# ESSENTIAL FORENSIC BIOLOGY

Second Edition

**ALAN GUNN** 

Companion Website

**WILEY-BLACKWELL** 

D919.1 G976 E.2

# **Essential Forensic Biology**

#### Second Edition

Alan Gunn Liverpool John Moores University, Liverpool, UK





**WILEY-BLACKWELL** 

A John Wiley & Sons, Ltd., Publication

This edition first published 2009, © 2009 by John Wiley & Sons Ltd.

Wiley-Blackwell is an imprint of John Wiley & Sons, formed by the merger of Wiley's global Scientific, Technical and Medical business with Blackwell Publishing.

Registered office: John Wiley & Sons Ltd, The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, UK

Other Editorial Offices: 9600 Garsington Road, Oxford, OX4 2DQ, UK 111 River Street, Hoboken, NJ 07030-5774, USA

For details of our global editorial offices, for customer services and for information about how to apply for permission to reuse the copyright material in this book please see our website at www.wiley.com/wiley-blackwell

The right of the author to be identified as the author of this work has been asserted in accordance with the Copyright, Designs and Patents Act 1988.

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, except as permitted by the UK Copyright, Designs and Patents Act 1988, without the prior permission of the publisher.

Wiley also publishes its books in a variety of electronic formats. Some content that appears in print may not be available in electronic books.

Designations used by companies to distinguish their products are often claimed as trademarks. All brand names and product names used in this book are trade names, service marks, trademarks or registered trademarks of their respective owners. The publisher is not associated with any product or vendor mentioned in this book. This publication is designed to provide accurate and authoritative information in regard to the subject matter covered. It is sold on the understanding that the publisher is not engaged in rendering professional services. If professional advice or other expert assistance is required, the services of a competent professional should be sought.

Library of Congress Cataloguing-in-Publication Data

Gunn, Alan.

Essential forensic biology / Alan Gunn. – 2nd ed. p.; cm.
Includes bibliographical references and index.
ISBN 978-0-470-75804-5 (HB) – ISBN 978-0-470-75803-8 (PB)
1. Forensic biology. I. Title.
[DNLM: 1. Forensic Medicine. W 700 G976e 2009]
QH313.5.F67G86 2009
363.25–dc22

2008040263

ISBN: 978-0-470-75804-5 (HB) 978-0-470-75803-8 (PB)

A catalogue record for this book is available from the British Library.

Set in 10/12 pt Sabon by SNP Best-set Typesetter Ltd., Hong Kong. Printed in Singapore by Fabulous Printers Pte Ltd.

1 2009

# Essential Forensic Biology Second Edition

To Sarah, who believes that no evidence is required in order to find a husband guilty.

## Acknowledgements

Thanks to Sarah and to all of the academic and technical staff at the School of Biological & Earth Sciences, Liverpool John Moores University who helped me along the way.

## **Contents**

Acknowledgements	X1
Introduction	1
PART A: HUMAN REMAINS: DECAY, DNA, TISSUES AND FLUIDS	9
Chapter One: The decay, discovery and recovery of human bodies The dead body The stages of decomposition Factors affecting the speed of decay Discovery and recovery of human remains Determining the age and provenance of skeletonized remains Future developments	11 11 12 28 35 39 41
Chapter Two: Body fluids and waste products Blood cells and blood typing Methods for detecting blood Confirming the presence of blood Bloodstain pattern analysis Artificial blood Post mortem toxicological analysis of blood Saliva and semen Vitreous humor Faeces and urine as forensic indicators Future directions	45 48 51 51 70 71 72 75 77 82
Chapter Three: Molecular biology The structure of DNA DNA sampling DNA profiling Polymerase chain reaction Short tandem repeat markers Single nucleotide polymorphism markers Determination of ethnicity Determination of physical appearance Determination of personality traits Mobile element insertion polymorphisms Mitochondrial DNA RNA DNA databases Future developments	85 86 87 88 92 97 107 108 109 110 112 115 116

