

Read Ch  
15

# The Use of Molecular Tools in Biological Control

# Why use Molecular tools?

- Species and Biotype Recognition
- Determine Phylogenetic Relationships Between Different Taxa
- Determine the Area of Origin of an Invasive Population

# Differentiation Between Species

- Only a single sex of species can be morphologically identified
- Immature stages cannot be identified to species
- Long rearing stages
- Morphological identification is expensive, time consuming, and there are few taxonomic experts

# Species and Biotype Recognition

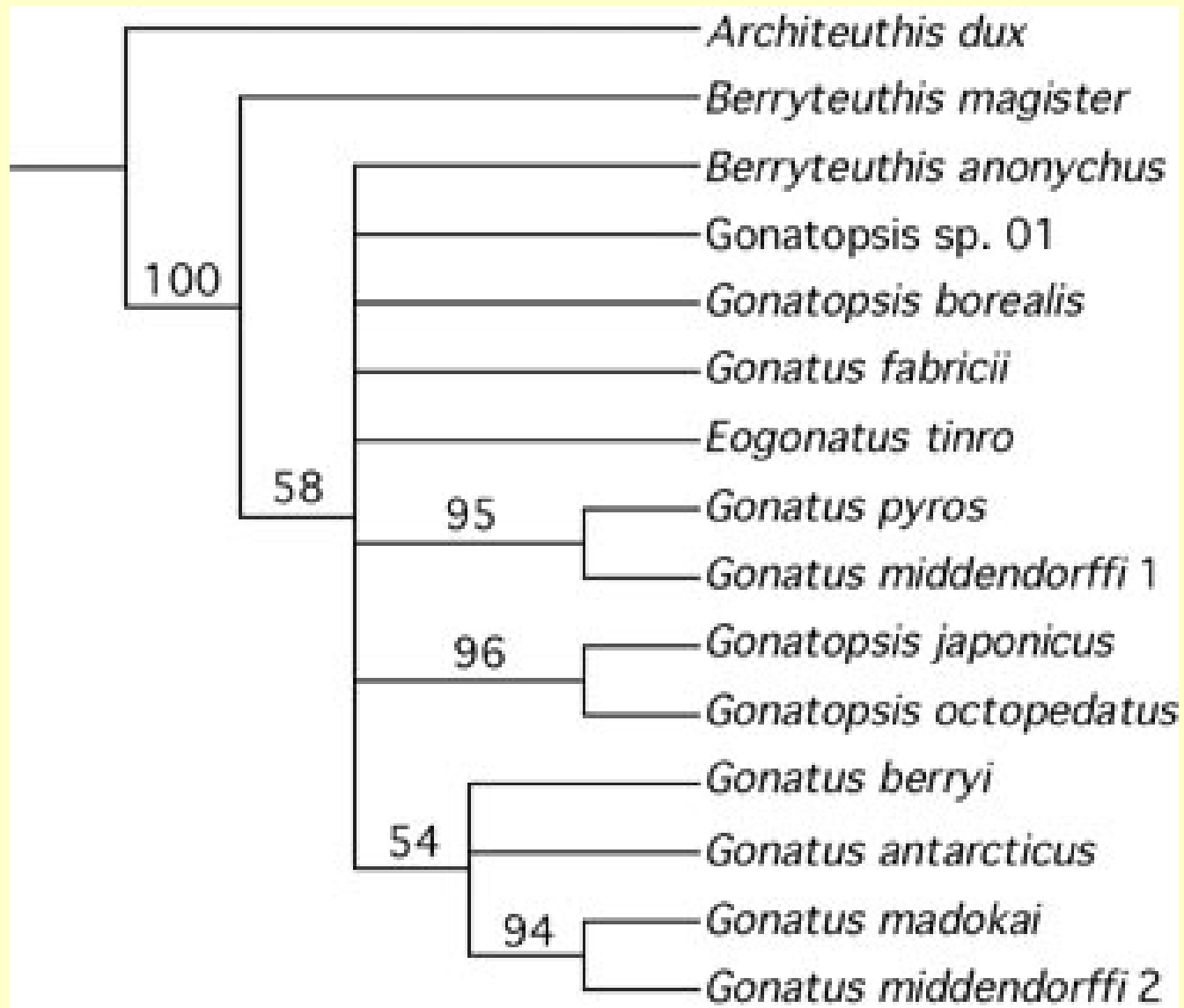


- Natural enemies are small and can have limited morphological characteristics



# Phylogenetic Relationships

- Extensive phylogenies of both the host and target pest can be useful in predicting life history characters of related species
- Help with selection of the most promising and host specific natural enemies



# Old World Climbing Fern



*Lygodium microphyllum*  
Old World Climbing Fern

- Fern and Mite have several different haplotypes
- Genetic relationship matched by geographical relationship



*Floracarus perrepae*  
Phytophagous mite

# Determining area of origin of invasive species

- Natural enemies adapted to source pest population are more likely to be synchronized with the pest
- Can lead to more efficient control of the pest

# Hemlock Woolly Adelgid

- Havill et. al (2005) used molecular methods to determine origin of introduction
- Phylogenetic analysis suggests that introduction to Eastern U.S. was from Japan



# Molecular Markers

- Isozymes
- RAPDS-Randomly Amplified Polymorphic DNA markers
- ISSR Markers-inter-simple sequence repeat markers
- Microsatellites
- Gene Sequences
  - DNA sequences of protein-coding, ribosomal RNA sequences, and mitochondrial genes

# Isozymes

- Enzymes that differ in amino acid sequence but catalyze the same reaction
- Typically related to each other because they originate through gene duplication
- Allozymes are different allelic forms of the same allozyme coding locus (a particular isozyme)

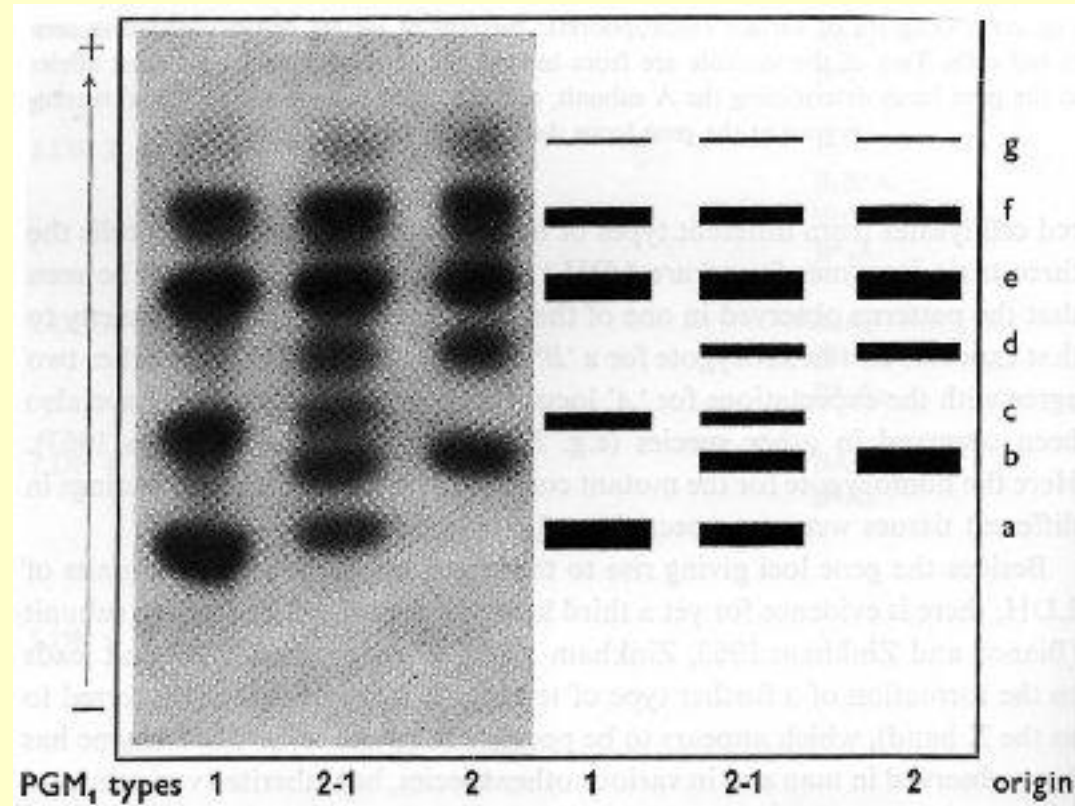
# Allozyme Markers

- Common in the 1970's and 80's
- Separate Allozymes on
  1. Gel Electrophoresis
  2. Isoelectric Focusing

