrow is taken in a tube and acid digestion is carried out by treating it with concentrated nitric acid (conc.  $\text{HNO}_3$ ) and heating it till a clear solution is obtained. This is followed by the addition of hydrogen peroxidase and boiling of the fluid. The fluid once cooled is then centrifuged, the supernatant is decanted off and the sediment is examined under the microscope. The fluid

**Table 17.5** Advancements in diatom analysis

Advancements	References
Detection of diatoms	Timperman (1972)
Detection of diatoms in bone marrow	Tamaska (1949)
Diatoms indicative of antimortem drowning	Porawski (1966)
Development of a method for diatom test for direct screening drowning deaths	Timperman (1972)
Acid digestion method	Pollanen (1998)
Use of a mixture of HNO <sub>3</sub> and H <sub>2</sub> O <sub>2</sub> for the digestion of tissue samples	Auer (1988)
Use of nitric acid for extraction of diatoms from bone marrow tissue	Hurlimann (2000)
Use of sulphuric acid for extraction of diatoms	Krstic (2002)
Use of nitric acid for extraction of diatoms from lungs and sternum bone	Bortolotti (2011)
Use of H <sub>2</sub> O <sub>2</sub> extraction technique for diatom samples from clothes	Scott (2014)

containing the diatoms is dropped on a glass slide, thermally fixed on a hot plate and mounted using Naphrax before visualizing it under the microscope. Comparison of all the species of diatoms recovered from the specimen and the suspected site of drowning can help in correlating the events. In absence of a reference or control water sample, the presence of a preset minimal number of diatoms in different vital organs can help in the establishment of ante-mortem drowning. By using Fluorimetry (luminescent properties), diatom samples can be located in the bone marrow and then can be differentiated from other diatoms found in nature by incorporating specific fluorescent tags. Using electric impedance spectroscopy, diagnosis of putrefactive corpses can also be done.

Advanced technique for diatom test includes visualization through scanning electron microscope and molecular analysis including PCR-based DNA sequencing. Molecular biology-based technique can be used for the detection of 16S rRNA subunits of ribosomal RNA (Rana and Manhas 2018; Zhou et al. 2020) (Table 17.5).

In cases of drowning, the identification of diatoms has been considered to be the “gold standard” (Khurshid et al. 2021). The diatoms can also be significant even occasionally if they have been recovered from the internal organs of non-drowning bodies. In the near future, Advanced technologies such as Nuclear Magnetic Resonance (NMR), Fluorimetry, Molecular biological techniques, Automatic Diatom Identification and Classification (ADIAC) etc. can be used for the detection of diatoms (Rana and Manhas 2018).

## 17.7 Wildlife Forensics

Wildlife forensics is an advancing field of criminal investigation which aims at using scientific procedures to examine, identify, and compare evidence from crime scenes related to plants and animals but also in monitoring the health and impact of environmental factors on the well-being of wildlife populations. Poaching is one of the most serious crimes investigated by wildlife forensic scientists. Other crime



**Fig. 17.8** Types of offences related to wildlife



against wildlife includes the illegal trade of protected animals and products made from protected animals (Bell 2011; Gouda et al. 2020).

Major types of evidence encountered and analyzed in a wildlife crime include any part of an animal such as whole animals (live or dead), skins or skeletons of vertebrate species, exoskeletons and shells of invertebrate species (such as butterflies, rhinoceros beetles and mollusc shells) and animal body parts (intact or processed, such as internal organs, whole feet/legs/wings/heads/fins, furs, feathers, scales, teeth, beaks, claws, muscle fillets, powdered shells/skeletons/skins and blood samples), carcasses, hair, teeth, claws, talons, tusks, hides, stomach contents etc. Wildlife forensic scientists may also investigate materials used to kill or harm animals, such as poisons, pesticides, projectiles, and weapons. Identification and individualization of products that are made from animals such as leather goods and medicines are also of interest (Fig. 17.8).

Variety of biological disciplines such as hair and fiber analysis, blood splatter analysis, DNA analysis; chemical, pathological as well as physical examination of various evidences can together be employed in wildlife caseworks as demonstrative evidences for linking the suspect with the crime.