viii CONTENTS

Chapter Four: Human tissues	123
The outer body surface	123
Hair	138
Bones	142
Teeth	151
Future developments	159
Chapter Five: Wounds	163
Definitions	163
Blunt force injuries	165
Sharp force traumas	172
Bone damage	180
Additional aspects of wound interpretation	183
Asphyxia	184
Pathology associated with drug use	190
Gunshot wounds	192
Bite marks	200
Burns and scalds	203
Ageing of wounds	205
Post mortem injuries	207
Future developments	209
PART B: INVERTEBRATES AND VERTEBRATES	211
Chapter Six: Invertebrates 1: biological aspects	213
An introduction to invertebrate biology	213
Invertebrates as forensic indicators in cases of murder or	213
suspicious death	214
Invertebrates as a cause of death	238
Invertebrates as forensic indicators in cases of neglect and animal	230
welfare	241
The role of invertebrates in food spoilage and hygiene litigation	243
The illegal trade in invertebrates	246
Invertebrate identification techniques	247
Future directions	250
Chapter Seven: Invertebrates 2: practical aspects	253
Calculating the PMI/time since infestation from invertebrate	20 50 8
development rates	254
Complicating factors affecting earliest oviposition date calculations	260
Determination of the PMI using invertebrate species composition	266
Determination of the PMI using ectoparasites	267
Determination of movement from invertebrate evidence	267
Invertebrate evidence in cases of wound myiasis and neglect	269
Detection of drugs, toxins and other chemicals in invertebrates	271
Obtaining human/vertebrate DNA evidence from invertebrates	271
Determining the source and duration of invertebrate infestations of food products	272
or rood products	272

CONTENTS	ìx

Collecting invertebrates for forensic analysis Killing and preserving techniques for invertebrates Future directions	273 276 279
Chapter Eight: Vertebrates Introduction Vertebrate scavenging of human corpses Vertebrates causing death and injury Neglect and abuse of vertebrates Vertebrates and drugs Vertebrates and food hygiene Illegal trade and killing of protected species of vertebrates Identification of vertebrates Future directions	283 284 284 291 292 293 295 295 298 309
PART C: PROTISTS, FUNGI, PLANTS AND MICROBES	313
Chapter Nine: Protists, fungi and plants Introduction Protists Fungi Plants Plants Plant secondary metabolites as sources of drugs and poisons Illegal trade in protected plant species Future directions	315 316 316 321 324 347 351 353
Chapter Ten: Bacteria and viruses Introduction The role of microorganisms in the decomposition process Microbial profiles as identification tools Microbial infections and human behaviour Microbial infections that can be mistaken for signs of criminal activity The use of microorganisms in bioterrorism Future directions	355 355 356 357 370 372 373 390
References	393
Index	417

### Introduction

The word 'forensic' derives from the Latin forum meaning 'a market place': in Roman times this was the where business transactions and some legal proceedings were conducted. For many years the term 'forensic' had a restricted definition and denoted a legal investigation but it is now commonly used for any detailed analysis of past events i.e. when one looks for evidence. For example, tracing the source of a pollution incident is now sometimes referred to as a 'forensic environmental analysis', determining past planetary configurations is referred to as 'forensic astronomy', whilst historians are said to examine documents in 'forensic detail'. For the purposes of this book, 'forensic biology' is defined broadly as 'the application of the science of biology to legal investigations' and therefore covers human anatomy and physiology, organisms ranging from viruses to vertebrates and topics from murder to the trade in protected plant species.

Although forensic medicine and forensic science only became specialised areas of study within the last 200 or so years, their origins can be traced back to the earliest civilisations. The first person in recorded history to have medico-legal responsibilities was Imhotep, Grand Vizier, Chief Justice, architect and personal physician to the Egyptian pharaoh Zozer (or Djoser). Zozer reigned from 2668–2649 BC and charged Imhotep with investigating deaths that occurred under suspicious circumstances. The codification of laws was begun by the Sumerian king Ur-Nammu (ca 2060 BC) with the eponymous 'Ur-Nammu Code' in which the penalties of various crimes were stipulated whilst the first record of a murder trial appears on clay tablets inscribed in 1850 BC at the Babylonian city of Nippur.