Have typically been replaced by PCR-based methods

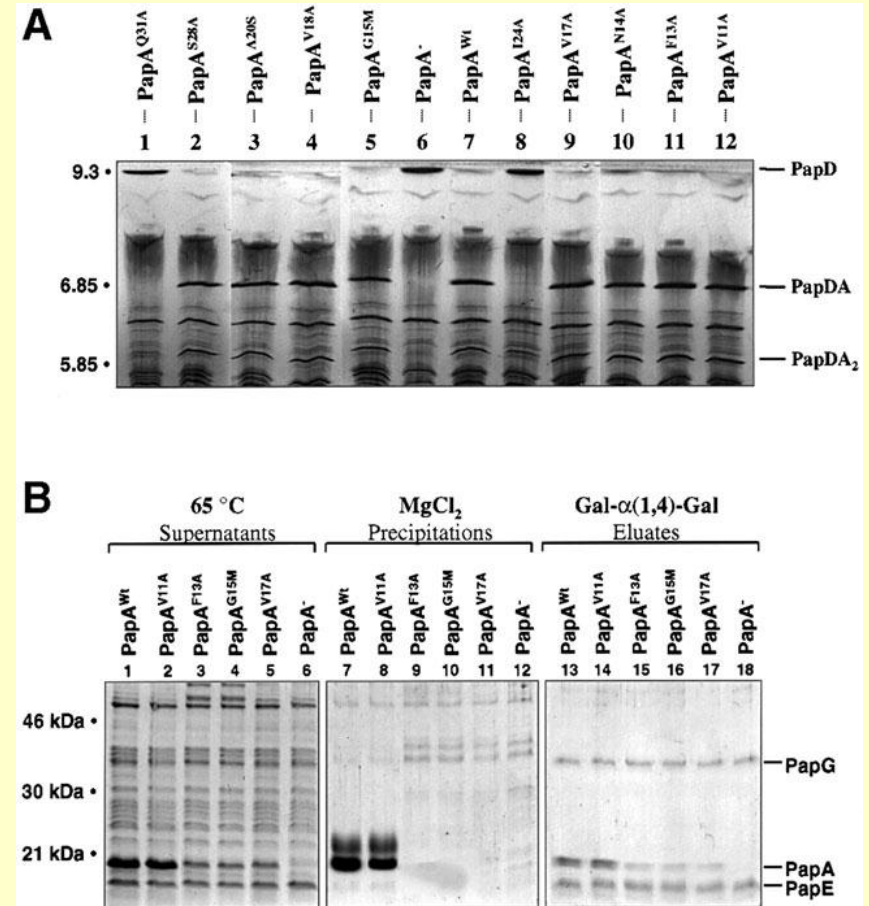
# Allozymes

- Gel Electrophoresis
- Freshly killed specimens homogenized and loaded on a gel
- Allozymes separated by size on a gel



# Isoelectric focusing

- Gradient of isoelectric points (in a buffer) on top of a membrane



# Allozymes use in Biocontrol

- Species/Biotype Recognition
- Population Genetic Studies
- Analysis of gut content of predators
- Determine if host insects are parasitized

# Allozyme use in Biocontrol

- Harwood and Obrycki (2005) evaluated levels of Bt toxin in gut contents of non-target herbivores
- Also evaluated levels of Bt in predators of non-target herbivores
- Looked for expression of Cry1Ab protein

- Detected significant quantities of Cry1Ab protein in both herbivores and predators



Herbivores

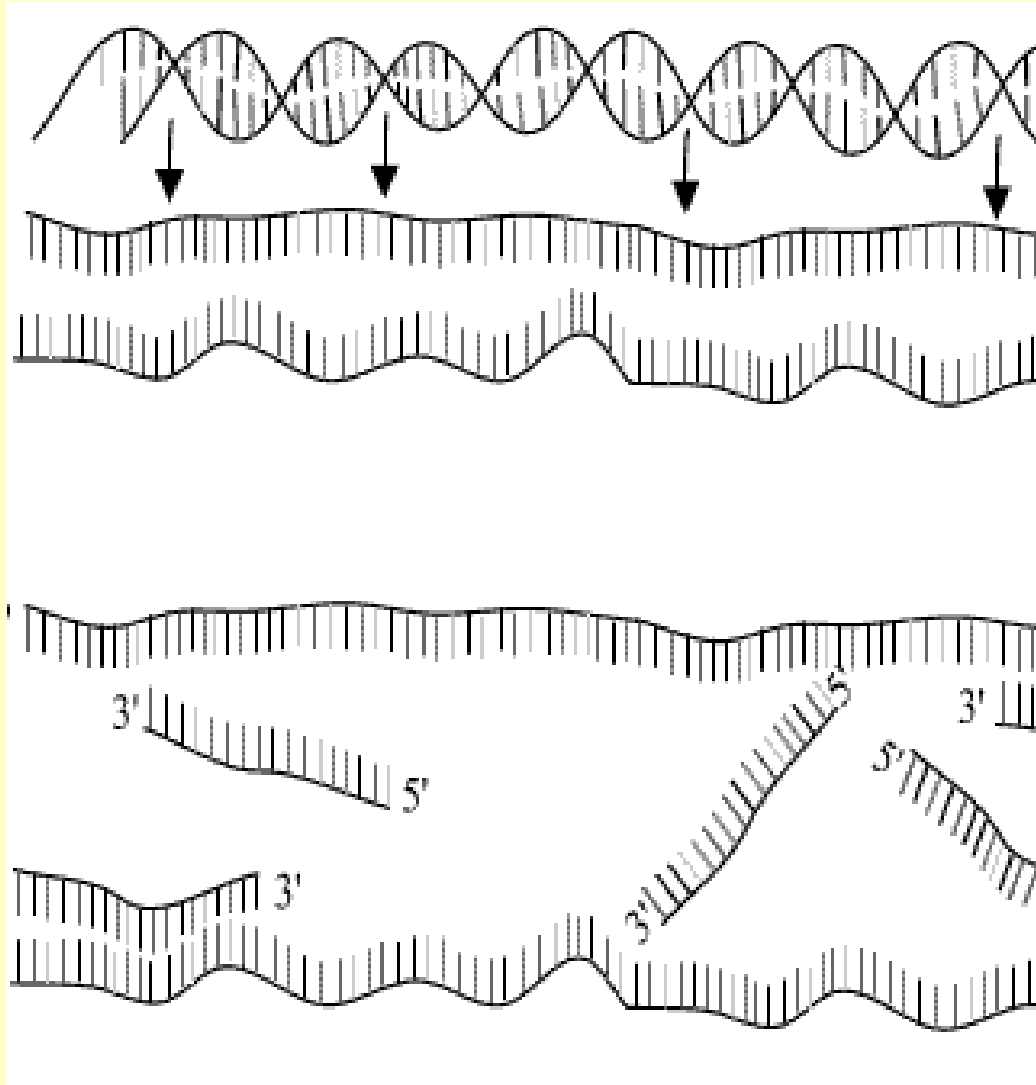


Predators

# Isozymes

- Pros
  - Quick
  - Cheap
  - Great for studies that are examining low levels of variation
- Cons
  - Out of date
  - Specimen must be extremely well preserved
  - Not Very informative

# How a PCR works





# What goes on in the lab?

## 1. DNA extraction-

break open cells (liquid nitrogen or a nasty chemical)  
remove all lipids (soap)  
remove all protein (phenol-chloroform)  
concentrate and wash genomic DNA (ethanol)

