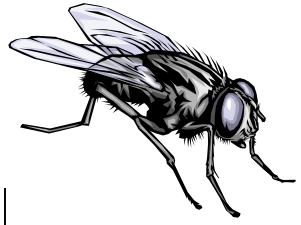


An Introduction to Forensic Entomology Part II

Dead do tell tales……

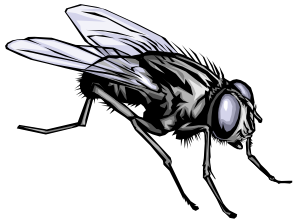
Prof. Abdelwahab A. Ibrahim

- Did you know…

The "Body Farm"

in Knoxville, Tennessee

is a university research facility to investigate human decomposition under various conditions in order to understand the factors which affect its rate.



Post-Mortem Interval (PMI)……

Post-Mortem Interval (PMI)……

- PMI = Time since death (Actually, time since initial colonization by insects).
- Most entomologically useful range is 2 – 4 weeks

- PMI estimation is essential in suspicious death investigation to reconstruct events and circumstances of death, to link a suspect to the victim, and to establish the credibility of statements made by witnesses.
- It is not just useful in criminal cases but also in civil cases.
- Indeed, even when the death is natural, accidental, or a suicide, such estimates can have judiciary implications in questions of insurance and inheritance

Post-Mortem Interval (PMI)…… (early PM period)

- A pathologist usually determines time since death in the early postmortem period based on the postmortem changes in soft tissues such as:
- **Stiffness or** rigidity of skeletal muscles **(rigor mortis),**
- skin discoloration caused by pooling of blood **(livor mortis),**
- **body cooling rate (algor mortis),**

$$\text{Hours since death} = \frac{98.4°F - \text{internal body temperature}}{1.5}$$

- **and stages of decomposition**.
- However, the sequence of such changes can only approximate how long the individual has been dead.
- Many factors affect human decomposition, some directly associated with the body (e.g. age, constitution, integrity of the corpse, cause of death) and others associated with the environment (e.g. temperature, ventilation, air humidity, clothing, access of the body to animals).

Time of Death can be broadly estimated up to about 36 hours

Temperature	Stiffness	Time of death
Warm	Not stiff	Dead less than three hours
Warm	Stiff	Dead between 3 to 8 hours
Cold	Stiff	Dead between 8 to 36 hours
Cold	Not stiff	Dead in more than 36 hours

