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ISTITUTO ZOOPROFILATTICO SPERIMENTALE DELL'ABRUZZO E DEL MOLISE "G. CAPORALE"

RVF vectors and entomological surveillance

Regional workshop on Rift Valley Fever surveillance in Northern African countries - PROVNA2

Tunis, Tunisia
12-14 November2024

Maria Goffredo & Silvio G. d'Alessio
IZSAM

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We will speak about:

- Role of vectors in transmitting RVF virus
- How entomological activities can serve surveillance

- Collection methods
- Ecology of Mediterranean mosquitoes, possible vectors of RVF

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RVF vectors

The RVF virus has been detected, in the field or under laboratory conditions, in more than 50 species of mosquitoes, belonging to at least seven genera

Two roles:

Maintenance vectors
i.e. *Aedes* mosquitoes
(Transovarian transmission)

Amplifying vectors
i.e. *Culex* mosquitoes

Aedes mcintoshi

RVFV isolated from unfed males and females hatched in dambos in Kenya (1982, 1984)

- ✓ Findings not confirmed
- ✓ Poorly studied

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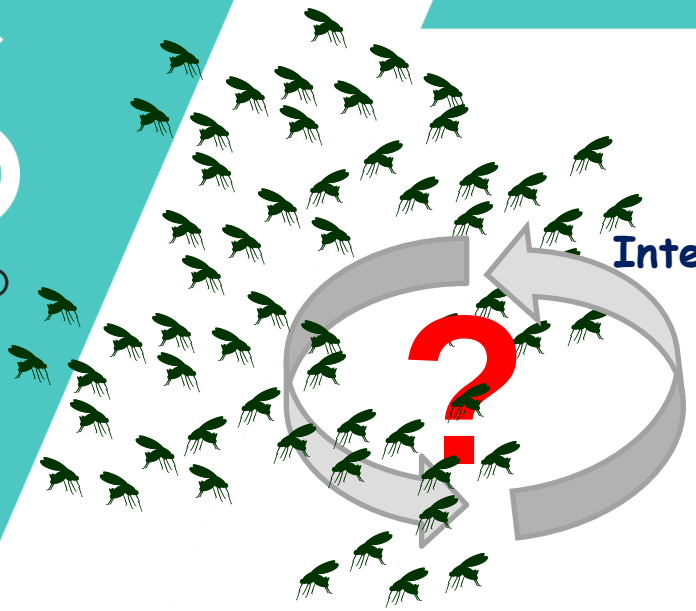
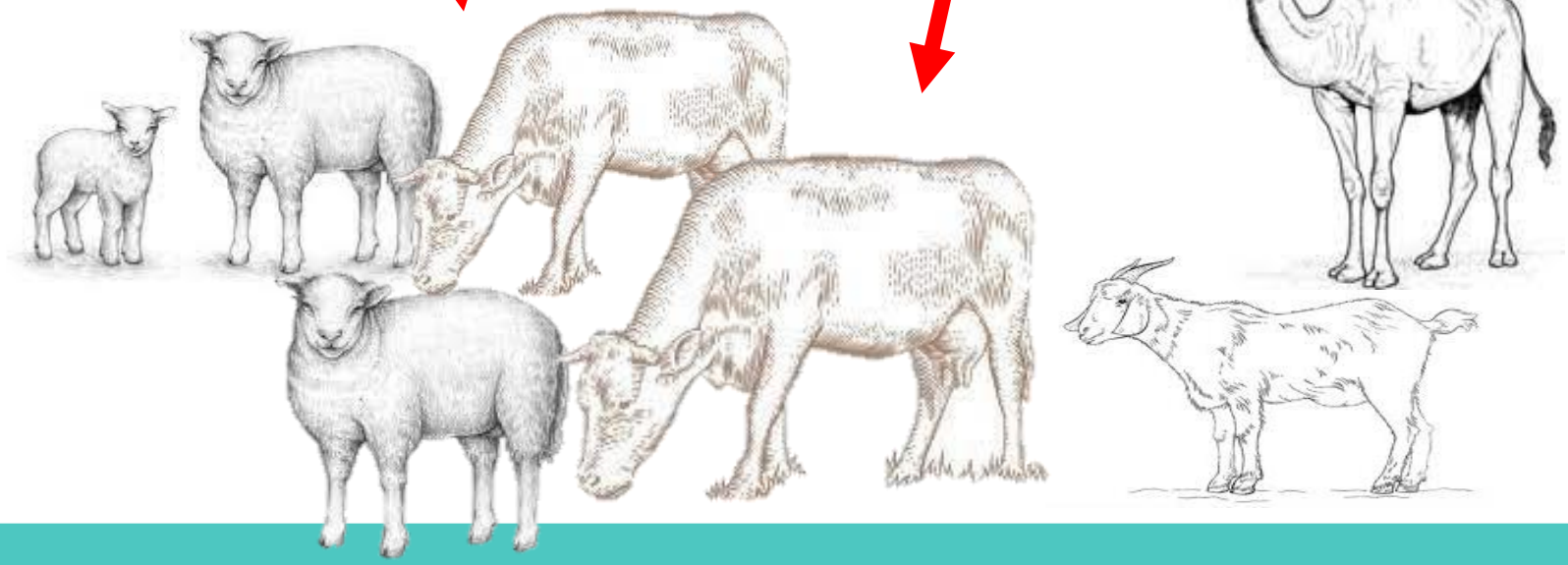
Rift Valley Fever



Interepidemic periods

- *Aedes* eggs?
- Other routes?

Amplification



Vectors
Aerosol
Direct contact
(infected tissues)

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Why entomological surveillance?

Generally speaking:

- ✓ To define the distribution and abundance of vector species
- ✓ To look for arboviruses in the vector population

To early detect virus circulation in a given area testing mosquitoes is **very difficult**, due to the low prevalence in vector population

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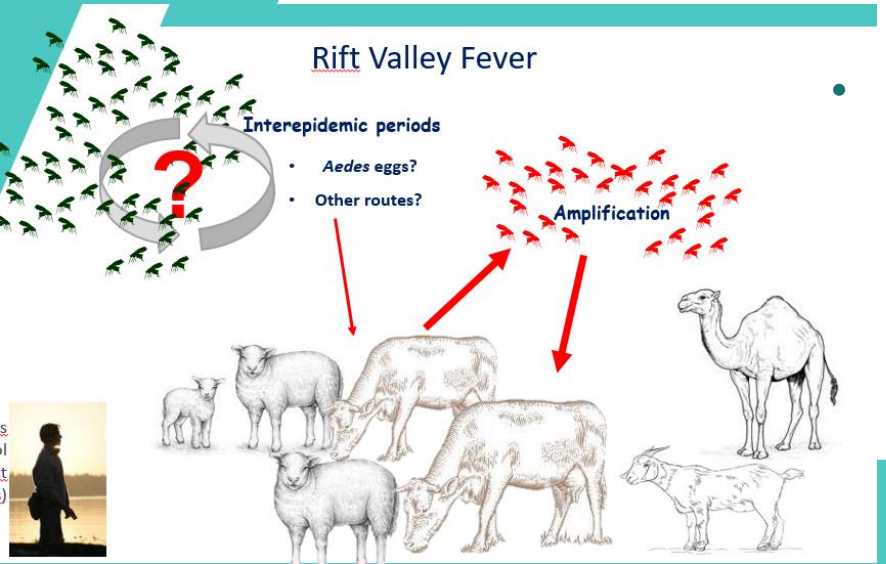
RVF: why entomological surveillance?