## 2. Polymerase Chain Reaction- (PCR)

increases the amount of a particular fragment of DNA

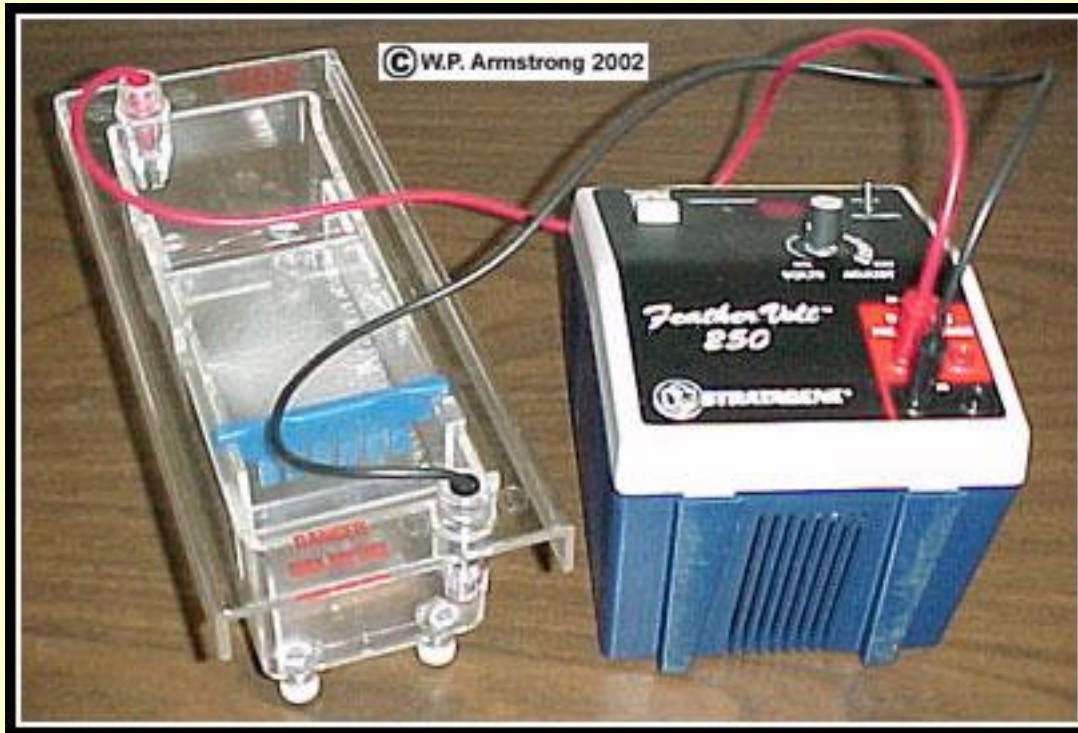
## 3. Genotyping



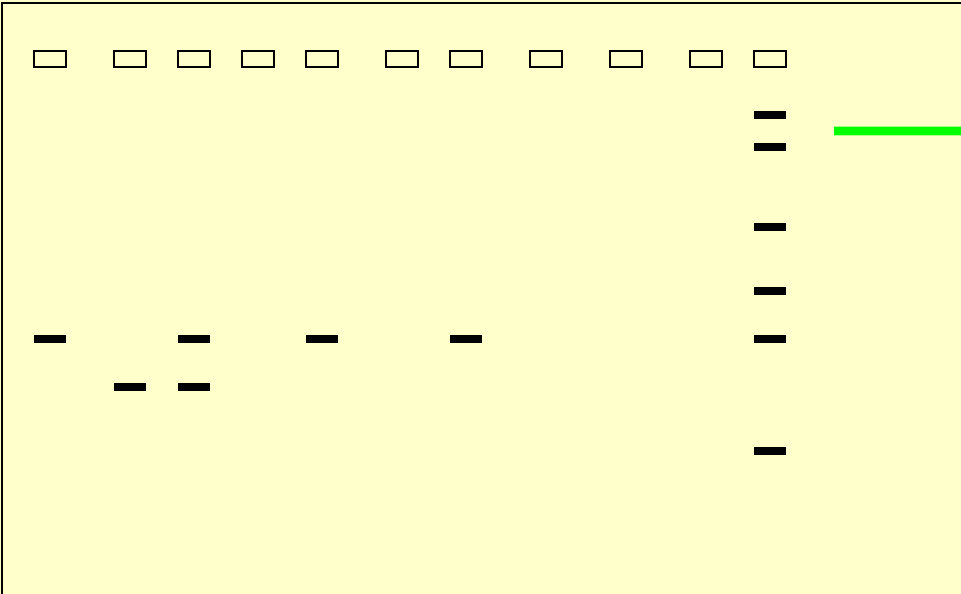
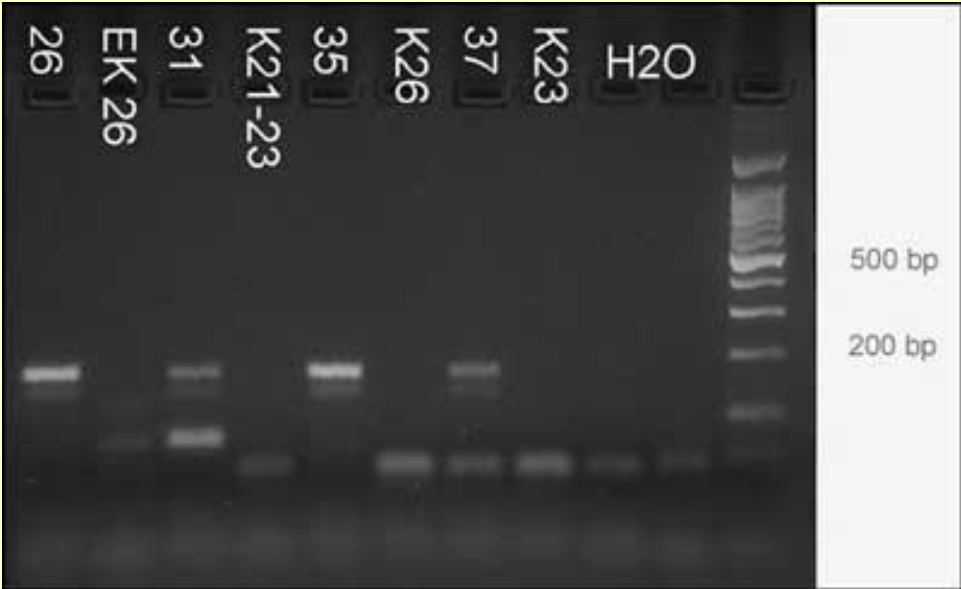








# Gel electrophoresis- visualizing the PCR products

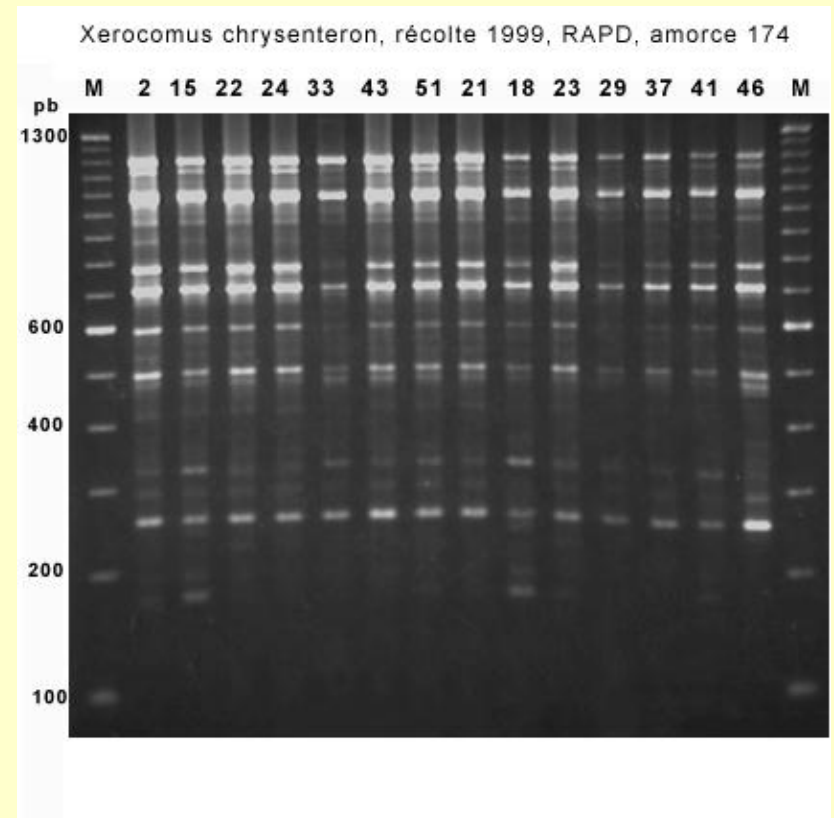


# RAPDs

- Obtained by PCR using RAPD primers (that are typically 10bps long)
- Single primer used
- Dominant markers (either present or absent)
- Primers bought in kits from specialized suppliers