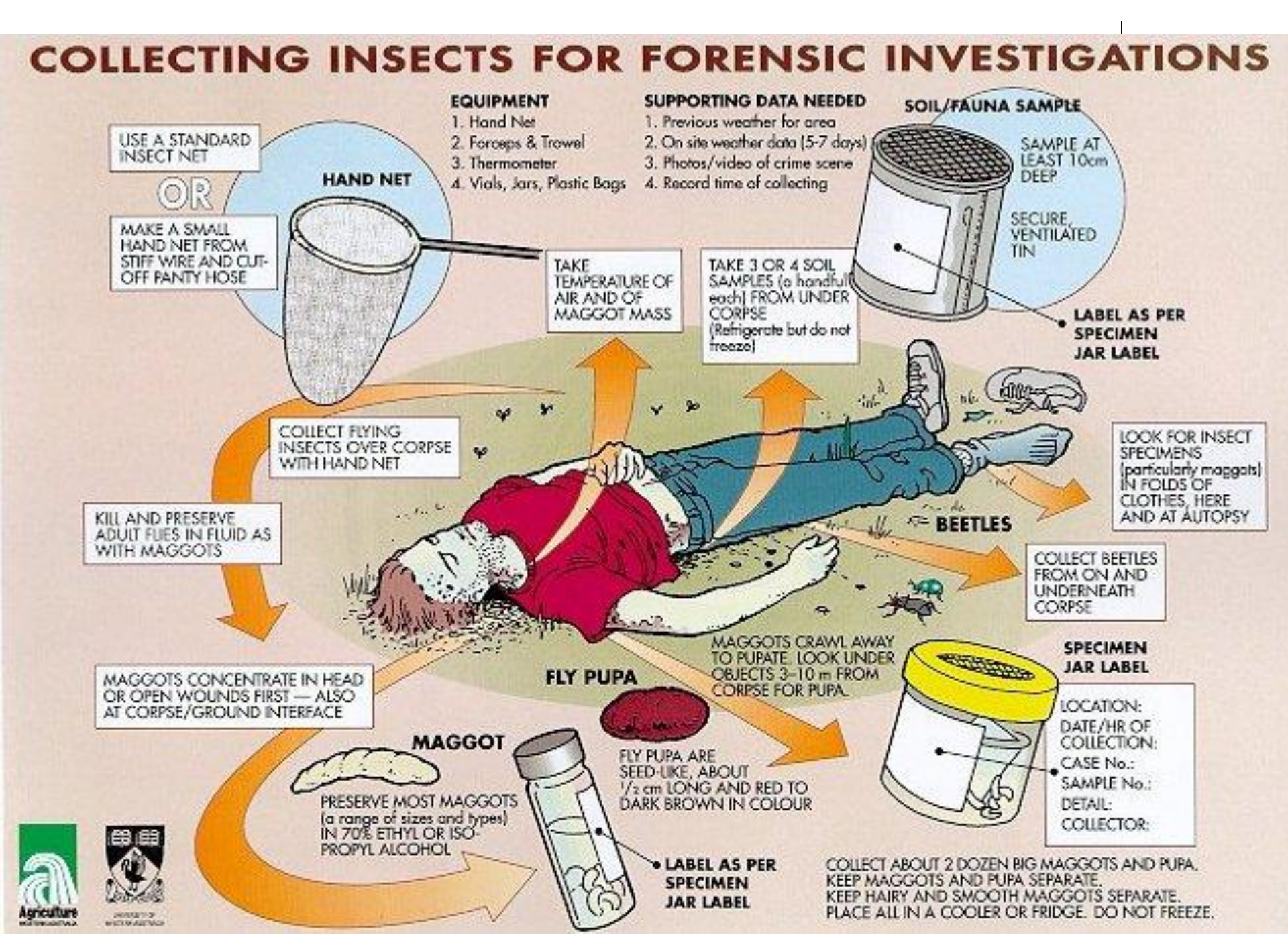
Post-Mortem Interval (PMI)……

- The accurate assessment of death chronology is not possible more than 1–3 days postmortem (i.e. the early postmortem period), depending on the availability of equipment, the nature of the circumstances of the death and the environment.
- The longer the actual PMI, the less accurate the estimate of the interval.
- Beyond that period, the entomological evidence associated with and around the corpse becomes much more important and can indicate the time elapsed since death up to a period of several weeks or even months.

Determining the PMI

- **Step 1. Collect samples of the insects present.**
  - Critical to collect the largest maggots on the cadaver, even if they are few.
  - Representative samples of other maggots present.
  - Preserve some immediately. Rear others to adults to confirm species ID's
- **Step 2. Determine temperature history at crime scene**
  - Air temperature for general area (airport readings, validate with micro data-loggers).
  - Look for 'windows' of insect opportunity
- **Step 3. Estimate time of egg laying**
  - Given the species present and their age (size), how long did it take them to develop to that point at the temperatures occurring in the area.
- **Step 4. What other insect evidence is available?**
  - Look for other evidence that might corroborate or contradict your PMI estimate.

COLLECTING INSECTS FOR FORENSIC INVESTIGATIONS




Maggot
Mass

Estimating the PMI

For each maggot mass:

- Kill ½ of the maggots collected to stops growth and Preserve in ethanol to prevents degradation
- Keep ½ of the maggots alive for rearing to adults for identification

?

Chrysomya megacephala

Estimating the PMI

Once insects and Climatological Data are collected (both adults and immature), they are used to estimate the postmortem interval (PMI).

Estimating the PMI

Analysis of Insects



Once evidence is received, the first step is to identify the species of samples.

Estimating the PMI
Identification of Insects

Question:

Why is species identification important?

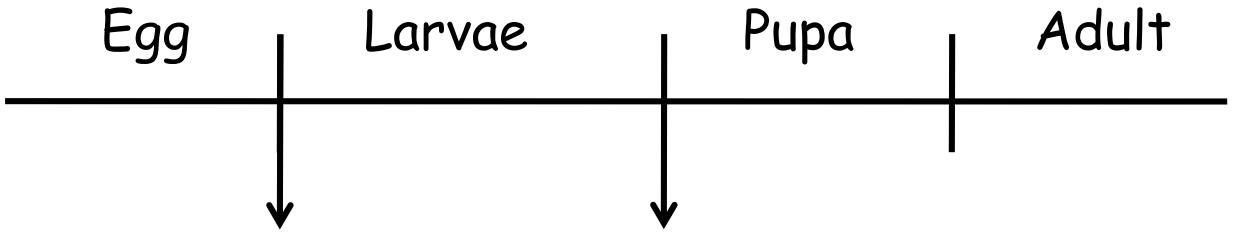
Answer:

Different species grow at different rates

7-mm maggot may be anywhere from 3 days old to 10 days old, depending on species

Estimating the PMI

Timeline:



Sarcophagid bullata	50 hours	110 hours
Sarcophagid cooleyi	40 hours	90 hours

Estimating the PMI

Identification of the specimen

Morphology

- Color, hairs and bristles, wing structure
- Male genitalia

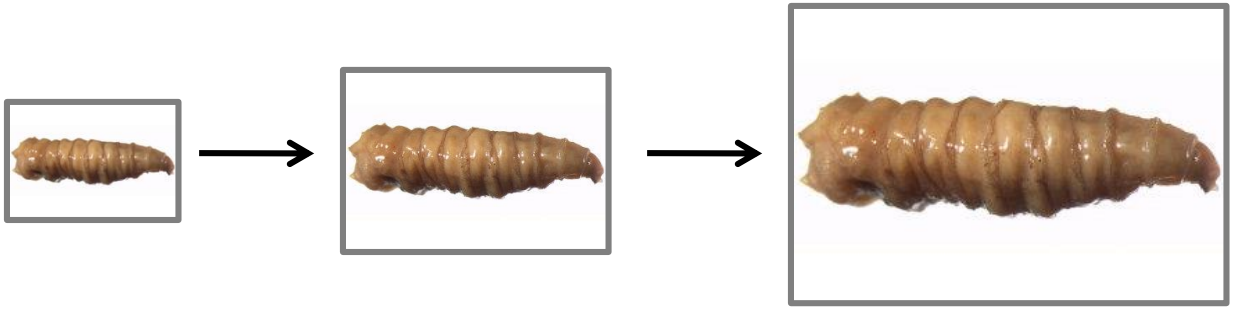
DNA barcoding

- Nuclear DNA versus Mitochondrial DNA
- Cytochrome c oxidase subunit I (COI) as DNA barcoding marker
- **Steps for DNA barcoding:** (DNA extraction, Amplification, Sequencing, Sequence analysis, Microarray

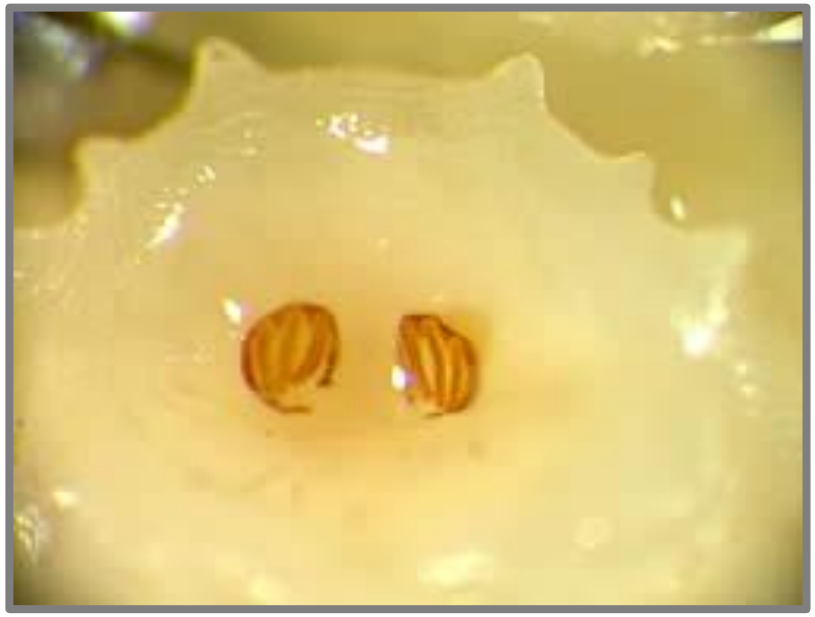
Estimating the PMI

If the samples are maggots, the molting stage can be identified.

Larvae

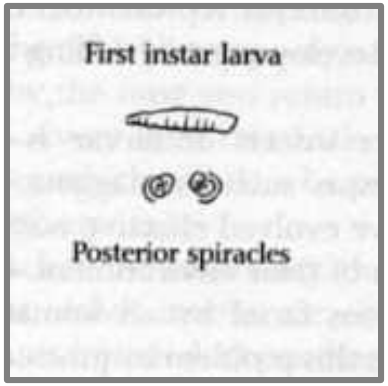


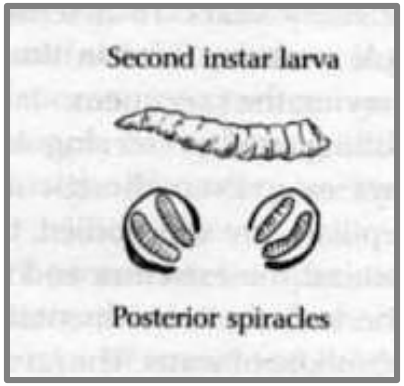
- Larvae grow in three stages called **instars**.
- During the 3rd instar, maggots will stop feeding, leave corpse, and pupate.

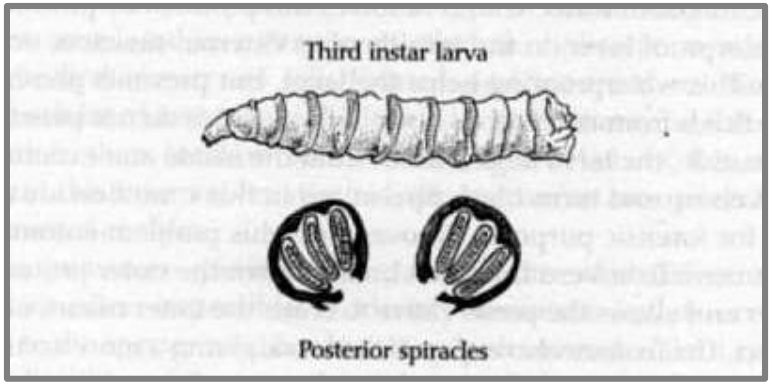


Many maggot instars can be determined by looking at spiracles.