IN AREAS WHERE RVF VIRUS CIRCULATION OCCURS

- **to investigate the mosquito species involved in the transmission**
  - species acting as vectors
  - overwintering routes
  - transovarial transmission

- **To investigate interepidemic manteinance routes**



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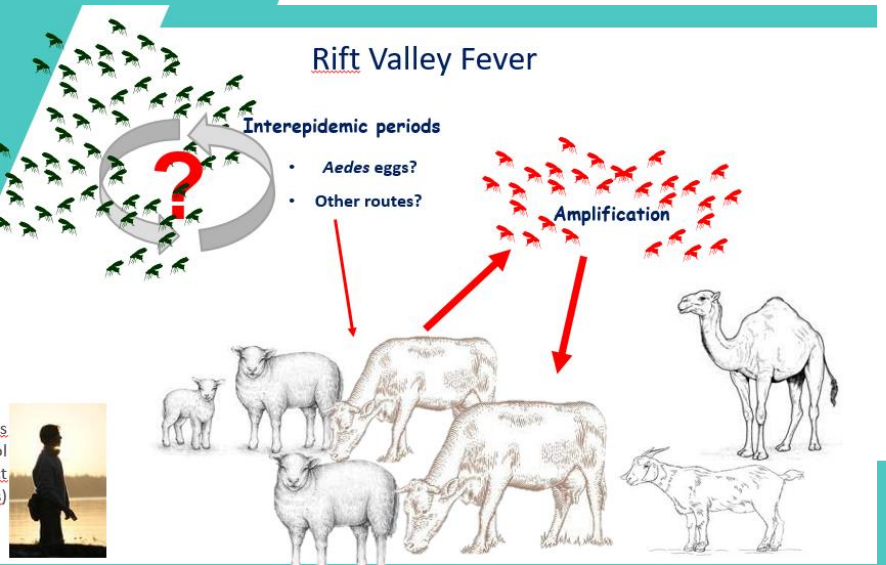
RVF: why entomological surveillance?

TO EVALUATE THE SUITABILITY FOR RVF OF DIFFERENT AREAS

- **To know** the local vector fauna:
  - Which mosquitoes are there, and how many, able to feed on susceptible animals?
  - Distribution (presence/absence) and abundance **maps**
  - **Seasonal** abundance



- To «feed» **models** for a risk-based surveillance of RVF

ECOREGIONS

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Sampling strategy
Collection protocols should be defined

- target vector species ← i.e.
  Diurnal – nocturnal
  Host preference (human biters, birdfeeders, etc)
  Breeding sites

- Where? ← i.e.
  Urban – rural – wild environment
  Selection of sampling areas

- When? ← i.e.
  Frequency (daily, weekly, monthly, etc)
  Sampling period (summer, all year, etc)

- How? ← i.e.
  Collection methods
  Samples management

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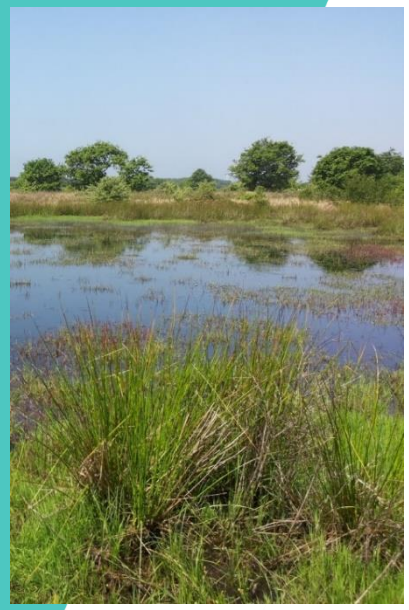
WHERE ?

Collection sites can be selected basing on:

- areas with **high probability** of mosquito presence

- Satellite imagery can help to select the collection sites

- Field trips are needed to select the exact location



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DELL'ABRUZZO
E DEL MOLISE
"G. CAPORALE"

WHEN ?

Sampling of a target species should be performed:

- during the mosquito seasonal peak period
- during the biting activity (nocturnal or diurnal)
- the frequency of trapping depends on the collection methods, on the aims (i.e. once or twice a month, or weekly)

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HOW ?

We can use a wide range of attractants:

CO_2	→	mammophilic species
Specific chemical lure (similar to human smell)	→	Human biter species
Light	→	Nocturnal species
Mixture for gravid mosquitoes (similar to breeding sites)	→	Adult gravid females
Animals (birds, mammals, humans)	→	Host-seeking mosquitoes, according to their host preference

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Surveillance protocols are defined according to the ecology of mosquito species….

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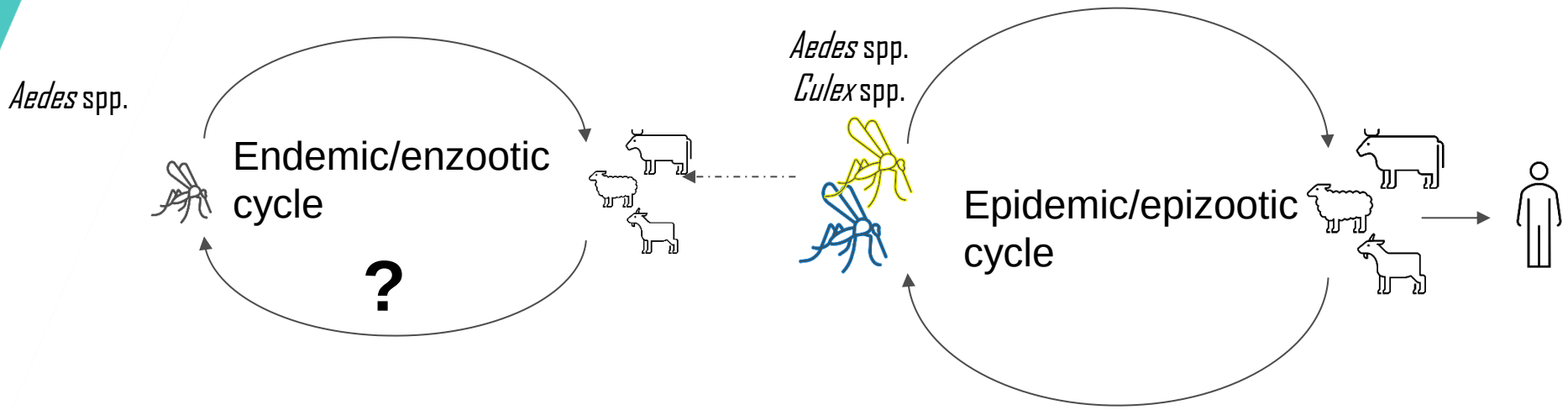

Mosquitoes can play two different roles:

Maintenance vectors
i.e. *Aedes* mosquitoes (transovarial transmission!?)

Amplifying vectors
i.e. *Culex* mosquitoes


Heavy rainfall

Climate factors and **land use changes** can affect the emergence and re-emergence of RVF


Irrigated areas


Dams

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More than 50 species of mosquitoes, belonging to **7 genera,** have been found infected in the field or under laboratory conditions

Of these at least 17 species are present in Mediterranean countries
Among these, the following 14 species belonging to *Aedes, Anopheles* and *Culex* genera (including the main potential vector species) are present in the area of interest:

- *Aedes (Adm.) vexans*
- *Aedes (Och.) caspius*
- *Aedes (Och.) detritus* s.l.
- *Aedes (Stg.) aegypthi*
- *Aedes (Stg.) albopictus*
- *Culex (Cux.) antennatus*
- *Culex (Cux.) perexiguus*
- *Culex (Cux.) pipiens* s.l.
- *Culex (Cux.) theileri*
- *Culex (Cux.) tritaeniorhynchus*
- *Culex (Ocu.) poicilipes*
- *Anopheles (Cel.) multicolor*
- *Anopheles (Cel.) pharoensis*
- *Anopheles (Cel.) sergentii*

Infected under laboratory conditions

The following species of our list have been infected under laboratory conditions

Species	Area of origin
Aedes albopictus	**Spain** Texas
Ae. caspius	**France** **Egypt**
***Ae. detritus* s.l.**	**United Kingdom** **France**
Ae. vexans	**Spain** **Senegal** Luisiana and Florida **Germany**