# RAPD gel

- Result in several different DNA sequences being amplified
- Cut bands and sequence specific length DNA sequences
- Examine pattern of amplified DNA



# What are RAPD's used for?

- Used as a finger printing technique for determining paternity of offspring
- Differentiate between species or biotypes
- Genetic mapping of traits
- Commonly used for recognition of biotypes

# ISSR's

- Similar to RAPD's as they are the result of PCR Amplification
- Are a series of dinucleotide repeats
  - CTCTCTCTCTTG (last two bases degenerate)
- Also amplify many different regions
- Extensive optimization of primers is needed to work in a repeatable fashion
- Genome region between microsatellite loci
- Dominant (band is present or lacking)

# What are ISSR's used for?

- Used as a finger printing technique for determining paternity of offspring
- Differentiate between species or biotypes
- Genetic mapping of traits
- Commonly used for recognition of biotypes
- Exactly the same uses as RAPD primers

# Example of ISSR's in biocontrol

- Used ISSR and RAPD markers to distinguish biotypes of the Russian thistle (*Salsola tragus* L.) in California
- Sobhian et al. (2003) found that both markers showed there were two biotypes (A and B)
- Found a gall midge from Uzbekistan as a biological control agent that preferred the type A biotype



# Microsatellites

- Simple Sequence Repeats or Short Tandem Repeats (STR)
- Consist of tandemly repeated DNA sequences (repeat unit consists of 1-6bp) and whole region is ~150bps
- Typically neutral and co-dominant

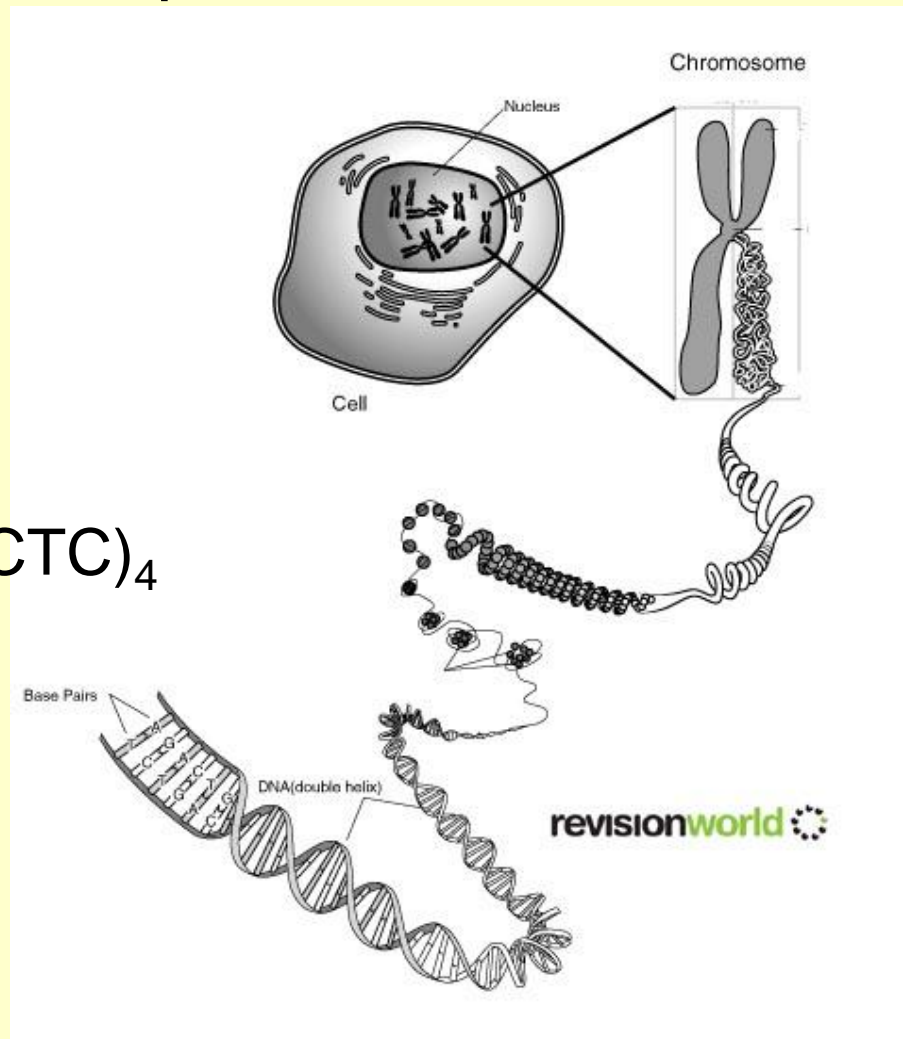
# Genetic marker

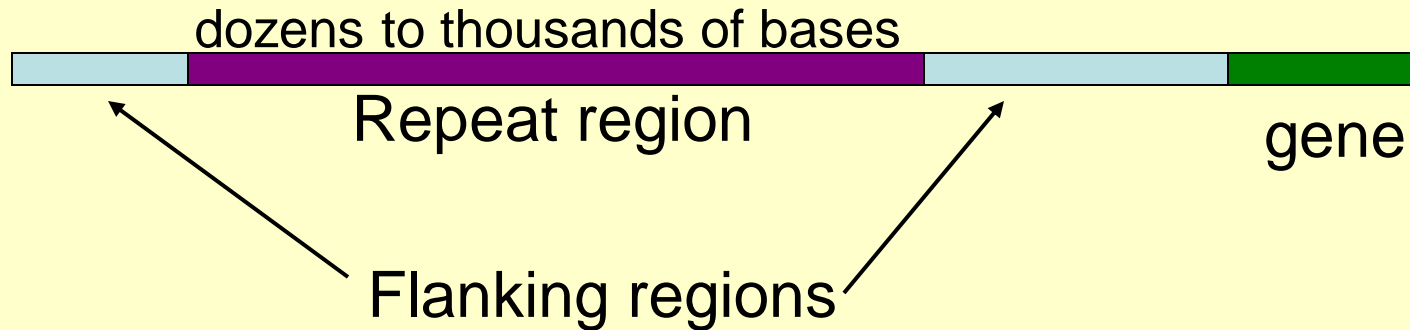
Microsatellites- consist of a specific sequence of DNA bases having tandem repeats

GTGTGTGTGTGT (GT)<sub>6</sub>

CTGCTGCTGCTG (CTG)<sub>4</sub>

ACTCACTCACTCACTC (ACTC)<sub>4</sub>





GGATCCCAAGTGTATGTGCATACACGTG**CACACACACACACACACACA**GGTGCATGCACACTCCAAGAGACAGTG

$(CA)_{11}$

**Some of the fastest evolving pieces of DNA (hyper-variable) why?**

1. “junk DNA” non-coding

About 80-90% of the human genome has been designated as "junk"

2. Replication errors  
Slipped strand mispairing

# Slipped strand mispairing during DNA replication

a)

