Binomial Nomenclature

Domain	Eukarya
Kingdom	Animalia
Phylum	Arthropoda
Class	Insecta
Order	Coleoptera
Family	Chrysomelidae
Genus	*Leptinotarsa*
Species	*Leptinotarsa decemlineata*

This example is for the Colorado potato beetle

Zoology - Ecdysozoa

Secretes the exoskeleton → discontinuous growth → molt (**ecdysis**), preceded by apolysis → displacement of the epidermis from the old exoskeleton

It includes:
Euarthropoda (Insecta, Crustacea, Myriapoda,
Chelicerata, Trilobita*)
arthropod-like **Onychophora,Tardigrada**
Nematoda
Nematomorpha
Priapulida
Kinorhyncha
Loricifera

Phylum Arthropoda

Subphyla	Trilobitomorpha, Chelicerata, Myriapoda, Crustacea, Hexapoda
Class	Entognata, Ectognata (Insecta)
Subclass	Apterygota, Pterygota

Origin of wings theories

Tergal origin hypothesis	Wings originated from an expansion of dorsal body wall (tergum)
Pleural origin hypothesis	Wings were derived from epicoxal hendites and exites

Origin of wings theories (cont)

Dual origin hypothesis	Contribution of both tergal and pleural components during the evolution of insect wings

Insects' Ecosystem services

Provisioning	material or energy outputs	Carbon absorption, control of pathogens, pest control, pollination, soil fertility
Supporting	maintenance of ecosystem	decomposition, seed dispersal, recycling
Regulating	directionality of ecosystem processes	
Cultural	educational, spiritual, aesthetic value	bioindicators

Pollination have an economic value of $235 to $577 billion per year worldwide

Ecological species concept

Species=	Group of organisms that occupy the same ecological niche
This means that	species are kept separated by the selection for niche adaptation, not by the reproductive isolation
Cons	Different developmental species inhabit different ecological niches

What is a DNA Barcode

Short standardized DNA markers for the taxonomic indentification

It has to be variable among species, not within species

They do not necessarily meet the requirements for DNA metabarcoding: many species have to be identified simultaneously

Primers:
1. annealing region highly conserved within the target group
2. annealing region not conserved in non-target organisms

Sampling

Community DNA	DNA extracted from a pool of individuals
Environmental DNA	Mixture of genomic DNA for example soil, litter, water

Homogenization and filtering

eDNA

Intercellular DNA	from living cellls or living multicellular organisms
Extracellular DNA	derives from cell death

Ecological factors

Stenoecious	→ organism that can tolerate a narrow range of variability, it can live only in a restricted range of habitats
Euriecious	→ organism that can tolerate a wide range of variability and can live in a wide range of habitats

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Published 24th September, 2021.
Last updated 30th September, 2021.

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Ecological factors (cont)

Ecological niche	→ how a specie interacts within an ecosystem

Hutchinson (1957) → 2 forms of niche:
Fundamental niche = focused in the abiotic conditions in which a specie could exist with no ecological interactions
Realized niche = population's existence in the presence of interactions

Biotic potential

It's the maximum reproductive capacity of an organism in optimal environmental conditions

It's limited by: unfavourable environmental conditions, inhibiting effects or predators, parasites, diseases

It's expressed as a % increase per year or as the doubling time

What limits the growth of populations

Ecoresistance	space, food resources, abiotic factors, biotic factors
Biotic factors:	Intraspecific or extraspecific
Intraspecific:	Competition for food or reproduction
Diapause	it's an endogenously regulated dormant state to survire seasons of adverse conditions. It can be obligatory (genetically determined) or facultative
Quiescence	immediate response to a change in the environment
Extraspecific:	parasitoids, predators, diseases

Insect-plant interactions

Direct defence	act directly on the phytophagous to reduce the feeding performance. Example: Anti-nutritional factors (induced) and Antixenosis or physical barriers (constitutive)
Indirect defence	attract natural enemies of the phytophagous. Example Synomones (induced)

1. insects sense plant's Volatile Organic Compounds by odorant binding proteins to select appropriate hosts

2. sucking insects cause minimal damage, while chewing insects cause wounding. They secrete proteins to suppress plant's defense response

3. many enzimes and transporters are involved. Plant derived toxic compound can be degraded by insect enzymes

Functional classification of pests

Not considered pests	their feeding activity does not cause economic damage
Occasional pests	occasionally cause damage due to abiotic factors
Key pests	relevant economic damage