Species	Area of origin
Culex antennatus	Kenya **Egypt**
Cx. perexiguus	**Egypt**
***Cx. pipiens* s.l.**	**United Kingdom** **Netherlands** **Spain** **Morocco, Algeria, Tunisia** Canada California, Colorado **South. France and Tunisia** **Egypt**
Cx. poicilipes	**Senegal** South Africa
Cx. theileri	South Africa
Cx. tritaeniorhynchus	Saudi Arabia
An. multicolor	**Egypt**
An. pharoensis	**Egypt**

References

Amraoui et al. 2012; Birnberg et al. 2019; Brustolin et al. 2017; Faran et al. 1988; Meegan et al. 1980; Gad et al. 1987; Iranpour et al. 2011; Jupp et al. 1988; Jupp et al. 2002; Lumley et al. 2018; McIntosh et al. 1973; McIntosh et al. 1980; Moutailler et al., 2008; Ndiaye et al. 2016; Turell et al., 1988; Turell et al. 1996; Turell et al. 2008; Turell et al., 2010; Turell et al. 2013; Vloet et al. 2017;

Found infected in field

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The following species of our list have been found to be infected in field

Species	Area	Main ecosystem
Aedes vexans	Arabian Peninsula Senegal Sudan	Irrigated area Semi-arid/Irrigated area Semi-arid/Irrigated area
Culex antennatus	Egypt Kenya Madagascar Nigeria	Irrigated area Dambos Rice field in highlands ?
***Cx. pipiens* s.l.**	Egypt	Irrigated area
Cx. poicilipes	Kenya Mauritania Senegal Sudan	Dambos Semi-arid/Irrigated area Semi-arid/Irrigated area Semi-arid/Irrigated area
Cx. theileri	Kenya Namibia South Africa Zimbabwe	Dambos Pans Vleis or pans ?
Cx. tritaeniorhynchus	Arabian Peninsula	Wadis/Irrigated area
An. pharoensis	Mauritania	River

References

Ba et al., 2012; Diallo et al. 2005; Diallo et al. 2005; Ba et al. 2012; EFSA (AHAW), 2013; El Hadi et al. 2013; Fontenille et al., 1998; Hanafi et al. 2011; Hoogstraal et al. 1979; Jupp et al. 2002; Jupp et al., 2002; Miller et al. 2002 Linthicum 1985; McIntosh et al. 1973; McIntosh et al. 1980; McIntosh et al. 1973; Ratovonjato et al. 2011; Sang et al. 2010; Seufi et al. 2010; Stoek et al. 2022; Traore-Lamizana et al., 2001; Zeller et al., 1997;

NATURAL HABITATS AND IRRIGATED AREAS

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Life cycle

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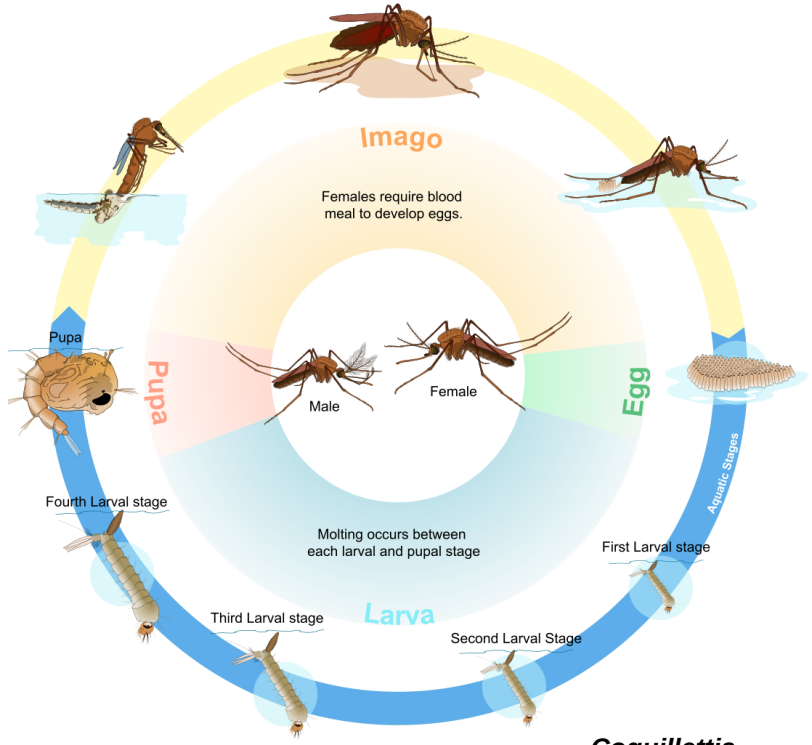
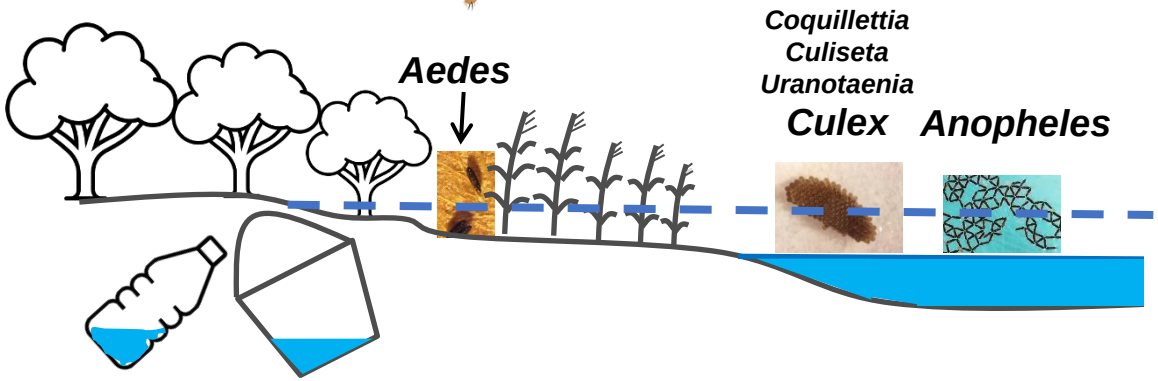
Life cycle depends on the temperature:
at 30°C it can last just 1 week





Larvae can inhabit nearly every type of water source

Breeding sites

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Four levels in classification of mosquito larval habitat

1. Ground habitats

1.1 Still waters

1.1.1 Permanent or semipermanent

- Marshes
- Swamps
- Exposed ponds, borrow pits
- Forest ponds
- Ditches
- Rice fields
- Wells
- Saltwater marshes
- Subterranean
- Polluted waters

Aedes ***Anopheles*** ***Culex***

Culex

1.1.2 Temporary

- Forest pools
- Exposed pools and puddles
- Hoofprints

Aedes -Anopheles - Culex

Aedes ***Anopheles***

- Saltwater pools

Aedes

1.2 Flowing waters

- Exposed streams, ditches, irrigation channels
- Forest streams
- Gravel streambeds

Anopheles ***Culex***

2. Container habitats

2.1 Natural

- Bamboo

Aedes, Anopheles

- Tree holes
- Leaf axils, bracts, bromeliads
- Pitcher plants
- Rock pools

Aedes ***Anopheles*** ***Culex***

- Fallen fruit husks, spathes
- Fallen leaves
- Crab holes

Aedes ***Culex***

- Empty snail shells

Aedes

2.2 Human made

- Water tanks, cisterns,

Aedes -Anopheles - Culex

- Domestic water storage pots
- Discarded tin cans, bottles, tires
- Miscellaneous

Aedes - Culex

- Latrines, septic tanks

Culex

Modified by: Service, M.W. (1993). Mosquitoes (Culicidae). In: Lane, R.P., Crosskey, R.W. (eds) Medical Insects and Arachnids. Springer, Dordrecht