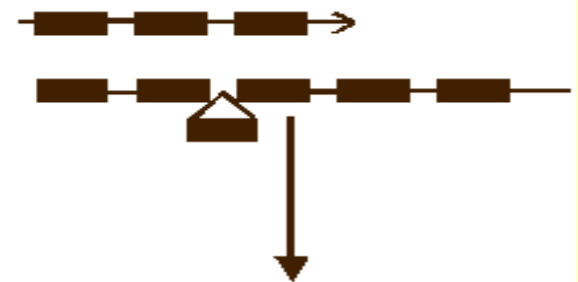
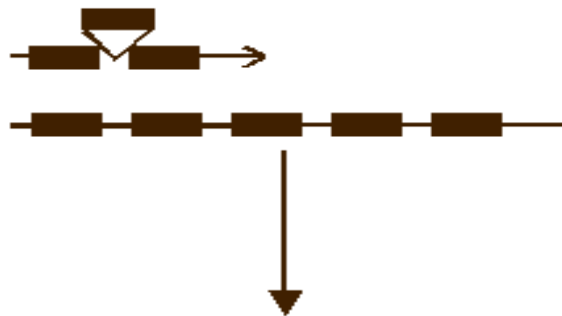


Slippage



b)

Misalignment



c)

-1 repeat



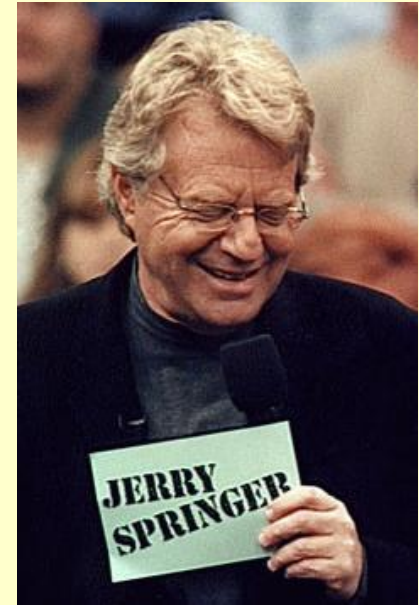
-1 repeat



# Microsatellites

- Used to determine from which populations does this individual originate.
- Evaluate the genetic relationships between individuals
- Examine the mating structure of a population
- Used to look at within population variance
- Used to determine paternity

# Who's your Daddy?



Paternity and crime scene analysis- how do they do it?



In human paternity cases 16 microsatellites are screened

Each of the 16 are located in different parts of the genome  
(**locus**, plural is loci)

At each of the 16 loci there are 2 **alleles**

11/14      11/11

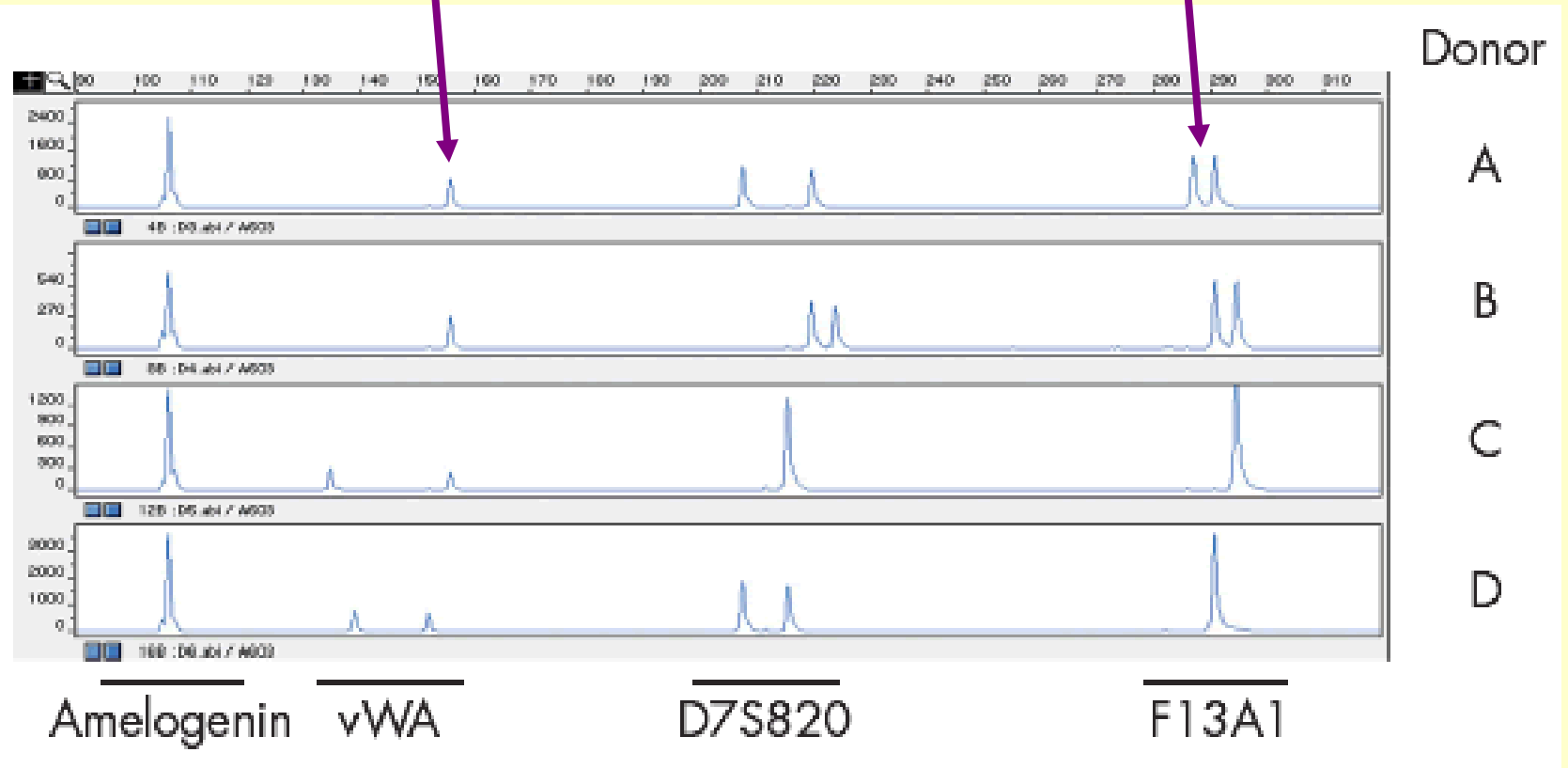
One allele at each locus is from the mother, and the other is from the father