Estimating the PMI







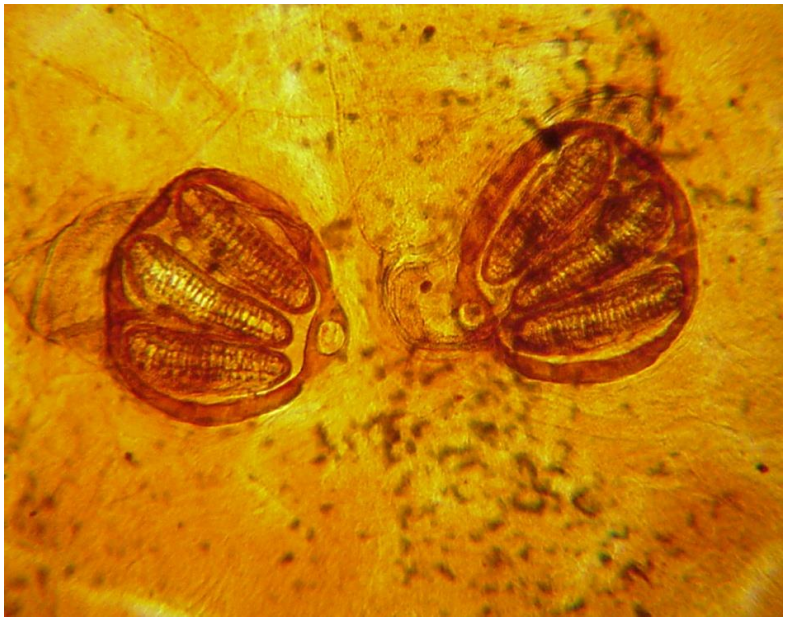
- 3 "footballs" = 3rd instar
- 2 "footballs" = 2nd instar
- Glob = 1st instar