Breeding sites

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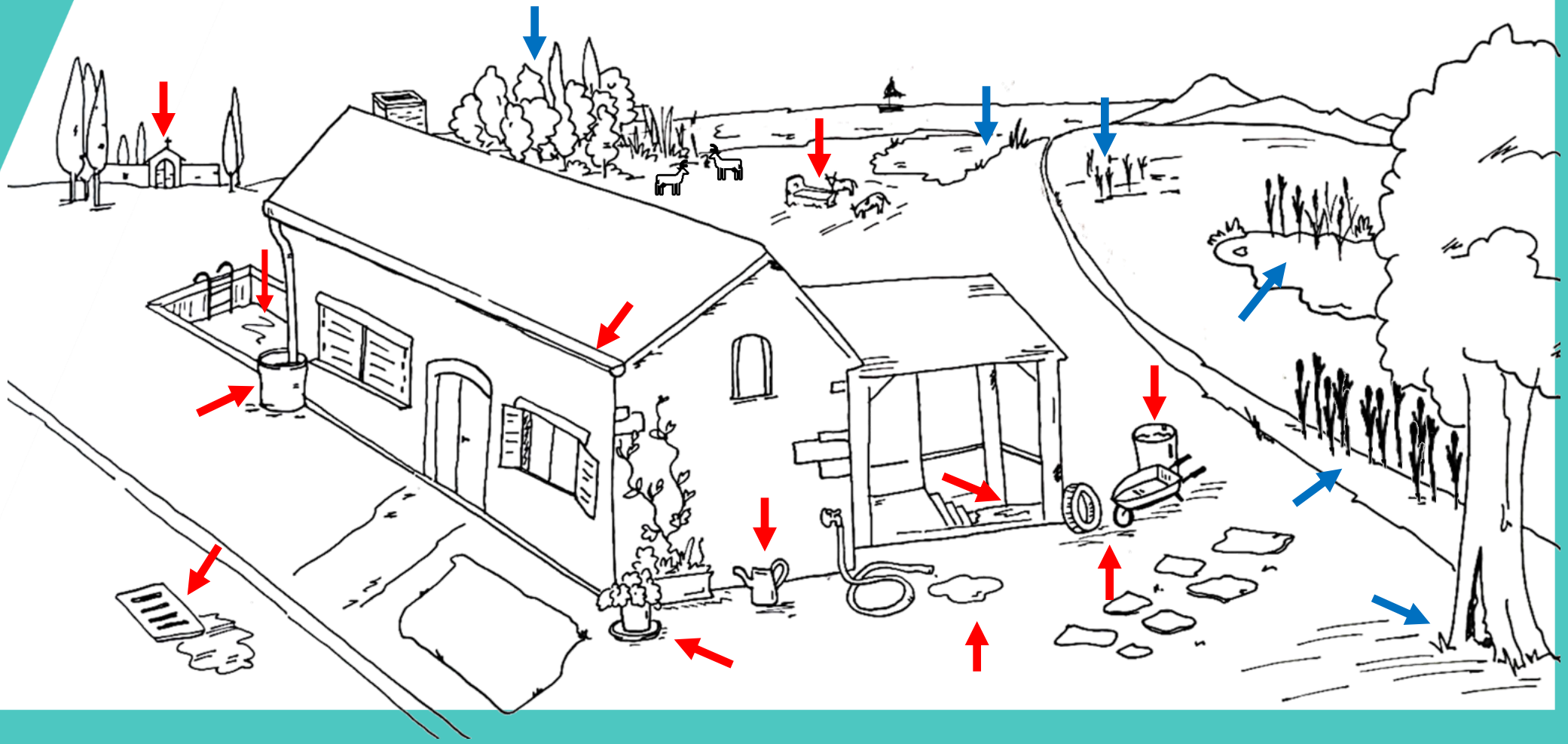
Natural habitats: ponds with vegetation, along river edges in still zones, in areas prone to inundation, in puddles and ruts, coastal marshes or rock pools, in water-filled tree-holes, leaf axils, etc.

Man-made water bodies: rice fields, flooded cellars, construction sites, road drains and pits, water barrels, metal tanks, ornamental ponds and any type of container (e.g. in gardens or cemeteries), etc.

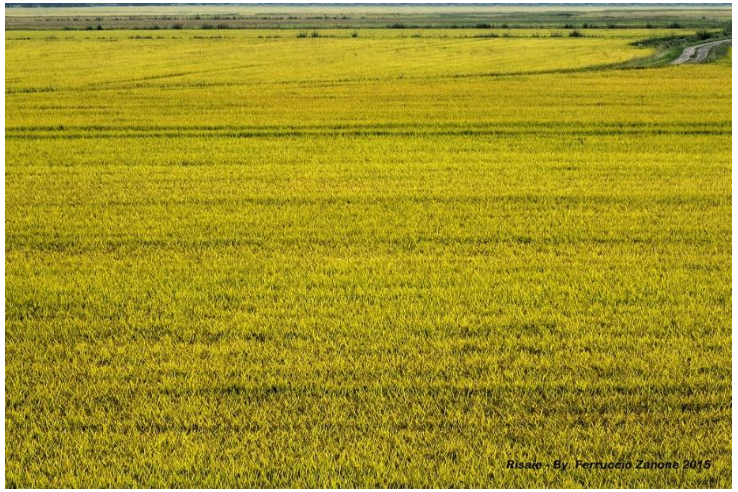


Breeding sites

Aedes geniculatus
Aedes berlandi
Aedes pulcritarsis
Anopheles plumbeus
Orthopodomyia pulcripalpis
…

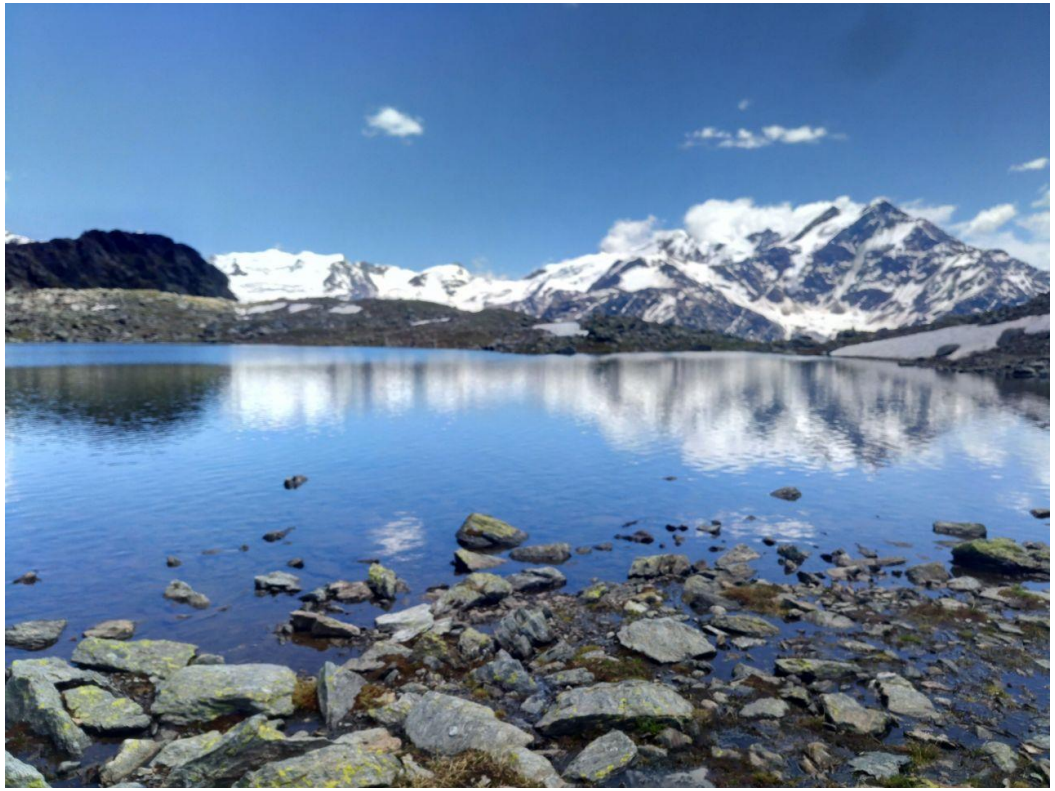
Aedes caspius
Aedes detritus
Aedes vexans

Anopheles spp.
Culex spp.