### 17.7.1 Morphological Analysis

The morphological or physical characteristics are the simplest way of wildlife forensic method in the identification process of evidences and the least expensive forensic analysis. The identification of species based on morphological characteristics of wildlife flora and fauna provides important clues based on external

appearance. Different species generally possess distinct physical appearances like skin coat color, pattern of coloration, eyes, pinna, tails, ivory etc. (Burnham-Curtis et al. 2015).

### **17.7.2 Footprints Analysis**

Footprints of wildlife species are one of the commonly encountered evidence in the forensic analysis of species. Structural and dimensional analysis of footprint impressions on the surface can be useful for determining the species of the animal as well as confirming the presence of the specific animal at the location.

### **17.7.3 Osteology**

It is the use of the morphology of bones of the skeleton to make identifications. The first question that always arises is whether it is human or not and if it is an animal then what animal is it. Identification of animals is really difficult because of the possibilities of various inter and intra-species. In cases when dentition is present, chances of identification become more (Bell 2011).

### **17.7.4 Microscopic Examination**

It includes the morphological, structural as well as elemental analysis of evidences to identify the species of origin. Analysis of hair, fur, skins, and ivory can provide leading information in case of metallic poisoning or other cause of death. Microscopy can be one of the most useful tools in wildlife forensics, especially while dealing with hair evidence. Scanning electron microscopy (SEM) can also be used in the study of surface morphology hairs. Scale patterns of wool fibers can be analyzed for species characterization (Sahajpal et al. 2021).

### **17.7.5 Molecular Examination**

In the past decades, advancements in molecular techniques had allowed forensic researchers to extract genomic DNA from different evidences encountered at the crime scene which may provide evidential link related to the individualization of any animal. DNA analysis of trace evidences generally exchanged during the crime can successfully link the suspect with the victim as well as the crime. Similar to human forensic analysis, molecular examination in wildlife crime can be useful in the identification of species, individualization of animal, age determination, gender determination, and paternity testing as well as in population studies such as phylogenetic evolution of animals (Sahajpal et al. 2021).

### 17.7.6 Isotopic Examination

The geographic origin of the unknown wildlife samples can also be identified using the study of elemental analysis. This becomes important in the cases where a particular species is endangered in one region and not in other. In this method, a comparison of the ratios of different isotopes can be done using inductively coupled plasma mass spectrometry (ICP-MS) and isotope ratio mass spectrometry (IRMS). The relative abundance of isotopes of various elements can also be measured using mass spectrometry and laser ablation–inductively coupled plasma–mass spectrometry (LA-ICP-MS) (Sahajpal et al. 2021).

## 17.8 Forensic Entomology

Forensic entomology is another interesting branch of forensic biology that incorporates the study of arthropods and other insects, their anatomy, life cycle, and other information to infer conclusions in legal caseworks. It particularly deals with the estimation of time since death or post-mortem interval (PMI), differentiation of primary and secondary crime-scene, determination of the cause of death, and an indication of physical abuse and illicit drug ingestions (Sharma et al. 2015; Harvey et al. 2016). Forensically significant group of insects includes flies (Order—Diptera) such as blowflies (Calliphoridae), flesh flies (Sarcophagidae), and beetles (Order—Coleoptera) such as rove beetles (Staphylinidae), Carrion Beetles (Silphidae) along with wasps, ants moths and other insects. Infestation and relative progression of these arthropods on cadavers at different stages of decomposition form the basis of forensic entomology (Rivers and Dahlem 2014). The famous ‘Ruxton’ case (1935) involved the application of forensic entomology for linking crime with the suspect. The third-instar larvae of blowflies on the body remains indicated disposal of bodies 12–14 days before their discovery that matched with the time-period of Mrs Ruxton and her nursemaid’s disappearance (Sharma and Singh 2015). For successful inference, detailed knowledge related to the colonization of insect, developmental phases and time along with the impact of insects on the decomposition of the corpse is essential.

### 17.8.1 Necrophagous Insects

Insects/arthropods that feed directly on the body remains, or the fluids released from the remains during the decomposition process are classified as Necrophagous species that include many species of the order Diptera from the families—Calliphoridae and Sarcophagidae.