Estimating the PMI

Phaenicia sp

Spiracles are complete

Third-instar larvae

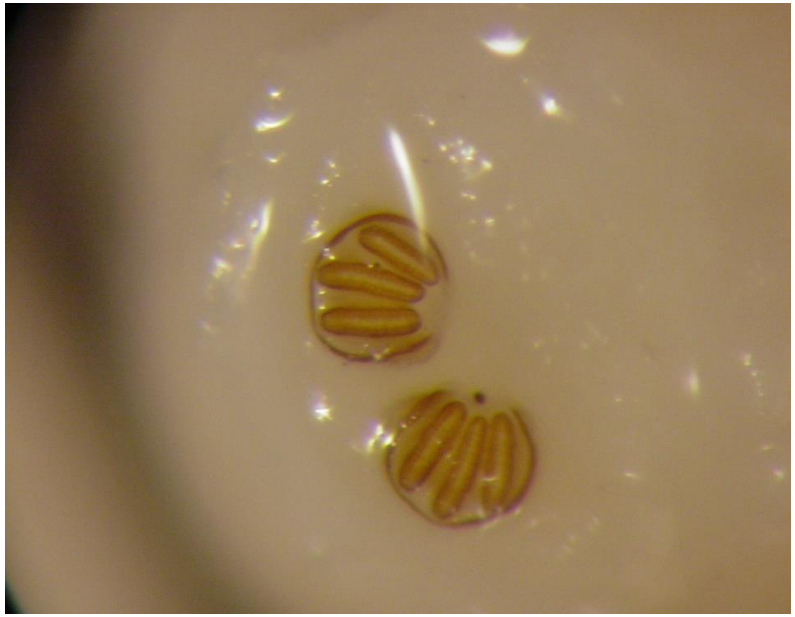


Estimating the PMI

Phormia regina

Spiracles are incomplete

Third-instar larvae



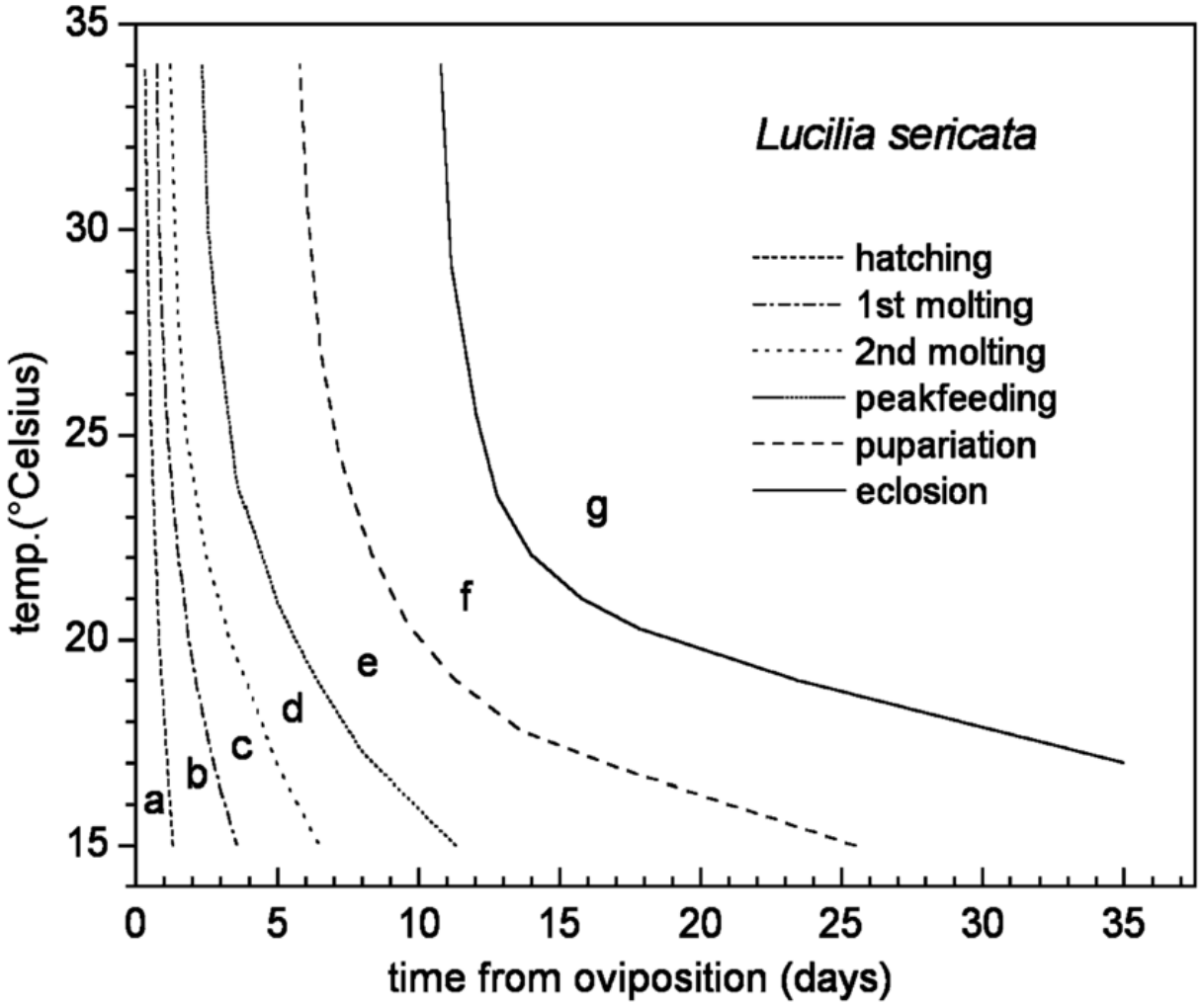
Determining PMI

- Forensic Entomologists use different ways in PMI determination
  - The isomegalen and isomorphen diagrams
  - Controlled rearing
  - Degree-Day Accumulation
  - Arthropod succession patterns
- Situation dictates which is best solution

Determining PMI (Isomegalen/isomorphen diagram)

- The development of insects can be visualized in growth curves for various constant temperatures, for example in the isomegalen and isomorphen diagrams that illustrate growth (length and weight) during development of the fly depending on time and temperature.
- If the temperature is roughly constant, as in the case of some corpses found indoors, the age of the maggot could be read off instantly from its length.
- If temperature is variable, as in the case of corpses found outdoors, an age range can be estimated between the points where the observed morphological change (hatching, pupariation, eclosion or particular size) cuts the graph at the maximum and minimum temperatures recorded.