Breeding sites

Aedes mariae
Aedes zammitii



Aedes cathaphylla
Aedes pullatus

Ecological and behavioral traits

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Flight range: distance from the breeding sites (from 200m to 10 km)

Biting activity: diurnal, nocturnal, crepuscolar (dusk and early night)

Feeding behaviuor: ornitophilic and batrachophilic; mammophilic and anthropophilic; opportunistic

Endophagy/exophagy: active search for the host indoor or outdoor

Endophily/exophily: indoor or outdoor resting sites

Overwintering stages: eggs/larvae/adult

(larvae in few species; mated females in diapausing)

Autogeny/Anautogeny: doesn't need/needs to perform a blood meal to lay eggs

Larval stages: wide variety of water bodies (stagnant or flowing, natural or artificial, unpolluted or polluted, small or large)

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Genus *Aedes*:

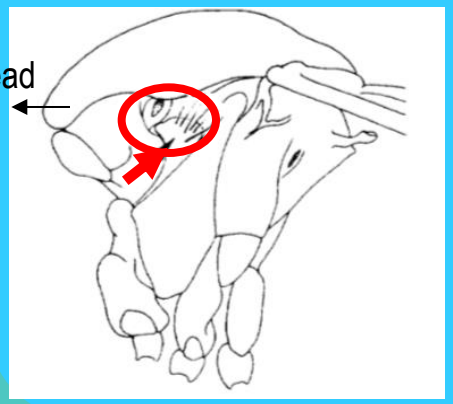
- Biting activities during the **day** and **night (**depending on the species)
- Include some of the most invasive (container) species: *Ae. aegypti*, *Ae. albopictus*, *Ae. japonicus*
- Floodwater mosquitoes: *Ae. caspius*, *Ae. detritus*, *Ae. vexans, …*

Ae. aegypti
Ae. albopictus
Ae. caspius
Ae. detritus
Ae. vexans

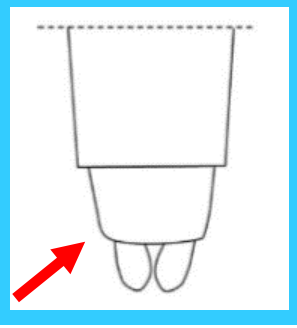
Aedes mosquitoes associated with RVF in Mediterranean basin:

- Seasonal temperature and photoperiodicity can lead to lay diapausing eggs (*Ae. albopictus*, *Ae. vexans*, *Ae. caspius, Ae. detritus*) as winter diapause; eggs can withstand desiccation
- The floodwater mosquitoes can show a bimodal seasonality caused by heavy rainfall following rainless period. Human activities (irrigation and water management) could modify this pattern.
- *Aedes vexans:* larval stages develop rapidly; adults have long lifespan and strong flight ability

Thorax
Postspiracular setae present



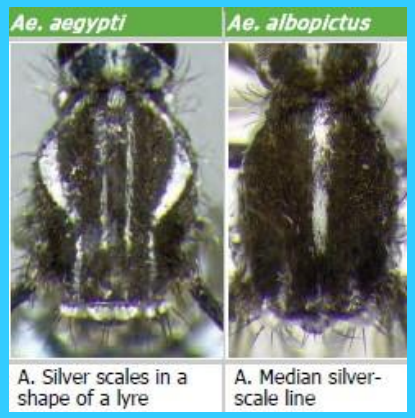
Abdomen
Apically pointed
Prominent cerci



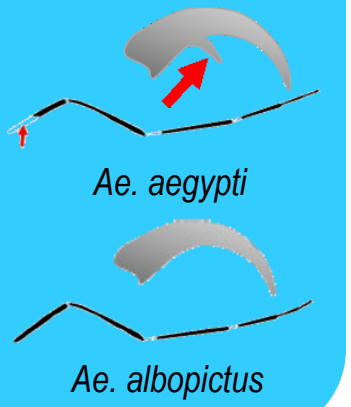
Abdomen

Ae. vexans *Ae. caspius*

Thorax



Claws –I,II; Fe-II



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GENUS *Culex*

Genus *Culex:*

- Adults feed on birds, reptiles, amphibians, and mammals (humans); exophilic/endophilic (depending on the species), biting activities mainly during the **dusk and night**
- lay eggs in fresh/brackish, clean/polluted **ground water**
- eggs are usually laid in raft

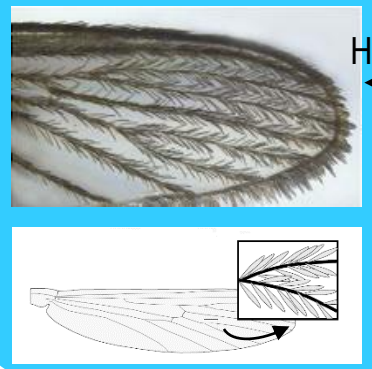
Cx. antennatus
Cx. perexiguus
Cx. pipiens s.l.
Cx. poicilipes
Cx. theileri
Cx. tritaeniorhynchus

Culex mosquitoes associated with RVF in Mediterranean basin:

- winter diapause at adult stage
- *Culex pipiens* has two biological forms (*pipiens* and *molestus*) and thei hibrids with different ecological needs
- Culex spp. reach very high population densities as the rainy season progresses

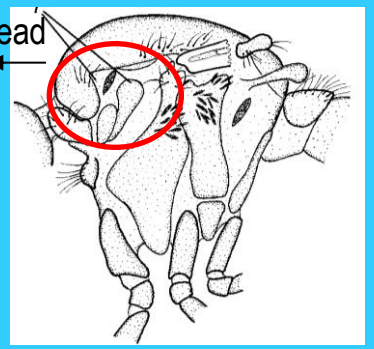
Wing

Scales generally narrow



Thorax

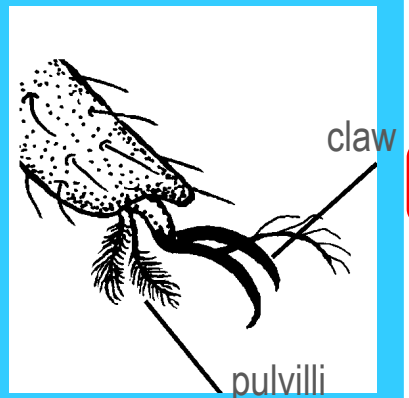
Pre and postspiracular setae absent



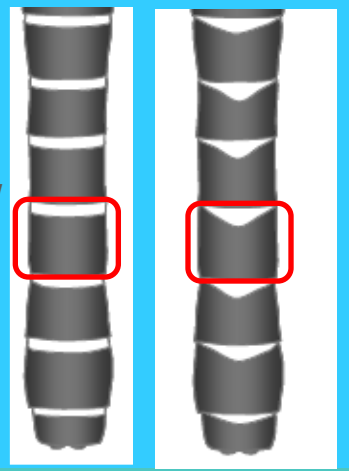
Abdomen

Apically rounded

Legs



Cx. pipiens *Cx. theileri*



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Culex pipiens s.s.

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In Spain *Cx. pipiens* hybrid strains (and *Ae. albopictus*) appear to be able to sustain the cycle of RVFV transmission.