In England, the office of coroner dates back to the era of Alfred the Great (871–899) although his precise functions at this time are not known. It was during the reign of Richard I (1189–1199) that the coroner became an established figure in the legal system. The early coroners had widespread powers and responsibilities that included the investigation of crimes ranging from burglary to cases of murder and suspicious death. The body of anyone dying unexpectedly had to be preserved for inspection by the coroner, even if the circumstances were not suspicious. Failure to do so meant that those responsible for the body would be fined, even though it might have putrefied and created a noisome stench by the time he arrived. It was therefore not unusual for unwanted bodies to be dragged away at night to become another village's problem. The coroner's responsibilities have changed considerably over the centuries but up until 1980 he was still expected to view the body of anyone dying in suspicious circumstances.

Although the coroner was required to observe the corpse he did not undertake an autopsy. In England and other European countries, the dissection of the human body was considered sinful and was banned or permitted only in exceptional circumstances until the nineteenth century. Most Christians believed that the body had to be buried whole otherwise the chances of material resurrection on Judgement Day were slight. The first authorized human dissections took place in 1240 when the Holy Roman Emperor Frederick II decreed that a corpse could be dissected at the University of Naples every five years to provide teaching material for medical students. Subsequently, other countries followed suit, albeit slowly. In 1540, King Henry VIII became the first English monarch to legislate for the provision of human dissections by allowing the Company of Barber Surgeons the corpses of four dead criminals per annum and in 1663, King James II increased this figure to six per annum. Subsequently, after passing the death sentence, judges were given the option of permitting the body of the convict to be buried (albeit without ceremony) or to be exposed on a gibbet or dissected. Nevertheless, the lack of bodies and an eager market among medical colleges created the trade of body snatching. Body snatchers were usually careful to leave behind the coffin and the burial shroud because taking these would count as a serious criminal offence - which was potentially punishable by hanging. Removing a body from its grave was classed as merely a misdemeanour. The modern day equivalent is the Internet market in human bones of uncertain provenance (Huxley & Finnegan, 2004; Kubiczek & Mellen, 2004). A recent notorious case arose when it was discovered that the body of the eminent journalist Alistair Cooke had been plundered whilst 'resting' in a funeral parlour in New York. Alistair Cooke died on March 30th 2004 and despite the fact that he was 95 yearsold at the time of his death and had been suffering from cancer, his arms, legs and pelvis were surreptitiously removed and sold to a tissue processing company. There is a perfectly legal market for bones and other body tissues for use in surgery or as dental filler but it is also highly lucrative and some people have been tempted into criminal behaviour.

Although the ancient Greeks are known to have performed human dissections, Julius Caesar (102/100 - 44 BC) has the dubious distinction of being the first recorded murder victim in history to have undergone an autopsy. After being assassinated, his body was examined by the physician Antistius who concluded that although Julius Caesar had been stabbed 23 times, only the second of these blows, struck between the 1st and 2nd ribs, was fatal. The first recorded post mortem to determine the cause of a suspicious death took place in Bologna in 1302. A local man called Azzolino collapsed and died suddenly after a meal and his body very quickly became bloated whilst his skin turned olive and then black. Azzolino had many enemies and his family believed that he had been poisoned. A famous surgeon, Bartolomeo de Varignana was called upon to determine the cause and he was permitted to undertake an autopsy. He concluded that Azzolino had died as a consequence of an accumulation of blood in veins of the liver and that the death was therefore not suspicious. Although this case set a precedent, there are few records from the following centuries of autopsies being undertaken to determine the cause of death in suspicious circumstances.