Isomorphen diagram



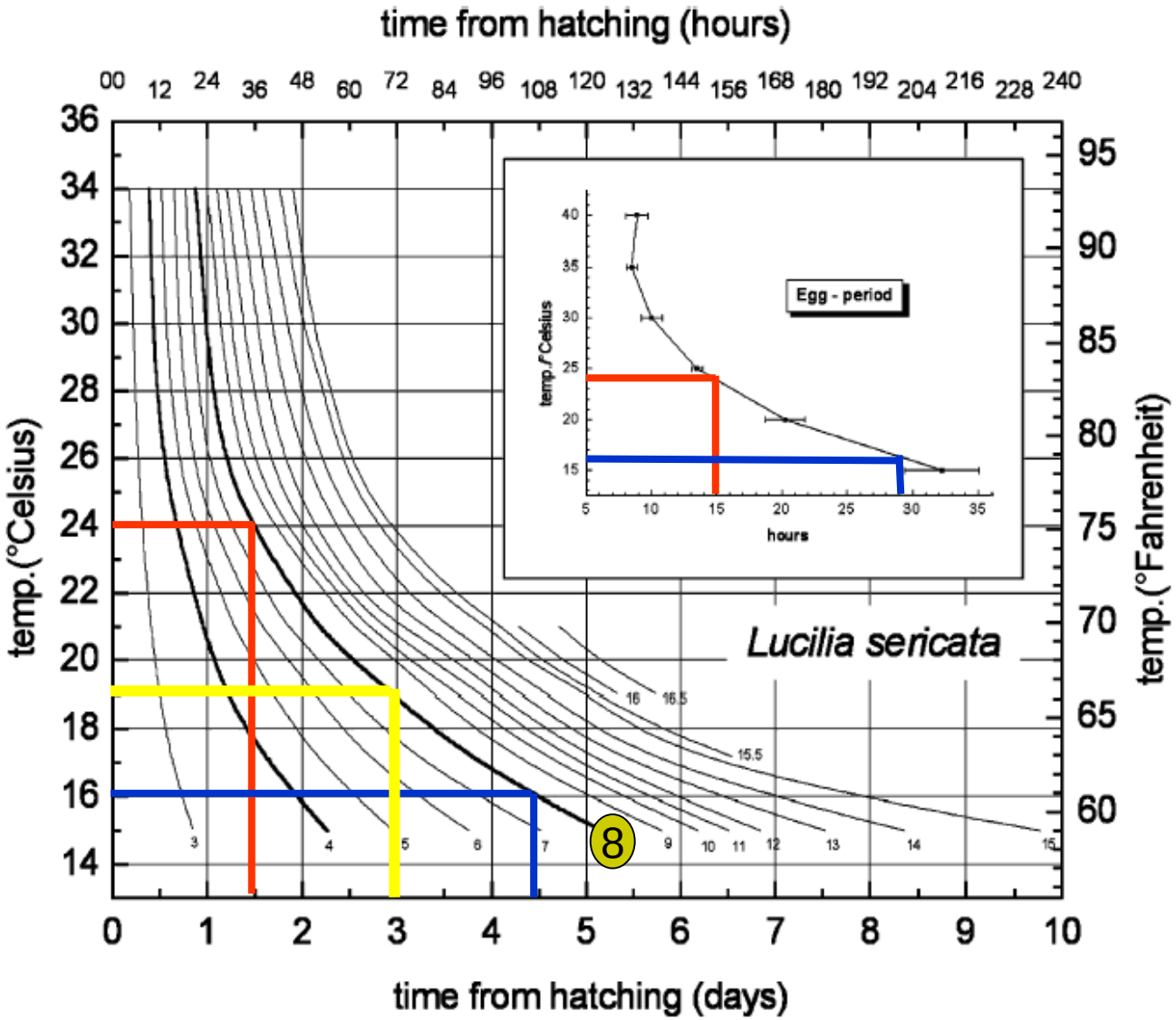
Determining PMI by using larval length (Isomegalen/isomorphen diagram)

- In the literature, there are several isomegalen/isomorphen diagrams available for the species of most common forensic insects such as *Calliphora, Lucilia, Chrysomya, Protophormia.*
- PMI estimate based on larval size can be misleading if account is not taken of the decrease in length preceding pupariation, or of the potential shrinkage effect of certain killing and preservative solutions.
- PMI also depends on the conditions in which the measurements of case specimens are carried out, for example, if data was prepared from boiled and fully extended specimens immediately after death, then the case specimens must be prepared in the same way.

Determining PMI by using larval length (Isomegalen/isomorphen diagram)

- A second type of graph can be used, which is derived where life cycle stages from egg hatch to the time of emergence of the adult (eclosion) have been plotted against time, at specific temperatures.
- Each line indicates a change in life cycle to the next stage.
- The areas between the lines relate to the identical morphological stages, these are called isomorphen diagrams.
- Isomorphen diagrams are useful when post-feeding larvae and/or puparia are collected from the crime scene.
- From these stages, the post mortem interval can be read directly off the graph, provided that the temperature has been constant.

Isomegalen Diagram for *Phaenicia* (= *Lucilia*) *sericata*



8 mm maggot
24 C daily max.
16 C daily min.