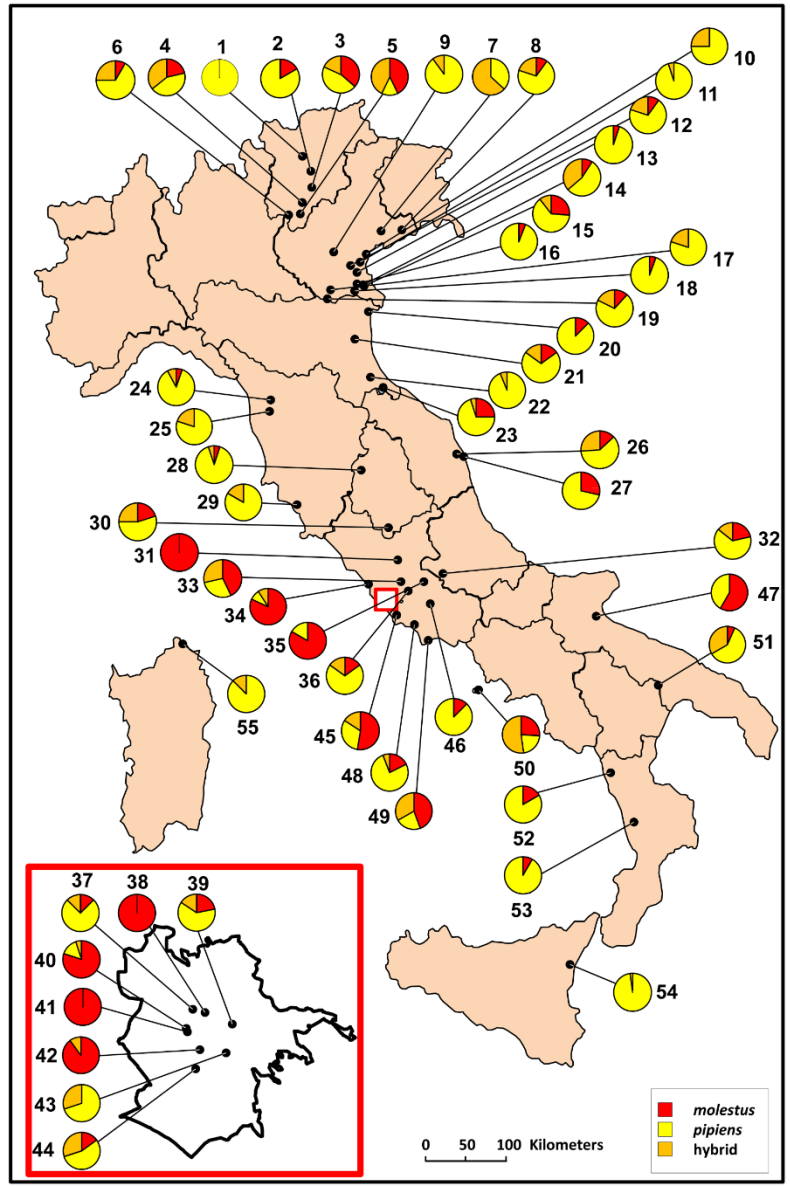
Cx. p. pipiens

- Rural
- Ornithophilic
- Anautogenous
- Eurygamous
- both aboveground and underground habitats
- wide variety of aboveground breeding sites
- heterodynamic

Hybrids

Cx. p. molestus

- Urban
- mammophilic (mammals and humans)
- Autogenous
- Stenogamous
- prefers underground water bodies with high organic contents
- homodynamic



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Di Luca et al. 2016. Ecological Distribution and CQ11 Genetic Structure of *Culex pipiens* Complex (Diptera: Culicidae) in Italy

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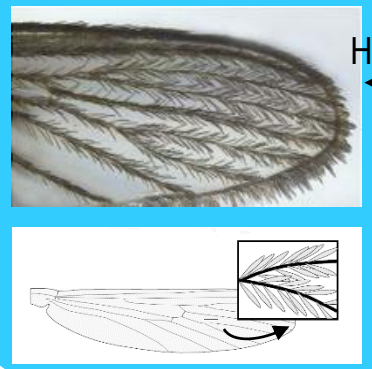
- Culex spp. reach very high population densities as the rainy season progresses

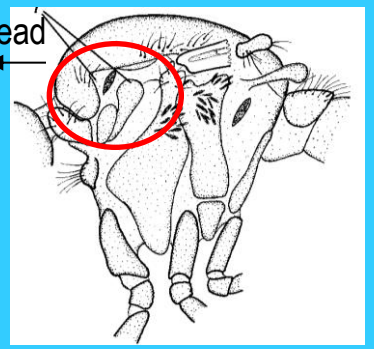
Wing

Scales generally narrow



Thorax

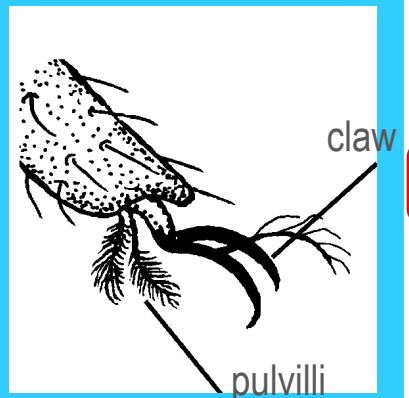
Pre and postspiracular setae absent



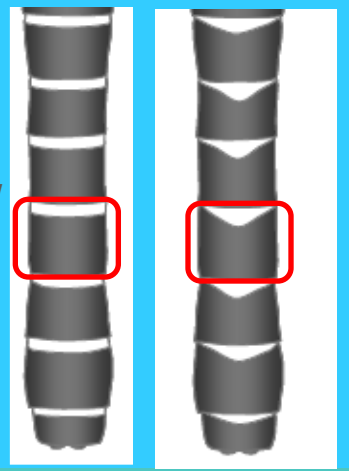
Abdomen

Apically rounded

Legs



Cx. pipiens *Cx. theileri*



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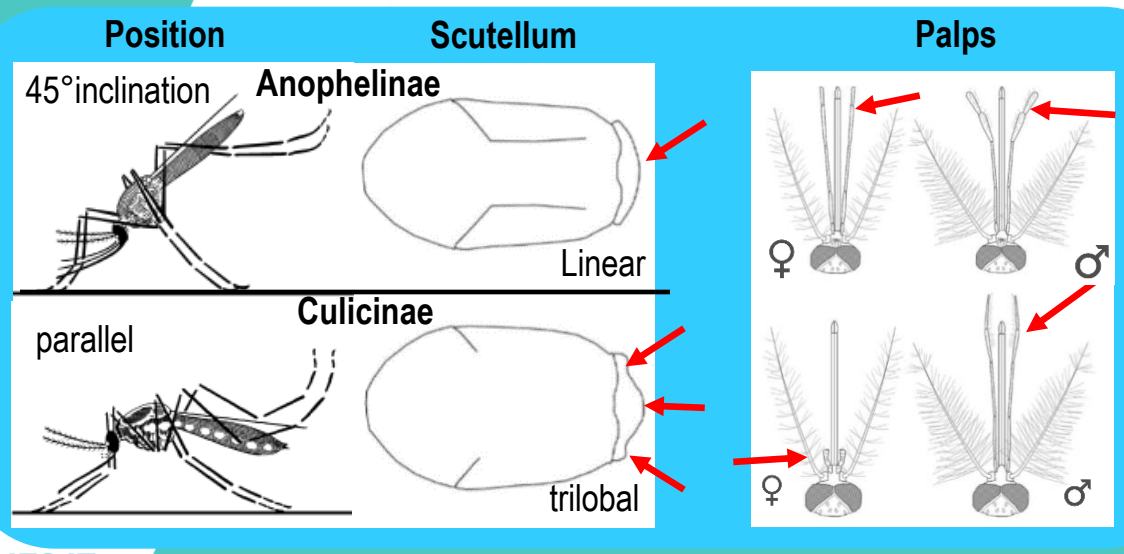
Genus *Anopheles:*