The first book on forensic medicine may have been that written by the Chinese physician Hsu Chich-Ts'si in the  $6^{th}$  century AD but this has since been lost. Subse-

quently, in 1247, the Chinese magistrate Sung Tz'u wrote a treatise entitled 'Xi Yuan Ji Lu' that is usually translated as 'The Washing Away of Wrongs', and this is generally accepted as being the first forensic textbook (Peng & Pounder, 1998). Sung Tz'u would also appear to be the first person to apply an understanding of biology to a criminal investigation as he relates how he identified the person guilty of a murder by observing the swarms of flies attracted to the bloodstains on the man's sickle. In Europe, medical knowledge advanced slowly over the centuries and forensic medicine really only started to be identified as a separate branch of medicine in the 1700s (Chapenoire & Benezech, 2003). The French physician Francois-Emanuel Foderé (1764–1835) produced a landmark 3 volume publication in 1799 entitled Les lois éclairées par les sciences physiques: ou Traité de médecine-légale et d'hygiène publique that is recognised as a major advancement in forensic medicine. In 1802, the first chair in Forensic Medicine in the UK was established at Edinburgh University and in 1821 John Gordon Smith wrote the first book on forensic medicine in the English language entitled 'The Principles of Forensic Medicine'.

Today, forensic medicine is a well-established branch of the medical profession. Clinical forensic medicine deals with cases in which the subject is living (e.g. non-accidental injuries, child abuse, rape) whilst forensic pathology deals with investigations into causes of death that might result in criminal proceedings (e.g. suspected homicide, fatal air accident). Pathology is the study of changes to tissues and organs caused by disease, trauma and toxins etc. Theoretically, any qualified medical doctor can perform an autopsy but in practise, at least in the UK, they are conducted by those who have received appropriate advanced training.

The majority of deaths are not suspicious so an autopsy is unlikely to take place. Indeed, even if a doctor requests an autopsy, the relatives of the dead person must give their permission. Some religious groups are opposed to autopsies and/or require a person to be buried within a very short period of death so this may be refused. For example, many Muslims, orthodox Jews and some Christian denominations remain opposed to autopsies. Some doctors are concerned about how few autopsies take place since it is estimated that 20-30% of death certificates incorrectly state the cause of death (Davies et al. 2004). The errors are seldom owing to incompetence or a 'cover-up' but a consequence of the difficulty of diagnosing the cause of death without a detailed examination of the dead body. Unfortunately, there are rogue elements in all professions and Dr Harold Shipman is believed to have murdered over 200 mostly elderly patients over the course of many years through the administration of morphine overdoses and then falsified their death certificates (Pounder, 2003). Dr Shipman's victims suffered from a range of chronic ailments and because of their age and infirmities nobody questioned the certificates he signed. In addition, he also falsified his computer patient records so that it would appear that the patient had suffered from the condition that he claimed had led to their death. He would sometimes do this within hours of administering a fatal dose of morphine. Ultimately, suspicions were aroused and several of his victims who had been buried were disinterred and subjected to an autopsy. The findings indicated that although they may have been infirm they had not died as a consequence of disease. They did, however, contain significant amounts morphine: morphine residues can be detected in buried bodies for several years after death. Dr Shipman had

therefore, surprisingly for a doctor, chosen one of the worst poisons in terms of leaving evidence behind. Dr Shipman was found guilty of murdering 15 of his victims in January 2000 and subsequently committed suicide whilst in prison.

In England and Wales, when a body is discovered in suspicious circumstances the doctor issuing the death certificate or the police will inform the coroner and they can then request that an autopsy is performed regardless of the wishes of the relatives. In this case, the autopsy will usually be undertaken by one of the doctors on the Home Office List of pathologists. As of 1 April 2006, there were 38 of these each of whom covered one of 8 regions of England and Wales. The name is a bit of a misnomer because although they are accredited by the Home Office, they are not employed by the Home Office. Scotland has its own laws and the Procurator Fiscal is the person who decides whether a death should be considered suspicious and also whether one or two pathologists should conduct the autopsy. [In England and Wales the pathologist usually works on their own.]. The situation in Northern Ireland is slightly different again with pathology services provided by The State Pathologist's department. Other countries have their own arrangements and there are calls for a thorough overhaul for the provision of forensic services in England and Wales and of the coroner system in particular (e.g. Whitwell, 2003).

