The sugar feeding behavior of *Anopheles gambiae*

a possibility for effective but environmentally friendly control?

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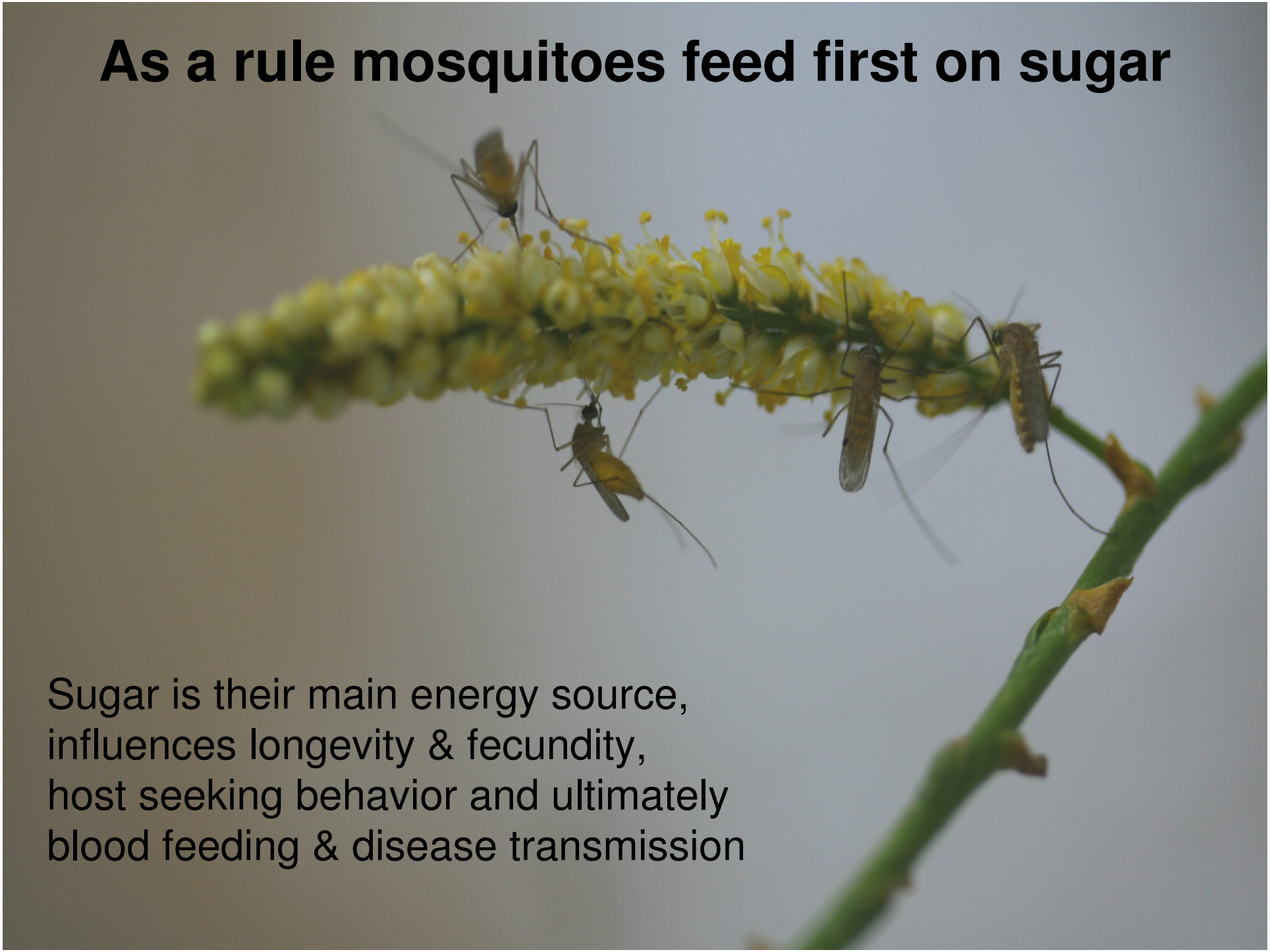
As a rule mosquitoes feed first on sugar



Sugar is their main energy source,
influences longevity & fecundity,
host seeking behavior and ultimately
blood feeding & disease transmission

.. but in many field studies the malaria vector *Anopheles gambiae* was found to be rarely fed on sugar and accordingly
it was in the past a common assumption that sugar feeding is not a major issue for this species.