*Calliphoridae*, also known as blowflies, carrion flies or cluster flies are the primary agent involved in the process of decomposition of organic matters. These are typically metallic blue, black, or green in color and possess antennae covered in

fine filaments or branches. A fully grown calliphoridae ranges in size from approximately 6–10 mm.

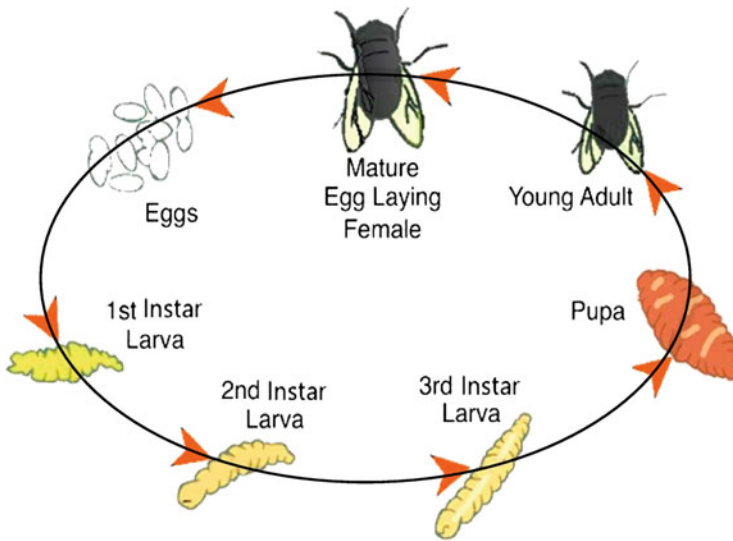
Sarcophagidae or flesh flies differ from most of the flies as they are ovoviviparous. They lay hatched or hatching maggots instead of eggs upon the carrion. An adult flesh fly range in size from 4 to 23 mm and has black and gray longitudinal stripes on the thorax and checkering on the stomach (Gennard 2012; Sharma et al. 2015).

### 17.8.2 Life Cycle of Blowflies

Flies, particularly Calliphoridae are among the pioneering insects to infest a corpse. The flies lay eggs usually in clumps on the surface of a cadaver that is white and slightly elongated, similar to a grain of rice in size, shape and color. On a fresh cadaver, these clumps are usually found on the area where the body's mucus membranes come into contact with the outside. The eggs hatch into larvae (maggots) in the duration of 8–23 h, depending upon the climate. The larvae undergo three instars or stages and develop through by feeding on the corpse, with a moulting event at the end of each one. The larvae at the first instar stage are typically around 2 mm long after nearly 2 days. The second instar stage is relatively quick, lasting only half a day and the larvae grow around 10 mm. Finally, the third instar lasts around 2.5 days and the larvae measure upto 17 mm. At the end of the third instar, the larvae undergo the pupal stage and will become mobile. The larvae moult again but do not shed their skin. Instead, the skin shrinks to form a cocoon which is hard and protective. During this stage, the larvae cannot move, feed, or defend themselves. It finds a safe place to shed its skin for the third time. At this stage, the larva forms rudimentary legs and wings, and an adult fly emerges out from hard cover on completion of the pupal stage, typically around 18–20 days from when the eggs are first laid. The adult that emerges from the pupa is pale and soft with crumbled wings and it takes around 2 days until its wings expand and its body color changes (Higley et al. 2001; Szpila 2009; Gennard 2012) (Fig. 17.9).

Life-cycle and age of an insect are useful for estimating PMI up to a week, before the completion of the whole cycle as identification of generations would become difficult in case of multiple generations infesting upon the cadaver. For such a situation, in which the dead bodies were disposed for a longer time, the technique of identifying insect succession must be employed. This involves information related to the succession of different orders of insects at different stages of body decompositions (Anderson and Byrd 2010).