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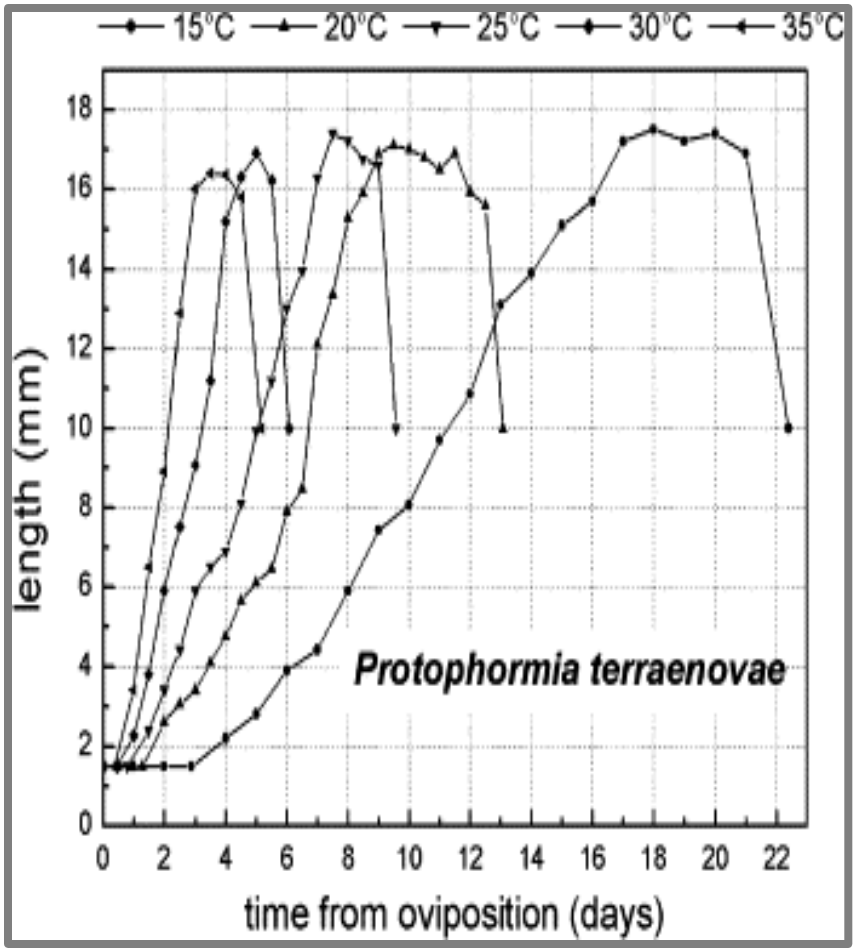
Estimating the PMI by Analysis of length of Immature stages



- PMI estimation can be made based on maggots size or development in pupa.
- Species and temperature must be known.

Determining PMI (Accumulated degree hours/days)

- Because the rate of development of the immature stages depends essentially on the ambient temperature, the age of specimens is positively correlated with the summed heat they accumulate during growth.
- The thermal budget of each specimen can be expressed in ADH or ADD, i.e. temperature (°C) above base temperature multiplied by time (hours or days).
- The relation between the rate of development and ambient temperature is represented by a curve that is essentially linear in the midrange of a sigmoidal curve, with UDT and LDT above and below which development ceases.
- The total thermal input required for an insect to develop from the time of oviposition to any stage in development, e.g. the time of adult emergence, can be calculated in terms of ADH or ADD.

Determining PMI (Accumulated degree hours/days)

- The use of ADH/ADD is based on the hypothesized linear relationship between temperature and development. However, its use only appears to be valid when experimental temperatures used to generate the ADHs/ADDs are similar to the temperatures encountered in any actual forensic case.
- As the temperature range under study increases, the relationship between temperature and development becomes less linear, particularly at the extremes of the optimum range and at low temperatures.
- Each developmental stage has its own total temperature requirement and each species requires a specific number of degree-hours/days to complete its development at a defined temperature.
- Data available in the literature for most common flies of forensic importance show differences of developmental times that can be attributed to variation, not only in extrinsic factors (e.g. experimental method) but also in intrinsic factors (e.g.geographic diversity of species physiology).

Determining PMI (Accumulated degree hours/days)

- Although the ADH/ADD concept can be very useful in estimating the age of larvae in forensic cases, it must be used with appropriate caution because of:
- the inherently large variations in ADH/ADD that have been measured across the temperature range, for example, the differences in ADH measured for *L. sericata* at different temperatures were as great within publications as between publications
- the variation that has been observed in ADD/ADH at particular temperatures, for example, at low temperatures for *C. vicina* .The variation seen in these figures could, in part, be due to variation in the base temperatures of different geographical populations of flies and it emphasizes the importance of using, where possible, local developmental data to estimate the age of insect evidence.

Degree-Day Accumulation

- Using a simple formula to calculate degree-day accumulation for a species with a 10 C° threshold

$$\frac{(DailyMax-10)+(DailyMin-10)}{2}$$

*If max or min is less than 10, then enter zero

Cmax	Cmin	dd,base10	cum,10			
26.11	14.44	10.28	10.28			
23.89	13.89	8.89	19.17			
25.56	15.56	10.56	29.72			
26.67	19.44	13.06	42.78			
27.78	18.89	13.33	56.11			
28.33	20.00	14.17	70.28			
28.33	21.67	15.00	85.28			

Another formula

Time (hours) × (temperature–base temperature) =ADH

Time (days) × (temperature–base temperature) =ADD

Determining PMI (Arthropod succession patterns)

- The study of arthropod succession enables scientists to associate each species or group to a well-established decomposition stage.
- Knowing the chronology of insects colonizing carrion in a certain area, analysis of the fauna on a carcass can be used to give a rough approximation of the PMI in the late postmortem period (cadavers in an advanced stage of decay).
- Forensically useful timetables indicating the relative abundance of different insect groups at different times are available, but much more work needs to be done in this area.
- Despite the efforts of researchers to master all the variables affecting the composition of the carrion arthropod community, the PMI assessed with such method is, however, still very approximate: the longer the PMI, the less precise the PMI estimate becomes.