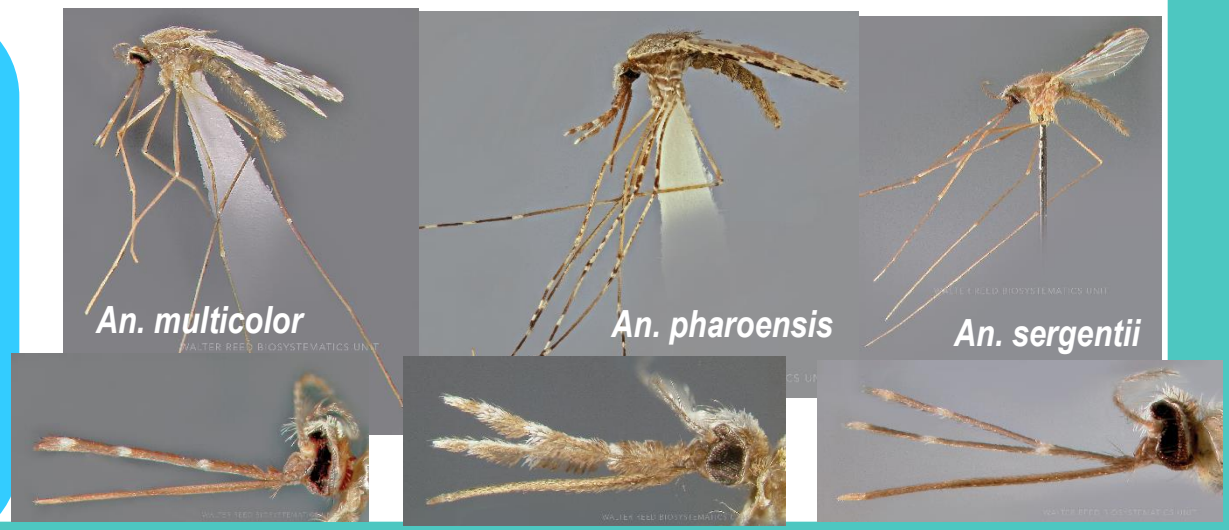
- Biting activities during the **dusk and night**, adults hide during the day in sheltered places
- Lay (individually) eggs (50-200) in clean, fresh (or brackish), **still or slow-moving waters** with floating vegetation
- winter diapause at larval or adult stages

An. multicolor
An. pharoensis
An. sergentii

Anopheles mosquitoes associated with RVF in Mediterranean basin:

- Gravid females of *An. pharoensis* were collected in aerial (40-290 m) samples in Mali;
- *An. multicolor* has strong flight ability
- *An. multicolor* and *An. sergentii* lay eggs also in brackish waters (Apr.-Jul.; Nov.-Dec.)





COLLECTION STRATEGY

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Mosquito ecology
Mosquito stages

Latitude

Temperature

Environment

Climate events

Human behavior

Cropland

Licvestock

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MOST COMMON COLLECTION METHODS

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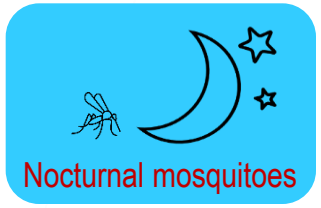
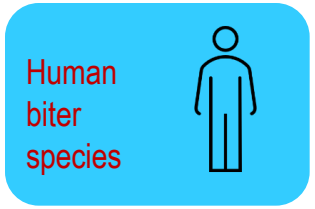
Based on the difference or the similarity in the ecology of the mosquitoes species, we can use a wide range of attractants:

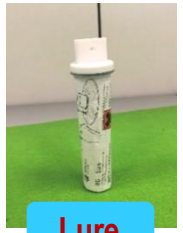
- light for nocturnal species
- olfactory stimuli, such as carbon dioxide and host odor components (e.g. lactic acid) for host-seeking mosquitoes
- water for oviposition



Light

CO_2 or dry ice





Lure

Human or animal baited

Water or infusion

Genus	Specie	Habitat	Biting activity	Feeding behaviour
Aedes	*aegypthi*	Container habitat	Diurnal	Mammals
Aedes	*albopictus*	Container habitat	Diurnal	Mammals
Aedes	*caspius*	Ground habitat	Diurnal/Nocturnal	Mammals
Aedes	*detritus*	Ground habitat	Diurnal/Nocturnal	Mammals
Aedes	*vexans*	Ground habitat	Diurnal/Nocturnal	Mammals
Anopheles	*pharoensis*	Ground habitat	Nocturnal	Mammals
Anopheles	*sergentii*	Ground habitat	Nocturnal	Mammals
Anopheles	*multicolor*	Ground habitat	Nocturnal	Mammals
Culex	*antennatus*	Ground habitat	Nocturnal	Mammals
Culex	*perexiguus*	Ground habitat	Nocturnal	Birds/Mammals
Culex	*poicilipes*	Ground habitat	Nocturnal	Birds/Mammals
Culex	*tritaeniorhynchus*	Ground habitat	Nocturnal	Mammals
Culex	*pipiens* s.l.	Ground and container	Nocturnal	Birds/Mammals
Culex	*theileri*	Ground and container	Nocturnal	Birds/Mammals

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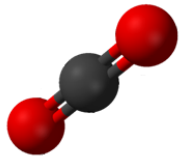
FLYING ADULT COLLECTION

Nocturnal mosquitoes

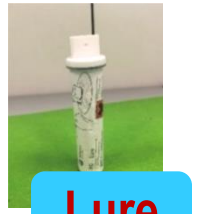
Light

Host-seeking mosquitoes



CO_2

Human biter species



Lure

CO_2 from gas bottle

CO_2 at a rate of 175 ml/min
10 kg of CO_2 lasts ~20 days

CO_2 from DRY ICE

1 kg of dry ice = CO_2 at a rate of 387 ml/min

CO_2 from yeast fermentation

CO_2 production for 24 hours

The most commonly used suction traps

CO_2 LIGHT TRAPS

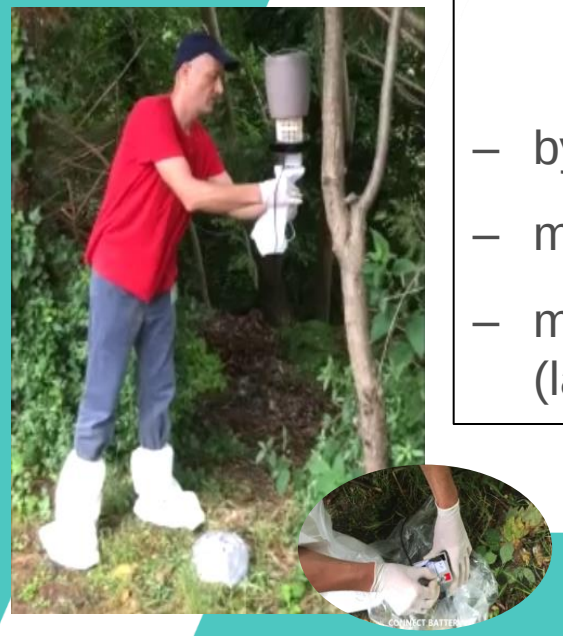
- The most commonly used traps
- Running overnight
- Baited with light and dry ice (CO_2)

Strong points

- efficient for nocturnal species

Weak points

- by-catch of other insect species
- mosquitoes damaged by the fan
- managing dry ice and batteries (last only one night)