Functional classification of pests (cont)

Induced pest	They can become dangerous usually after changes in the environment dued to human activities, example: allochthonous insects, monoculture, broad range pesticides

Economic damage can be:
1. proportional to physiological damage
2. less than proportional to the physiological damage
3. starts at a certain point of the physiological damage

Supplement of metabolism

Symbionts provide
→ B vitamins (Blood is deficient)
→ sterols
→ essential amino acids (N recycling capability)

Common traits in p-symbionts:
- genome reduction (~ 5.5 Mb)
- High AT content
Functional complementarity with host and co-symbiont genomes
- Evolutionary stasis

Integrated Pest Management

Definition	ecosystem approach to crop production and protection that adopts a combination of strategies
Key points	identification of key pest
	monitoring strategy
	define thresholds
	implement control strategies
	evaluate results

Economic injury level = cost of control / (market value x loss)

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IPM control categories

Agronomical practices	Crop rotarion
	Cultivar choice
	soil management practices
	fertilization
Physical and mechanical approaches	Heat to treat food and seeds
	Microwave to trear wood
	Mechanically remove insects: light, traps
Agrochemicals	pheromone based suppression: mass traps
	pheromone based attract and kill
	mating disruption with sexual pheromones
	Auto confusion, auto sterilization
Biological control	introduction of natural predators or parasitoids
	pathogens: bacillus thuringiensis, fungi, nematodes

Inoculative biological control: natural enemies are released in the environment
Inondative biological control: natural enemies are released repeately
Conservation biological control: habitat management to let natural enemies live

Evolutionary background

Nothing in biology makes sense except in the light of evolution (Theodosius Dobzhansky, 1973)
+
...Nothing in evolution makes sense without a good (true) phylogeny
=
Nothing in biology makes sense without a phylogeny

Cuticle synthesis

Insecta and Crustacea have an **exoskeleton**

Tyr and **Phe** are precursor of an essential component of cuticle systhesis

An **Endosymbiont** provides Tyr and Phe to the host

The host **regulates** the load of Endosymbiont to achieve cuticle, then eliminates it through apoptosis and autophagy

Zoology - Insecta

4 pairs of cephalic appendages (1 pre + 3 post oral)

Exposed mouthparts

Size: 0.2 - 300 mm

30 orders: Archaeognata + Zygentoma (Apterigota) and Pterygota

Insect orders

Apterygota	*Archaeognata*, *Zygentoma*
Pterygota	*Ephemeroptera*, *Odonata*, *Plecoptera*, *Isoptera*, *Blattodea*, *Mantodea*, *Grylloblattodea*, *Mantophasmatodea*, *Phasmatodea*, *Embiidina*, *Orthoptera*, *Dermaptera*, *Zoraptera*, *Psocoptera*, *Phthiraptera*, *Thysanoptera*, *Hemiptera*

DNA Taxonomy

Definition	Process of naming and classifying organisms into groups, according to their similarities and differences
DNA barcoding	a standardized approach to identify organisms by the use of a DNA barcode
DNA barcode	Short DNA sequence taken from stardadized portions of the genome, coding or not a protein
Origin	1977: *the idea;* 1996: *the first DNA metabarcoding;* 2003: *use of the term DNA barcoding;* present: *DNA barcoding, metabarcoding, eDNA*

Cryptic species

Morphologically indistinguishable species that can be recognized only by molecular data

Molecular taxonomy

It merges BSC, MSC and Phylogenetic species concept PSC
PSC considers monophyletic groups as the unique real entities of the speciation process

Types of taxon

Monophyletic taxon	A group of organisms including the *most recent common ancestor and its descendants*
Polyphyletic taxon	A group of organisms in which the most recent common ancestor *is not included*

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Types of taxon (cont)

Paraphyletic taxon	A group of organisms including the *most recent common ancestor* but **not** its descendants

DNA barcoding workflow

1. Sample collection
2. DNA extraction
3. PCRs
4. Sanger sequencing
5. Electropherograms
6. Comparison with database (BOLD, BLAST)
7.Identification!

DNA metabarcoding workflow

1. Sample collection
2. Sample processing
3. DNA extraction
4. Libraries preparation
5. Sequencing
6. Bioinformatic analyses
7. Results

Note!

A reference database is mandatory in DNA analysis!