Animals and plants have always played a role in human affairs, quite literally in the case of pubic lice, and have been involved in legal wrangles ever since the first courts were convened. Disputes over ownership, the destruction of crops and the stealing or killing of domestic animals can be found in many of the earliest records. For example, Hammurabi, who reigned over Babylonia during 1792-1750 BC, codified many laws relating to property and injury that subsequently became the basis of Mosaic Law. Amongst these laws it was stated that anyone stealing an animal belonging to a freedman must pay back ten fold whilst if the animal belonged to the court or a god, then he had to pay back thirty fold. Animals have also found themselves in the dock accused of various crimes. In the Middle Ages there were several cases in which pigs, donkeys and other animals were executed by the public hangman following their trial for murder or sodomy. The judicial process was considered important and the animals were appointed a lawyer to defend them and they were tried and punished like any human. In 1576, the hangman brought shame on the German town of Schweinfurt by publicly hanging a pig in the custody of the court before due process had taken place. He never worked in the town again and his behaviour is said to have given rise to the term 'Schweinfurter Sauhenker' (Schweinfurt sow hangman) to describe a disreputable scoundrel (Evans, 1906). However, the phrase has now fallen out of fashion. Today, it is the owner of a dangerous animal who is prosecuted when it wounds or kills someone, although it may still find itself facing the death penalty.

During the nineteenth century, a number of French workers made detailed observations on the sequence of invertebrate colonisation of human corpses in cemeteries and attempts were made to use this knowledge to determine the time since death in murder investigations (Benecke, 2001). Thereafter, invertebrates were used to provide evidence in a sporadic number of murder investigations but it was not until the 1980s that their potential was widely recognised. Part of the reason for the slow development is the problem of carrying out research that can be applied to real case situations. The body of the traditional experimental animal, the laboratory rat,

bears so little resemblance to that of a human being that it is difficult to draw meaningful comparisons from its decay and colonisation by invertebrates. Pigs, and in particular foetal pigs are therefore the forensic scientists' usual choice of corpse although America (where else?) has a 'Body Farm' in which dead humans can be observed decaying under a variety of 'real life (death?) situations' (Bass & Jefferson, 2003). Leaving any animal to decay inevitably results in a bad smell and attracts flies – so it requires access to land far from human habitation. It also often requires the body to be protected from birds, dogs, and rats that would drag it away. Consequently, it is difficult both to obtain meaningful replicates and to leave the bodies in a 'normal' environment. Even more importantly, these types of experiments conflict with European Union Animal By Products Regulations that require the bodies of dead farm and domestic animals to be disposed of appropriately to avoid the spread of disease – and leaving a dead pig to moulder on the ground clearly contravenes these.

The use of animals other than insects in forensic investigations has proceeded more slowly and that of plant-based evidence has been slower still. The first use of pollen analysis in a criminal trial appears to have taken place in 1959 (Erdtman, 1969) and although not widely used in criminal trials since then its potential is now being increasingly recognised (Coyle, 2004). By contrast, the use of plants and other organisms in archaeological investigations has been routine for many years. Microbial evidence has seldom featured in criminal trials although this is likely to change with the development of new methods of detection and identification and the concerns over bioterrorism.

By contrast to the slow progress in the use of animal and plant-based evidence, the use of molecular biology in forensic science is now well established and it is an accepted procedure for the identification of individuals. This is usually on the basis of DNA recovered from blood and other body fluids or tissues such as bone marrow and Jobling & Gill (2004) provide a thorough review of current procedures and how things may develop in the future. The use of molecular biology for forensic examination of non-human DNA is less advanced, although this situation will probably improve in the near future as DNA databases become established (Coyle, 2007). When this happens, animals and plants can be expected to play a larger part in legal proceedings.

One of the major stumbling blocks to the use of biological evidence in English trials is the nature of the legal system (Pamplin, 2004). In a criminal prosecution case, the court has to be sure 'beyond all reasonable doubt' before it can return a guilty verdict. The court therefore requires a level of certainty that science can rarely provide. Indeed, science is based upon hypotheses and a scientific hypothesis is one that can be proved wrong – provided that one can find the evidence. Organisms are affected by numerous internal and external factors and therefore the evidence based upon them usually has to have qualifications attached to it. For example, suppose the pollen profile found on mud attached to the suspect's shoes was similar to that found at the site of the crime: this suggests a possible association but it would be impossible to state beyond reasonable doubt that there are not other sites that might have similar profiles – unlikely perhaps, but not beyond doubt. Lawyers are, quite correctly, experts at exploiting the potential weaknesses of biological evidence because it is seldom possible for one to state there is no alternative explanation for