Size of fragment increases



homozygous 11/11

heterozygous 100/104



**Locus 1**

**Locus 2**

**Locus 3**

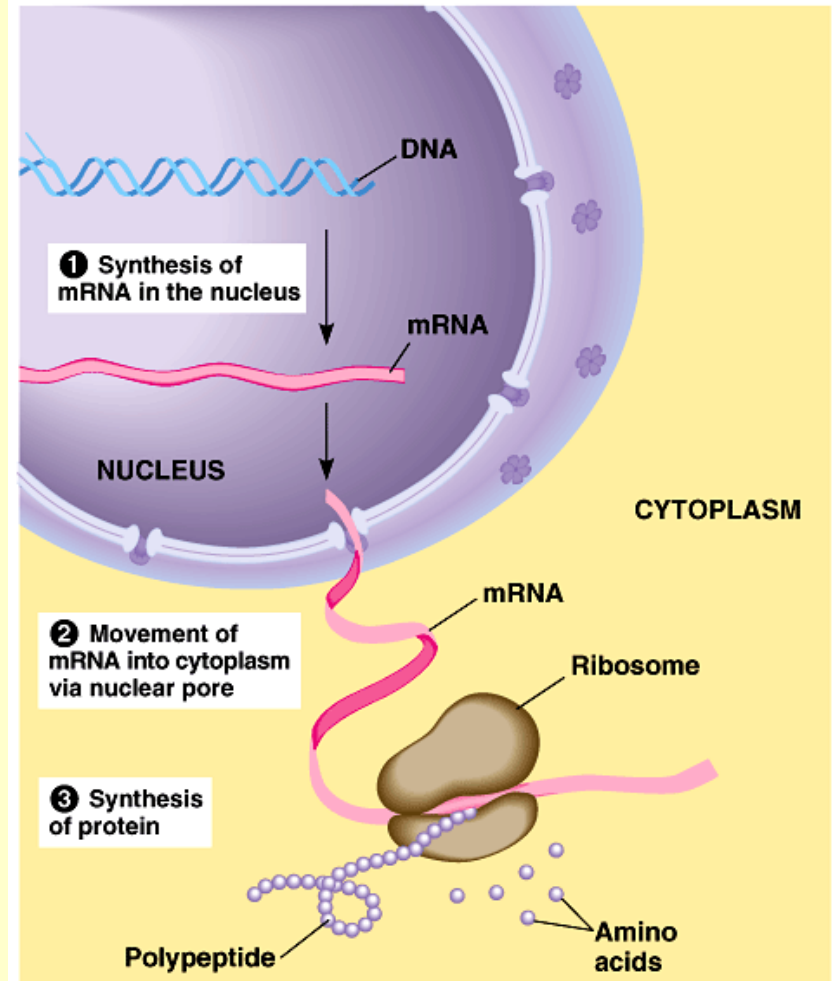
# Gene Sequences

Three main types of gene sequences

1. DNA sequences coding for proteins (nuclear genes)
2. Ribosomal RNA sequences
3. Mitochondrial Genomes

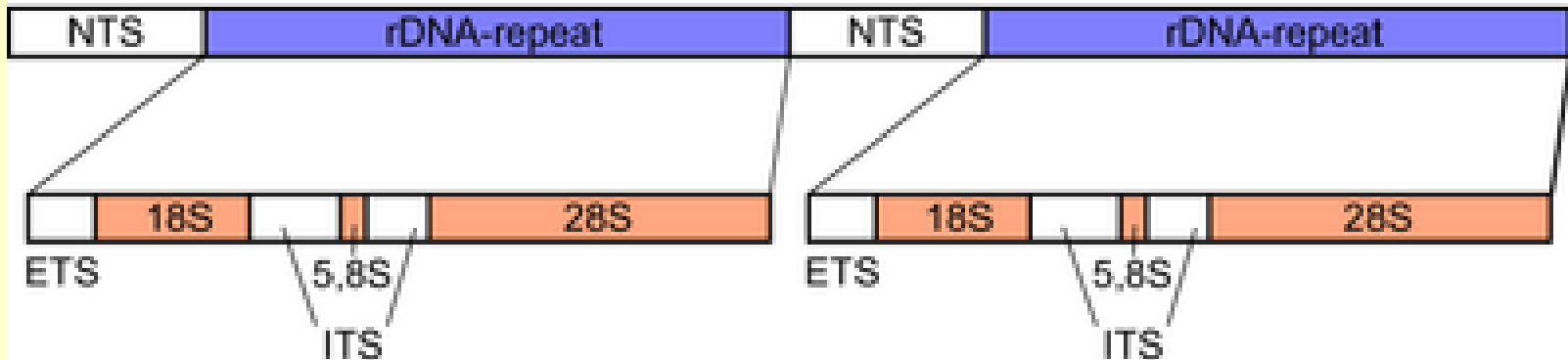
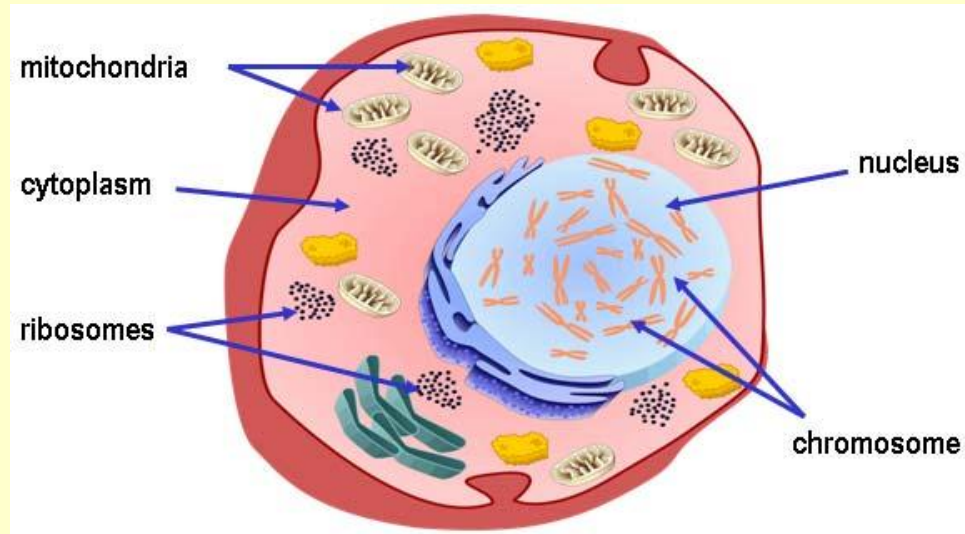
# DNA sequences

- Single copy in genome of organism
- Most commonly used to determine phylogenetic relationships
- No studies of direct importance to biological control
- Nuclear genes not used as much because of low level of variation
- Ex: EF1-alpha



# Ribosomal RNA Sequences

- Ribosomes-where mRNA is translated to proteins
- Major genes
  - 5.8s, 18s, 28s,
  - 1 spacer region-ITS



# Ribosomal RNA Sequences

- 18s, 5.8s, and 28s evolve slowly and typically used to determine higher classification (like order)
- ITS not used in phylogenetic studies because alignment is uncertain
  - However used extensively for species recognition

# ITS in Biocontrol

- Zhu et al. (2000) developed species specific primers (ITS1) to detect 2 parasitoids of Russian wheat aphid

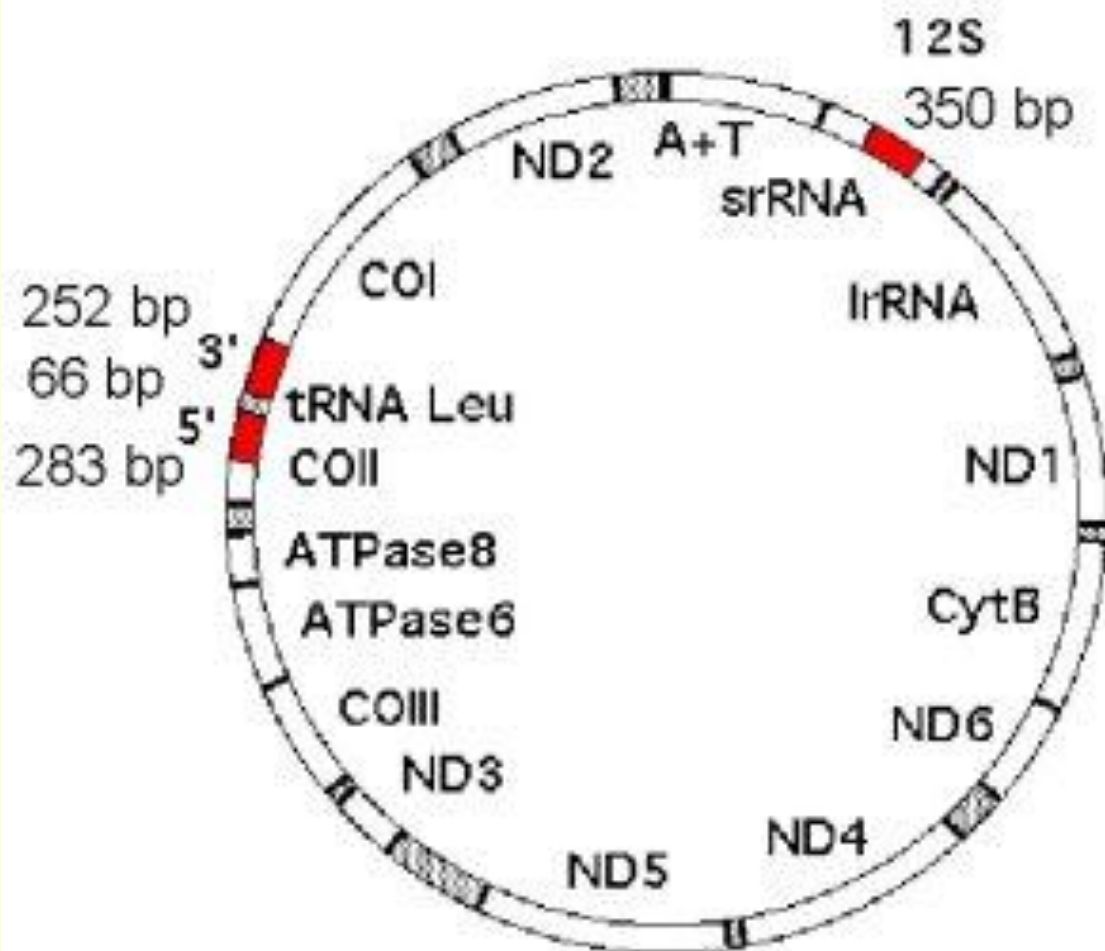


# Mitochondrial genes

- Purely maternal transmission
- Relatively small genome
  - Focus primarily on COI and COII
- Most commonly used in Phylogeography studies (geographic distribution of genealogical lineages)
- Barcoding project