Homology, Orthology, Paralogy

Homology	2 genes that share a common ancestor (evolutionary hypothesis)
Orthology	homologous that have diverged after a speciation event
Paralogy	homologous that have diverged after a duplication event
Xenology	homologous that derived through lateral transfer

Insects environments

1. Caves
2. Forests
3. Meadows
4. Deserts
5. Urban environments
6. Lakes and rivers
7. Agroecosystem

Agroecosystem → many species interact.
They're natural ecosystem modified for the production of food and fiber
Planned diversity: plants and animals farmed + benefical organisms added
Unplanned diversity: weeds, pests, other organisms

Interactions: demoecology

Demoecology	studies the demography of a population (density, structure, dynamics) and predicts future population in a given scenario
Metapopulation	when the individuals live in a fragmented habitat
Structural properties	density, distribution, size, age classes, sex ratio, genetic variability
Functional properties	behaviour, birth rate, mortality, genetic variability

r vs k strategy

r:
1.exponential growth
2. short life cycle
3. small size
4. collapse dued to abiotic factors

r vs k strategy (cont)

5. related to ephemeral environments, can cause serious damage
6. large offspring, null parental care

k:
1. logistic growth
2. long life cycle
3. medium-large size
4. rarely collapse because of abiotic factors
5. related to stable environments
6. reduced offspring, parental care

Multitrophic interactions

Green leaf volatiles	esters, aldehydes, alcohols C6

terpenoids C10, C15, indoles

These are volatiles induced by the insect injuries.

They:
1. have a repellent action
2. induce plant-defence genes
3. attract other phytophagous (kairomones)
4. attract parasitoids and predators (synomones)

Insects defense towards GLS

1. Detox of isothiocyanates by conjugationo with Glutathione
2. Hydrolysis of GLS to obtain less toxic compounds (nitriles)
3. GLS sequestration to use against predators

Symbiosis

Blockmann	observed Bacteriocytes in the body fat cells of cockroaches
Sulc	described aggregations of bacteriocytes in the body cavity of cicads

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Endosymbiont

Primary	Obliged association, associated with their insect for long time, host-symbiont cocladogenesis, vertically transmitted (ie roaches and blattabacterium)
Secondary	Facultative symbiont, roles from mutualism to manipulation of reproduction, recently associated with their host

FUNCTIONS:
→ supplement of metabolic functions
→ manipulation of host reproduction
→ protection against pathogens or toxins

Manipulation of host reproduction

The hereditary, vertically transmitted symbiont does:	help hosts that trasmit it OR sterilize hosts that don't trasmit it (kill male embryos, induce cytoplasmatic incompatibility, feminize males, induce parthenogenesis)
Wolbachia	kills male embryos, feminizes male, induces parthenogenesis, induces cytoplasmatic incompatibility (CI)

CI:
infected sperm + *infected egg* = ✓☐
infected sperm + healthy egg = X☐
healthy sperm + *infected egg* = ✓☐
healthy sperm + healthy egg = ✓☐

Insects species in Italy

Allochtonous	*Aedes albopictus, Trichopoda pennipes,* ***Scaphoideus titanus****, Leptoglossus occidentalis, Metcalfa pruinosa, Icerya purchasi,* ***Halyomorpha halys****, Vespa velutina,*
Others	***Drosophila suzuki****,* ***Diabrotica virgifera****, Empoasca vitis, Lobesia botrana, Cysia ambiguella, Argyrotenia pulchellana*

Key points in evolution

1	Cambrian explosion
2	Origin of Insecta
3	Vascular plants
4	Seed plants
5	End-permiam mass extinction
6	Flowering plants
7	Angiosperm

Remember the Cambrian explosion thanks to *Opabinia*, the weirdest animal ever

Zoology - Arthropoda

arthron = articulated ; *podos* = foot
1.200.000 described species
Exoskeleton
Bilateria, protostomes and triploblastic
Metameric segmentation; tagma
High adaptability
Ectotherms

General Morphology

Heteronomous metamery: 20 metamers

Each metamer: ventral sternite, dorsal tergite, pleura (lateral sclerite), one pair of spiracle, one pair of ganglia, a couple of appendixes

3 morphological regions (tagma) → *Head* (6 metamers fused), *Thorax* (3 regions: pro- meso- meta-), *Abdomen* (11 metamers or urites + telson)

Pterygota

Endopterygota
Neuroptera
Megaloptera
Raphidioptera
→Coleoptera
Strepsiptera
→Diptera
Mecoptera
Siphonaptera
Trichoptera
→Lepidoptera
→Hymenoptera

DNA Taxonomy is useful because:

It's a standardized approach to identification
Solves limitations of morphological approaches
Identifies organisms also from fragments or juvenile stages
Solves the "taxonomic impediment"

Morphological approach

Easy on the most cases	but what about fragments or juvenile stages?