Table A Questions arising when a body or stains are found in suspicious circumstances

Are the remains or stains of human origin?
Who is the victim?
What was the cause of death?
How long ago did the victim die?
Did the victim die immediately or after a period of time – and if so, how long?
Did the person die at the spot where their body was found?
Did the person die of natural causes, an accident or a criminal act?
If the person was killed as a result of a criminal act who was responsible?

the findings or an event would never happen. Within civil courts, biological evidence has greater potential since here the 'burden of proof' is based upon 'the balance of probabilities'.

Although all biological evidence has its limitations, it can prove extremely useful in answering many of the questions that arise whenever a body is found under suspicious circumstances. The first question is, of course, are the remains human? This might be obvious if the body is whole and fresh or even if there is just a skull but sometimes there may be no more than a single bone or some old bloodstains. Assuming that the remains are human, biological evidence can also help to answer the subsequent questions (Table A).

Similar sorts of questions arise in the cases of wildlife crime (e.g. killing of / trade in protected species), neglect of humans and domestic animals, miss-selling of animal products, and food contamination. This book is intended to demonstrate how an understanding of biology can answer all these questions and is designed for undergraduates who may have a limited background in biology and not the practicing forensic scientist. I have therefore attempted to keep the terminology simple whilst still explaining how an understanding of biological characteristics can be used to provide evidence. Descriptions of potential sources of biological evidence and tests that could be performed upon it continue to grow at a bewildering rate. However, to be truly useful any test / source of evidence should be accurate, simple, affordable, and deliver results within an acceptable time period (Table B). With such a large subject base, it is impossible to cover all topics in depth and readers wishing to identify a maggot or undertake DNA analysis should consult one of the more advanced specialist texts in the appropriate area. Similarly, those wishing more detailed coverage of individual cases would be advised to consult the excellent books by Erzinclioglu (2000), Goff (2000), Greenberg & Kunich (2002) and Smith (1986). Where information would not otherwise be easily accessible to undergraduate students, I have made use of web-based material although the usual caveats apply to such sources.

At the start of each chapter, I have produced a series of 'objectives' to illustrate the material covered. They are written in the style of examination essay questions, so that the reader might use them as part of a self-assessment revision exercise. Similarly, at the end of each chapter I have produced a number of questions to test knowledge and recall of factual information. Also at the end of each chapter, I have made some suggestions for undergraduate projects. Because the usefulness of bio-

#### Table B Characteristics of an ideal forensic test

Accurate: The results must stand up to intense scrutiny in court

Sensitive: Many forensic samples are extremely small and are finite (i.e. one cannot collect more material once it used up)

Specific: If the test also cross-reacts with other materials then its accuracy will be compromised.

Quick: Investigations cannot be allowed to drag on. If there is a chance that a criminal might offend again they must be apprehended and charged as soon as possible. It is also unfair to deprive a suspect of their liberty for long periods whilst time-consuming tests are conducted.

Simple: The more complex a test becomes the more opportunity there is for mistakes to be made. It also becomes expensive to train people to conduct the tests.

**Reliable and repeatable:** It is essential that a test can be replicated by other workers at other laboratories.

**Affordable:** Financial considerations are important. Exceedingly expensive tests cannot be used on a routine basis.

Equipment and reagents are readily available: The effectiveness of the test will be compromised if equipment becomes unusable through lack of spare parts or reagents are difficult to obtain.

logical material as forensic evidence depends on a thorough understanding of basic biological processes and the factors that affect them, there is plenty of scope for simple projects based upon identifying species composition or that measure growth rates. Obviously, for the majority of student projects cost, time and facilities will be serious constraints; DNA analysis can be extremely expensive and requires specialist equipment. Similarly, the opportunities to work with human tissues or suitably sized pigs may not exist. However, worthwhile work can still be done using the bodies of laboratory rats and mice or meat and bones bought from a butcher as substitute corpses with plants and invertebrates as sources of evidence.