# Locusta migratoria

1.58 Kb

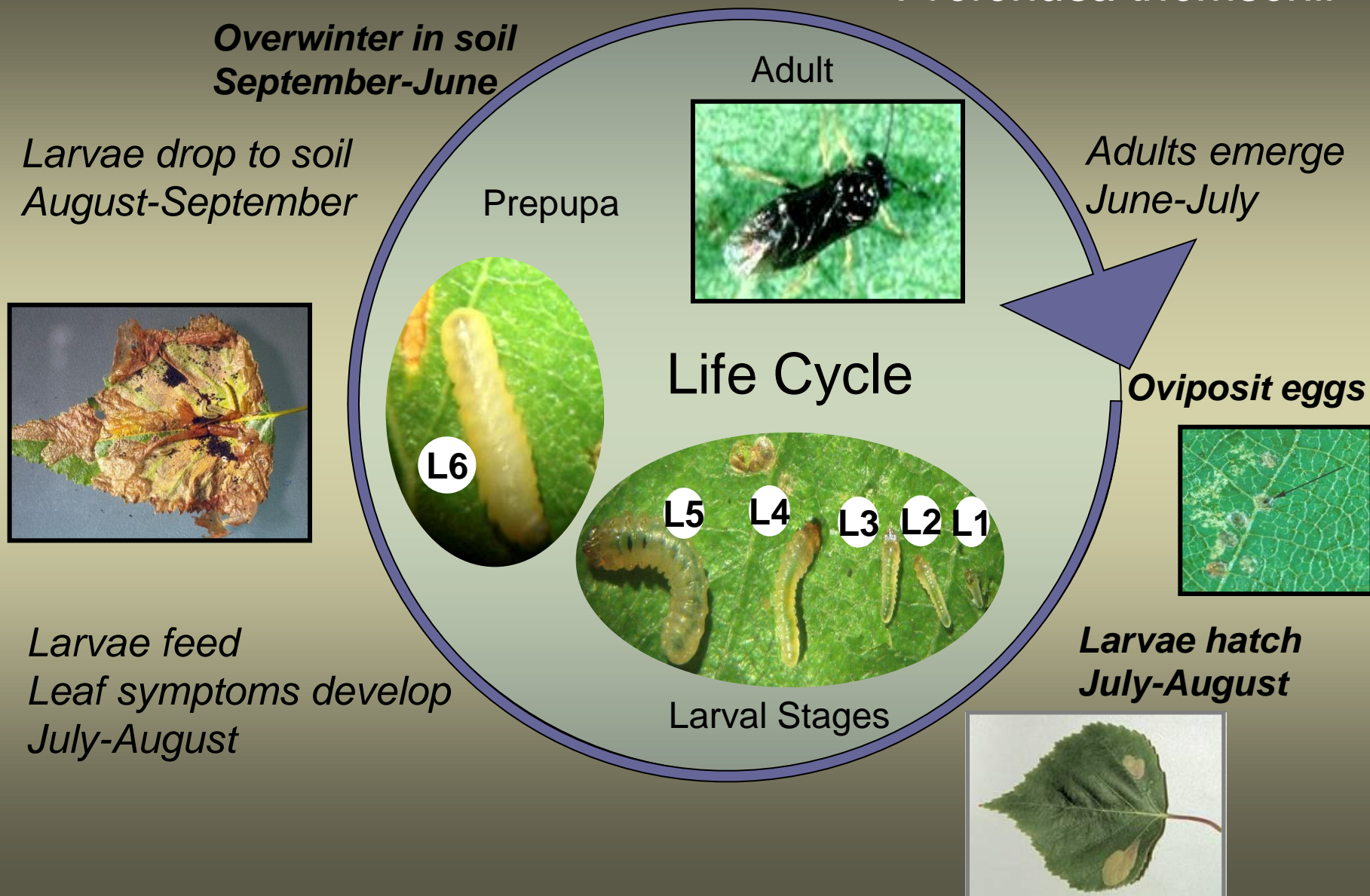


# Barcoding Project

- Hebert et al. (2003) developed biological identifications through DNA barcodes
- Taxonomic experts are becoming fewer
- Targeted a ~700bp region of the COI gene to identify organisms to species
- Widely criticized