BG SENTINEL TRAPS

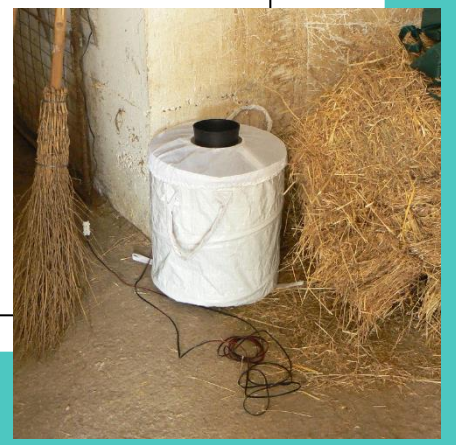
- Running during day and night
- Baited with BG-lure; with CO_2 (dry ice) attractive for wider range of mosquitoes

Strong points

- efficient for diurnal anthropophilic species
- catch bag above the fan reduces mosquito damage
- Automatic shutter ensures catch integrity

Weak points

- designed to attract only some *Aedes* sp.
- managing batteries



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Suction traps

BG – Pro: 2 in 1

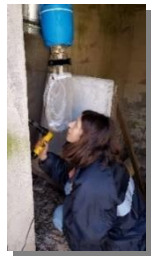
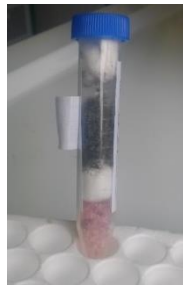
- **BG sentinel style:** in standing choice, used with BG-Lure (*Ae. albopictus*, *Ae. aegypti*)
- Used with CO_2, it can capture different mosquito species
- **CO_2 light trap-style:** in hanging choice used with dry ice or yeast fermentation and LED light

- Multiple power options (battery, power supply, powerbank)
- Low power consumption for longer battery life (5 or 6 V), powered for 2 days with a 10,000-mAh power bank (W180 gr.)
- Catch bag above the fan reduces mosquito damage
- Automatic shutter ensures catch integrity

SUMMARIZING COLLECTION METHODOLOGY

Methodology	Target	Attractants	Sites	Sample management
CO_2 Light trap 	Nocturnal mosquitoes 	Light and dry ice  	Shaded and protected from the wind 	Sample between cotton wool in tube with silica gel 
BG – Sentinel (CO_2) 	Diurnal (and nocturnal) mosquitoes 	Chemical lure (and dry ice)  	Protected from the rainfall 	Sample between cotton wool in tube with silica gel 
Gravid trap 	Eggs laid directly in the water 	Infusions of dead oak leaves or grass 	Shaded 	Sample between cotton wool in tube with silica gel 

SUMMARIZING COLLECTION METHODOLOGY

Methodology	Target	Attractants	Sites	Sample management
Ovitrap 	Container mosquitoes: some *Aedes* sp. 	Water 	Close to or under vegetation or near buildings 	In plastic bags In water for rearing larvae
Netting, dipping, aspirating 	Larvae 	None	Breeding sites 	Rearing L1-L3 larvae to L4 larvae or until adult emergence
Aspirator 	Endophilic or exophilic mosquitoes 	None (indooor) or human/animal bait (indoor/outdoor)	Indoor or outdoor  	Sample between cotton wool in tube with silica gel 

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SPERIMENTALE
DELL'ABRUZZO
E DEL MOLISE
"G. CAPORALE"

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Culex pipiens complex (or assemblage)

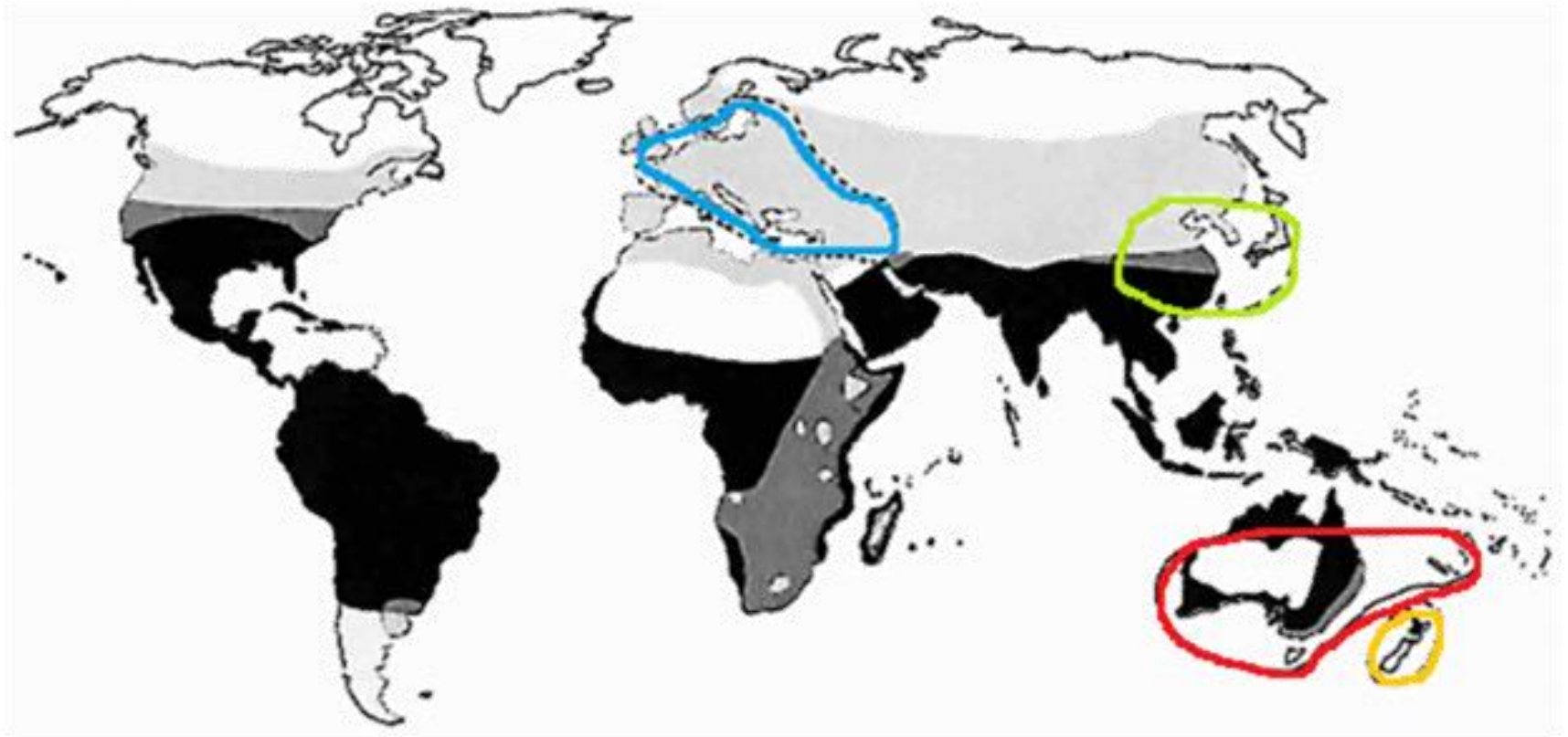

FIGURE 1. Distribution of the *Culex pipiens* complex and its sibling species based on maps of Dahl,[35] Belkin,[36] Mattingly and others,[37] and available literature.[12,38,39] Light gray = *Cx. pipiens*; black = *Cx. quinquefasciatus*; dark gray = overlapping ranges of *Cx. pipiens* and *Cx. quinquefasciatus*; region marked by dotted line = *Cx. torrentium*; region marked by solid line = *Cx. australicus*; region marked by dashed line = *Cx. pipiens pallens*; New Zealand marked by dotted and dashed line = *Cx. pervigilans*.

Smith and Fonseca, 2004. Rapid assays for identification of members of the Culex (Culex) pipiens complex, their hybrids, and other sibling species (Diptera: Culicidae). Am. J. Trop. Med. Hyg., 70(4), 2004, pp. 339–345