Besides the morphological characterization of various insects, molecular analysis of these insects can be of great importance. In cases like Sarcophaga, microscopic examination of insects cannot infer the difference between the insects. Molecular examination of PCR-based DNA analysis makes use of cytochrome oxidase (CO-I) to differentiate two individual insects from the same colonies (Wells et al. 2009; Tarone et al. 2015). Entomotoxicology is an advancing field of entomology that deals with ante-mortem consumption of drugs and absorption of drugs by the insects



**Fig. 17.9** Life cycle of Calliphoridae

infesting on the corpse. These absorbed drugs may have an accelerating or degenerating impact on the development of insects thereby hampering the correlation of the insect's developmental stage and the actual post-mortem interval. Therefore, precise knowledge about ecology, distribution pattern and the developmental pattern is essential for the successful employment of entomology in forensic caseworks (Goff and Lord 2001; Vasudeva Murthy and Mohanty 2010).

## 17.9 Forensic Mycology

Besides insects, fungal species infesting upon a cadaver can be potentially employed to estimate the source of information about post-mortem interval (PMI), cause of toxicity or poisoning and death, determining time since deposition. In addition, the small size of fungal spores and their abundance assists in linking the suspect with crime-scene and/or victim in the form of trace evidence. The application of fungi in the legal context is dealt under the subject of forensic mycology.

### 17.9.1 Structure of a Fungus

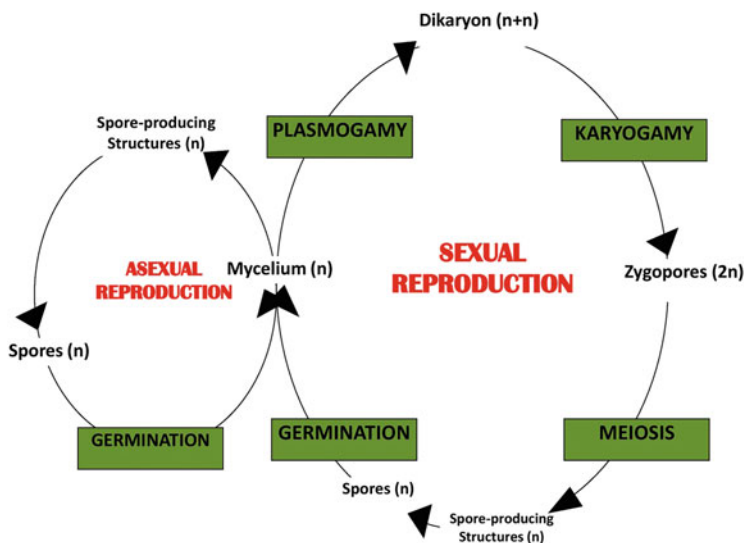
Fungi (singular—Fungus) are filamentous multicellular eukaryotic organisms that are heterotrophic in nature with cell walls composed of chitin and polysaccharides. The body of a fungus known as thallus is formed of small tube-like cells interconnected to each other that are known as hyphae. The bulk of such tubular hyphae is called mycelium. Some fungi have septae or cross walls in their hyphae

(Webster and Weber 2007; Raghukumar 2017). On the basis of their roles in the ecosystem, the fungi can be categorized into three- pathogens or the disease-causing fungi; symbionts or the mutualistic beneficial fungi such as lichen, mycorrhiza, etc., and the saprobes or the decomposing fungi that decay the organic matter from the ecosystem. Based on their morphology, life cycle, and physiology, the major groups of fungi are Phycomycetes, Ascomycetes, Basidiomycetes and Deuteromycetes (Brandt and Warnock 2015).

Cells of a fungus are similar to those of eukaryotes and typically possess cell walls, cell membranes, endoplasmic reticulum, mitochondria, ribosomes, vacuoles, and microtubules. Besides a membranous structure, lomasomes are presently attached to the plasma membrane that facilitates vesicular transport and enlarge the surface area (Zadworny and Eissenstat 2011). Cells of fungus may be uninucleate or multinucleate. Hyphae that are continuous and filled with multinucleated cytoplasm are called coenocytic hyphae. A variety of decay fungi possess binucleate cells. The stage of cells in binucleate condition can be termed as dikaryon stage on the condition of the nuclei being genetically different and can result from the fusion of two hyphae cells with an incomplete fusion of their nuclei. Cross walls or septae in the hyphae are present in higher orders of fungi and help in strengthening the cells as well as maintaining turgor pressure (Clark and Anderson 2004).