Only recently this perception is changing.

Some basics on sugar feeding:

Cx. pipiens feeding on a rotten fruit

An. sergentii feeding on nectar

- Floral nectar
- Rotten or damaged fruits
- Honeydew
- Extra floral nectaries
- Tree sap


An. sergentii feeding on leaf


Culex sp. feeding on seed pod

Measured attraction distances for female *An. sergentii*

• Non-flowering plants (+/-) honeydew	<2m
• annual flowers ranged from	<2-4m
• *Acacia raddiana*	22m
• *Ochradenus baccatus*	44m
• Chicken	32m
• Human	40m
• ATSB	8m

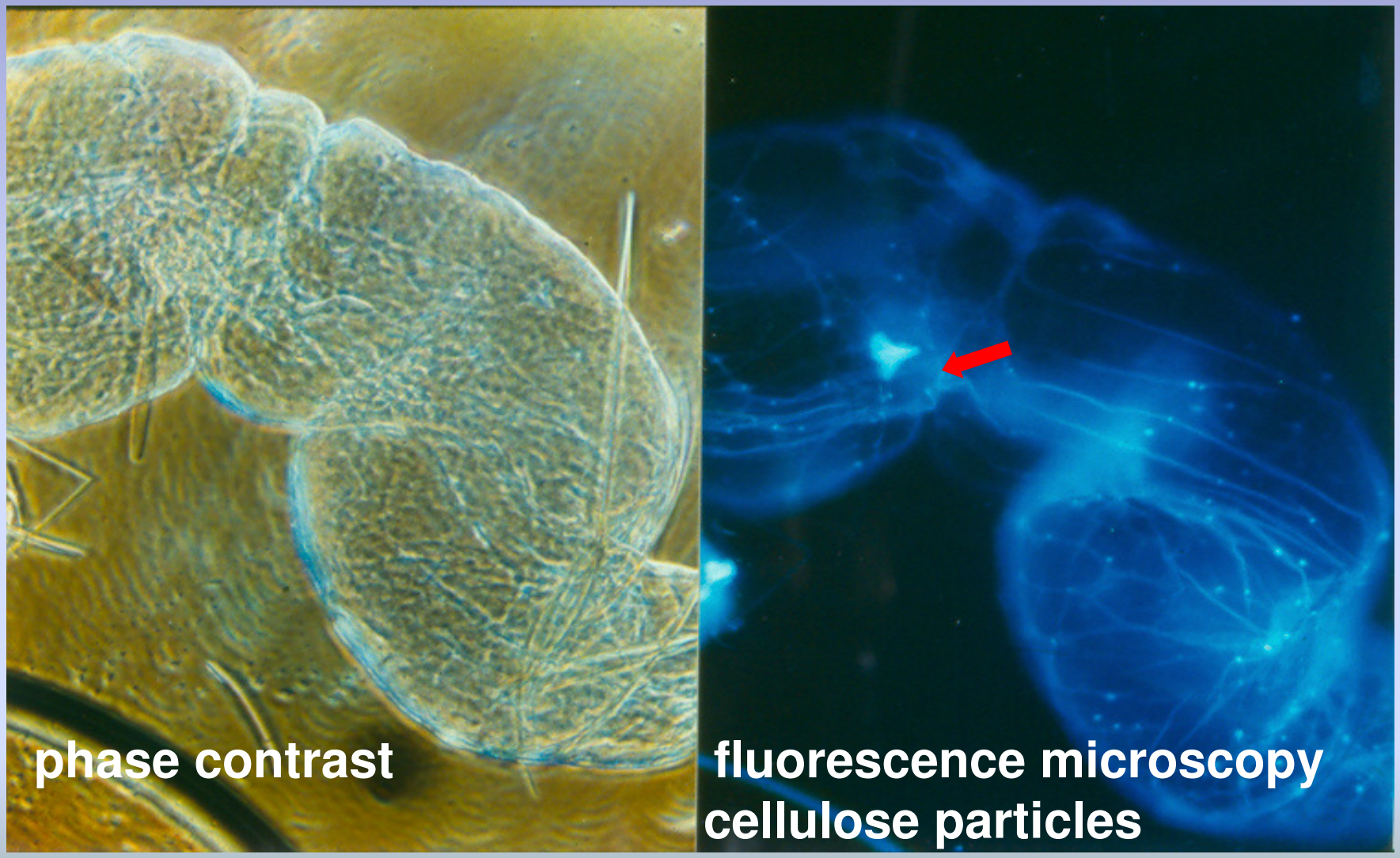
How to find sugar in a mosquito gut?