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Morphological approach (cont)

Species:	groups of interbreeding natural populations that are reproductively isolated from other such groups
The morphological species concept:	Operational tool of the biological species concept → the existence of reproductive isolation is deduced by the analysis of morphological traits
Cons:	Subjective (*the specialist decides*); Intraspecific varability; Cryptic species

Application os DNA taxonomy

Outside entomology	Food safety
Inside entomology	

Application of DNA taxonomy

Outside entomology	Food safety, veterinary application
Inside entomology	Forensic science, biomonitoring, biodiversity surveys, investigate multitrophic relationships

DNA barcoding vs DNA Metabarcoding

DNA barcoding	sequences 1 DNA to identify 1 organism
DNA metabarcoding	sequences 10^5-10^7 DNA to identify organisms, simbionts, parasites, dead remnants and extracellular DNA

Sequence

Definition: S, is an order of *n* characters (Si)

DNA is composed of 4 nucleotides (A, C, G, T)

RNA is composed of 4 nucleotides (A, C, G, U)

Proteins are composed of 20 aminoacids

Ecosystem

It's a **structural and functional unit** consisting of a biological community of **living organisms interacting** with themselves and their **physical environment**, in a unit of space

Ecological factor: every environmental element interacting directly on living organisms

Biotic factors: influence organisms' fitness and distribution

Sampling

Direct	qualitative, useful for presence/-absence analyses, depends by the human experience
Indirect	quantitative, useful for hypotesis testing, do not depend by human

Interactions: autoecology

Autoecology	Study of the individuals: range of tolerances, thermoregulation, water balance

Autoecology studies guilds:
-Herbivores
- Predators
- Scavengers
- Parasites and parasitoids

Trophic guilds

Scavengers	Feed on dead or dying plants, dead or dying animals, excrements of other animals

Trophic guilds (cont)

Phytophagous	phyllophagous (leaves), carpophagous (fruit), plant--sucking (xylem, phloem), anthophagous (flowers), rhizophagous (roots), xylophagous (wood)
Zoophagous	feed on other animals (predators and parasitoids)

Interactions: Sinecology

It studies the interactions among species in a definited space

Competition, Predation, Symbiosis

Competition is higher in a limited environment (ie leafminers). It can be symmetric or asymmetric (if one species is more competitive than the other)

Predation: prays evolve features to defense, predators to overcome the preys strategies

Symbiosis: parasitism, mutualism, commensalism

Detox of plant's defense compounds

1. reduction of toxicity by **ctyochrome P450 monooxygenases (CYPs)**. Heme-containing enzimes catalyzing
2. hydrophobic compounds are converted in hydrophilic by **Glutathione S-transferases (GSTs)** and **Uridine 5'-diphospho-glucuronosyltransferases (UGTs)** catalysing the conjugation of GSH from uridine 5diP glucoronic acid to the xenobiotic substance
3. ATP binding cassette **(ABC)** transports xenobiotics outside the cell

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Damages caused by insects

Direct	Insect directly attacks the commercial parts
Indirect	Insect attacks non-commercial parts but reduces plant efficiency
Physiological damage	Phyllophagous insects **reduce leaves surface**, reducing photosynthesys, of aphids can induce **presence of black sooty mold** or **viruses**, rhizophagous and xylophagous insects
Productive damage	measurable plant loss (qualitative and quantitative), or aesthetic damage
Economic damage	monetary value of the productive damage

Causes promoting pest damage

Abiotic factors

Biotic factors (plant genotype, insect preference, phenological phase)

Random factors

Relationships

/	Parasitic	Mutualistic
Facultative	male killing, feminisation	increase fecundity
Obligate	oogenesis	nutrient provisioining

Vertically transmitted symbionts:
- reproductive parasitism
- mutualistic symbiosis
- standard parasitism

Open questions about symbiosis

Understand molecular basis of insect-symbiont interactions

Microbiota and microbioma associate to non-model insects

Impact of commensal bacteria in niche colonization

Impact of ecological determinants on the microbiota structure

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