### 17.9.2 Life Cycles of Fungi

The life cycle of fungi has many different patterns based on the species of the fungi. They may reproduce vegetatively, sexually or asexually. Vegetative reproduction can be of three types—fragmentation, budding and fission. Asexual reproduction is the most common method of reproduction and ensures the dispersal of species to different locations. Sexual reproduction is beneficial in the condition of extreme environments with limited resources. The life cycle of fungi consists of three stages—Spores, Mycelium and Germ cells. Life of all fungi begins with the spore stage which is in haploid condition and genetically identical to their parent fungus. They may be motile or non-motile, thin or thick-walled propagules that separate from parent bodies when ready for dispersal. After reaching a suitable location, mass of spores club together and forms a bunch of root-like structure known as mycelium. At the mycelium stage, the fungi can opt for reproducing sexually or asexually. For asexual reproduction, the mycelium grows into spore-producing bodies. For sexual reproduction, the mycelium undergoes meiotic phase. Meiotic phase involves two steps—plasmogamy or the fusion of cytoplasm and karyogamy or the fusion of nuclei. Haploid gametangia conjugate to form diploid zygospores. The zygospores are genetically different from the parent fungi due to the phenomenon of crossing over during meiosis. Sexual reproduction occurs by different methods, namely planogametic copulation, gametangial contact, gametangial copulation, spermatogamy, somatogamy (Moore-Landecker 1972; Bridge and Spooner 2001; Gruninger et al. 2014) (Fig. 17.10).



**Fig. 17.10** Schematic diagram demonstrating the life cycle of fungi

### 17.9.3 Roles of Fungi in Forensic Sciences

There are more than 1.5 million of fungal species known worldwide. A small locality may possess thousands of fungal species generating a unique microbiota of that location. Identification and differentiation of multiple crime scenes can be relied on the unique pattern of fungal distribution or particular pattern of microbiota belonging to a specific location. As fungal spores are produced in abundance and are light-weight, they can easily be dispersed by wind. Also, the anatomical structure of fungal spores reveals the presence of spike-like structure on the outer wall that helps the spore in adhering to the clothes, hair, or body of an individual. As per Locard's principle of exchange, the spores may get picked up from the surface of an object by another object that comes in contact with it, thereby forming a link between both objects (Menezes et al. 2007; Hawksworth and Wiltshire 2015).

Information related to the fungal life cycle and development pattern can be useful in taphonomical study of a cadaver, particularly in determining the time since death, cause of death, etc. Fungal colonies infesting on dead bodies are different from the ones found in living tissues, often pathogenic in nature. Studies reveal accession of soil fungi on the surface of cadavers deposited on the soil upon reaching the stage of decomposition. These fungi found on cadavers are medically-insignificant but include the majority of decomposer or spoilage fungi. Details related to the growth rate of such fungal colonies found on the cadaver, along with the impact of various environmental factors are essential for determining the postmortem interval (Bridge and Spooner 2001; Tranchida et al. 2014). However, precise identification of the fungus, knowledge of biotic and abiotic factors around the cadaver, storage conditions etc. are crucial criteria for counting on such methods. Time and location

of deposition of any biotic substance can be identified in a similar manner, depending upon the study related to the colonization pattern of the fungus.

In addition to microscopic-based examination of fungal morphology and colonization, DNA-based identification of fungus can be utilized in forensic mycology. Singleplex and multiplex PCR-based DNA profiling and DNA barcoding are some of the DNA-based taxonomic tools employed for the accurate identification of fungal colonies (Sharma et al. 2020a).

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## 17.10 Conclusion

Biological specimens are one of the most valuable evidences encountered at the crime scene. Widely ranging from macroscopic to microscopic evidences, these can significantly help in linking perpetrator-victim-scene of crime. Being organic in nature, biological evidences is prone to contamination, deterioration and decomposition, therefore proper handling, collection, and storage condition are demanded for the successful application of such samples. Forensic biology is one of the highly recognized disciplines of forensic science and is thriving day by day with the introduction of molecular biology and instrumental advancements. Further advancement in these techniques is highly expected for fulfilling future demands.

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