Controlled rearing

- Collect sample from crime scene
- In the lab, rear until adults emergence under conditions similar to that of maggot mass.
- Determine the ADD (or ADH) required to complete development after collection.
- Subtract this value from the total required for the species to determine unknown amount accumulated since oviposition.
- Count back the days (or hours) prior to collection necessary for the maggot to reach the stage at which it was sampled.

Data for Controlled Rearing

Species	Lower Threshold (°C)	ADH	ADD
Phaenicia sericata	10	4140 - 5812	173 - 242
Phormia regina	10	4038 - 6100	168 - 254
Calliphora vomitoria	6	17678	737
Cynomyopsis cadaverina	6	5511	379

Complete lifecycle: egg-adult

Calculating PMI from Accumulated Degree Hours (ADH)

From	To	Temp	Hours	ADH	Cumulative ADH
Egg	1st Instar	70° F	23	23 x 70= 1610 ADH	1610
1st Instar	2nd Instar	70 ° F	27	27 x 70= 1890 ADH	1610+ 1890
2nd Instar	3rd Instar	70 ° F	22	22 x 70= 1540 ADH	1610+1890+ 1540
3rd Instar	Pupa	70 ° F	130	130 x 70= 9100 ADH	1610+1890+ 1540+9100
Pupa	Adult Fly	70 ° F	143	143 x 70= 10010 ADH	1610+1890+ 1540+9100 +10010

Using the Data

- If ADH in three days (952+1488+1488) is 3928 .
- How many ADH of 70° are there in these 3 days?
- 3928/70=56.11 hours
- At 70° the insects have 72 hours this means that the insects are passing to the 3rd instar. But 72 hours at colder temperatures and insects will only be at 2nd instar stage.

Notice:

- It is important to consider using the temperature of the maggot mass as the temperature for larval development in particular instars.
- If maggot mass temperature was recorded as greater than the ambient temperature, the temperature of the mass should be used in the calculations. This is true where third or potentially late second instar larvae are recovered from the body, as the maggot mass temperature may be the highest temperature experienced by the larvae.
- If puparia are recorded, the crime scene soil temperature at 5, 10 and 20 cm depth should be used to adjust the estimated crime scene air temperatures, for the period likely to reflect the time the insect was in pupariation.
- Converting temperatures :
- $T_c = (5/9) \times (T_f - 32)$
- $T_f = [(9/5) \times T_c] + 32$

PMI Calculation Example.

Body is found with *Lucilia sericata* larvae, prepupae, and pupae (in soil next to body). Temps at site had averaged 16° C. Pupae brought into the lab & held at that temp. began to eclose after 112 hours

Development rate of sheep blowflies, *Lucilia sericata,* (in hours) at three different temperatures

Temp(°C)	Egg	Larva 1st Instar	Larva 2nd Instar	Larva 3rd Instar	Pre-pupa	Pupa	Total time (days)
16	41	53	42	98	148	393	32
21	21	31	26	50	118	240	20
27	18	20	12	40	90	168	14

PMI = (41 + 53 + 42 + 98 + 148 + 393 – 112)/24 = **26.5 days**

The relationship between time of death and physical breakdown of the body

- Giertsen (1977) cited Casper's Dictum as a means of determining the length of the post mortem interval. This rule says that:
- The degree of putrefaction present in a body lying in the open air for one week (month) corresponds to that found in a body after lying in the water for two weeks (months), or lying in the earth in the usual manner for eight weeks (months)'.
- The reason for this difference in decomposition is that the speed at which the body loses heat in water is twice the speed at which the body will lose heat in air.

Succession of Insects on the Corpse

- Estimates of postmortem intervals based on insects present on the remains are based on:
  - The time required for a given species to reach a particular stage of development.
  - Comparisons of all insect species present on the remains at the time of examination.
- Ecological succession occurs as an unexploited habitat (like a corpse) is invaded by a series of different organisms.
- The first invasion is by insect species which will alter the habitat in some form by their activities. These changes make the habitat attractive to a second wave of organisms which, in turn, alter the habitat for use by yet another organisms.

Succession of Insects on the Corpse

- Beetles- **generally found on the corpse when it is more decomposed.**
  - **Rove Beetles - Family Staphylinidae**
  - **Hister Beetles - Family Histeridae**
  - **Carrion Beetles - Family Silphidae**
  - **Ham Beetles - Family Cleridae**
  - **Carcass Beetles - Family Trogidae**
  - **Skin/Hide Beetles - Family Dermestidae**
  - **Scarab Beetless - Family Scarabaeidae**
  - **Sap beetles - Family Nitidulidae**

THE END

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