# Life cycle corresponds to symptom development...

*Profenusa thomsonii*



# Damage caused by *Profenusa* *thomsoni*



Damaged leaves

# Parasitism



*Lathrolestes sp.*





Three wasps in the  
system parasitize  
Ambermarked  
Birch Leafminer

*Lathrolestes thompsoni*

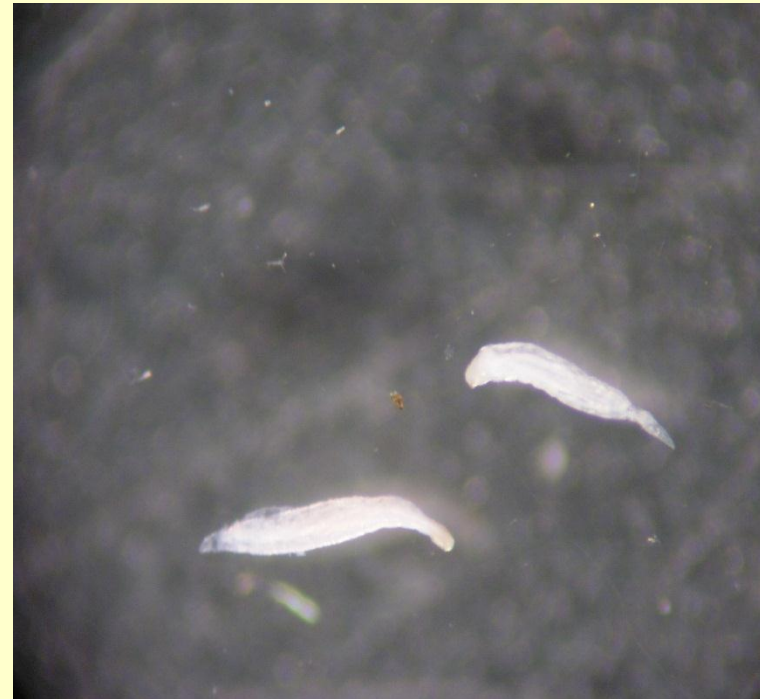


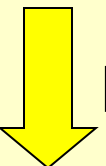
*Aptesis segnis*



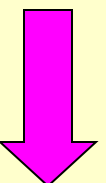
*Lathrolestes soperi*

# Dissection

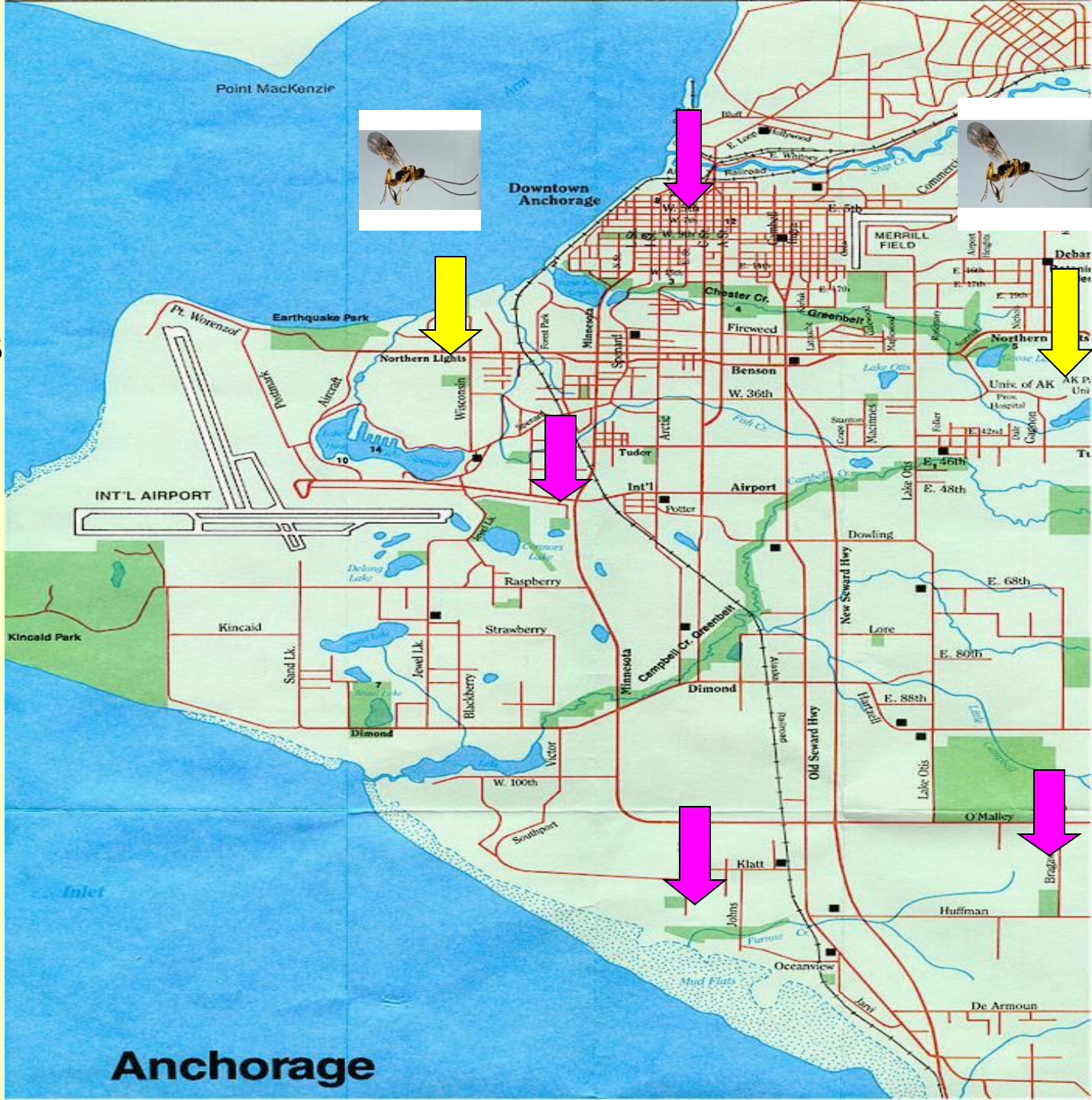




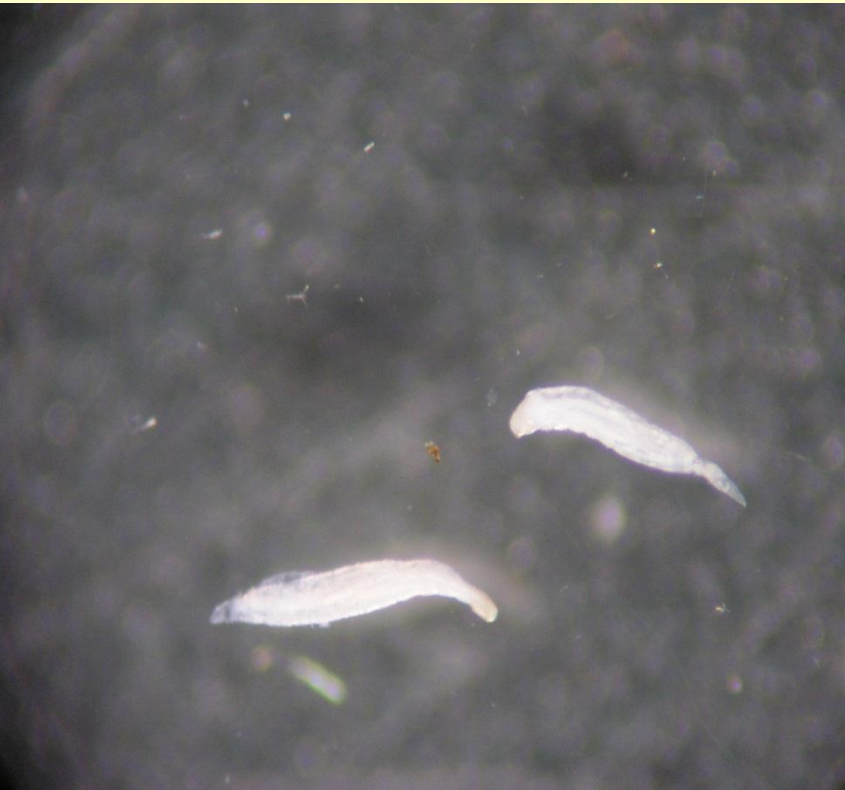
Release Sites



Parasitism observed



# The Big Question



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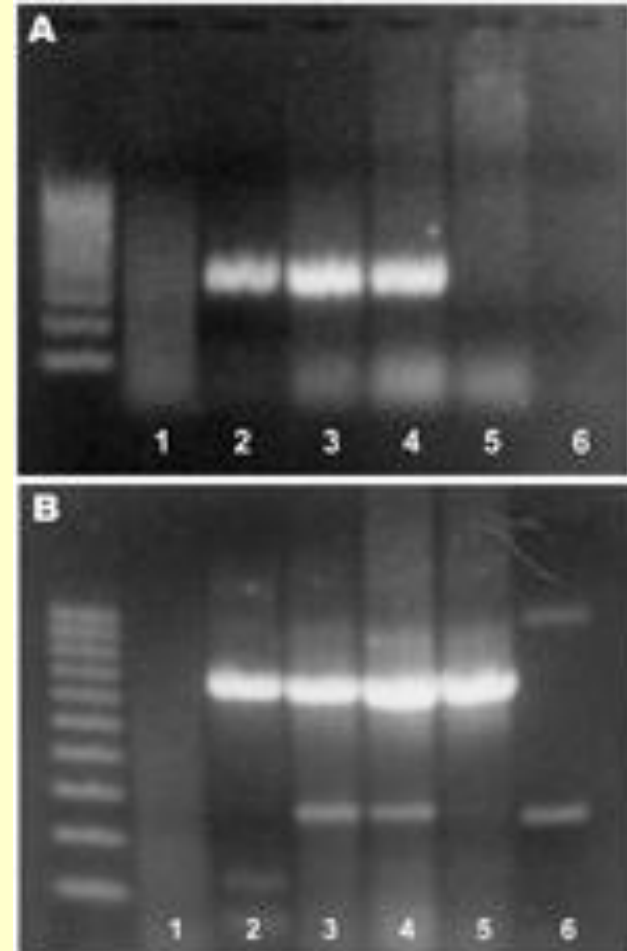


# Methods

- DNA extracted from unknown larvae
- DNA extracted from all three adult wasps
- Ran a PCR in the barcoding region of COI, and Nuclear Ribosomal DNA regions 28s and 18s

# Results

- Adult wasps amplified in COI and 18s;
- Larval DNA amplified in COI and 18s



# Summary

- Molecular tools have practical applications in biological control for:
  1. Species Identification
  2. Determining pest areas of origin
  3. Studying efficacy of natural enemy biotypes
  4. Determining non-target impacts