Mosquitoes are born sugar negative

- **Mosquitoes are placed in a microtiter plate**
- **Reaction agent is added**
- **Microplate Reader 590nm**
- **OD's are converted to sucrose equivalents according to a calibration curve**

Sugar negative results were defined by assays of unfed mosquitoes

How to know if mosquitoes fed on plant tissue?



To understand the influence of sugar shortage on mosquitoes

first some **basics** of mosquito physiology

How long does it take until an *Anopheles* is infective after a blood meal?

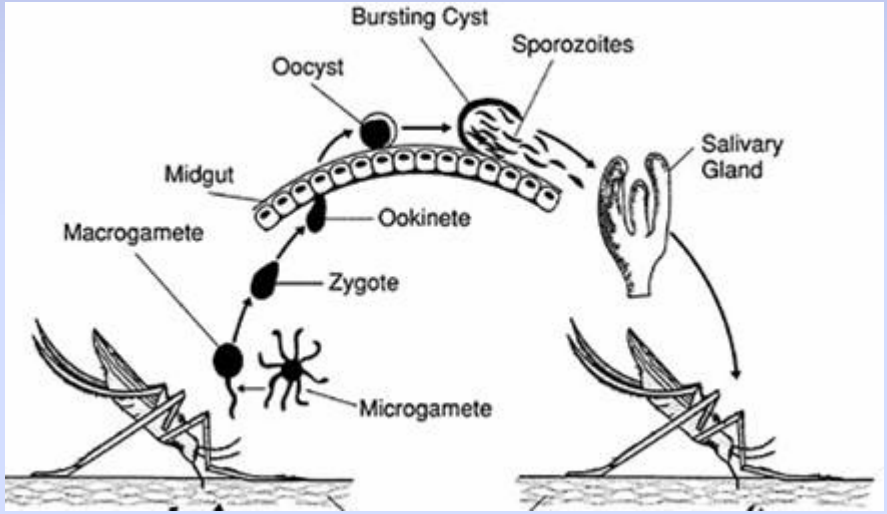
The cycle in the mosquito

takes in nature about:

9 days for *P. vivax*

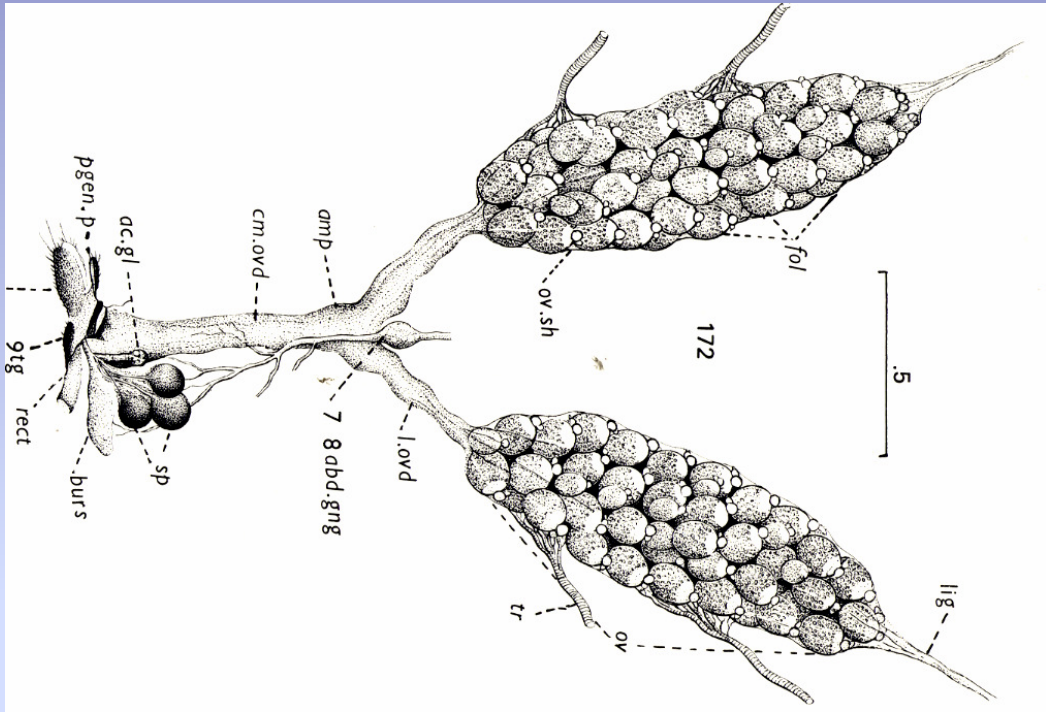
11 days for *P. falciparum*

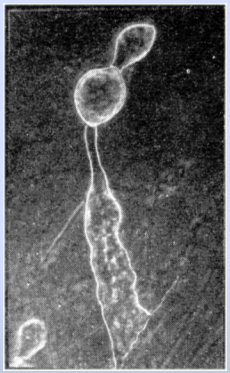
21 days for *P. malariae*



Age grading of mosquitoes

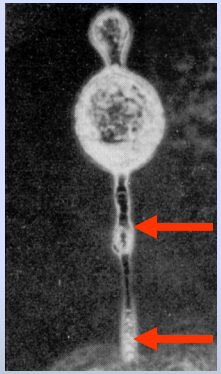
The physiological age of females can be determined by the counts of ovarian dilatations.



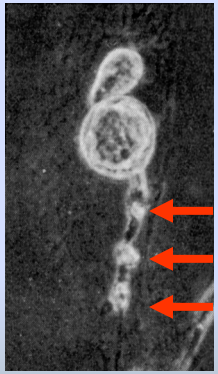


Pedicel stretched after oviposition

ovariole with single dilatation

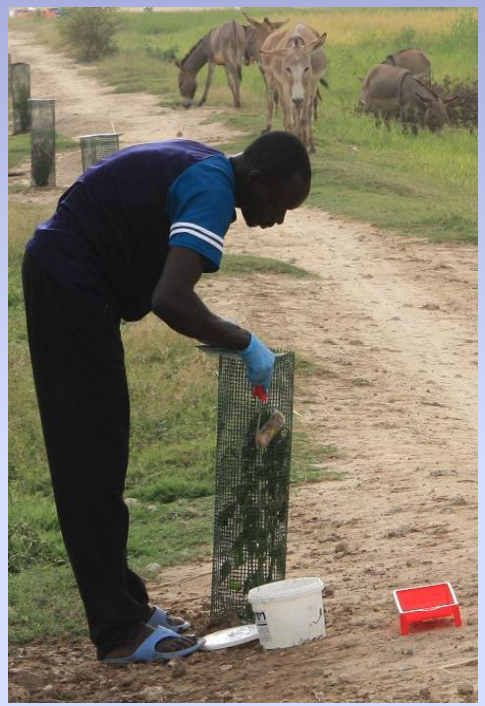
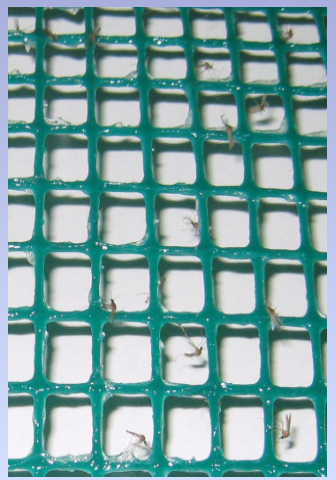


ovariole with two dilatations



ovariole with three dilatations

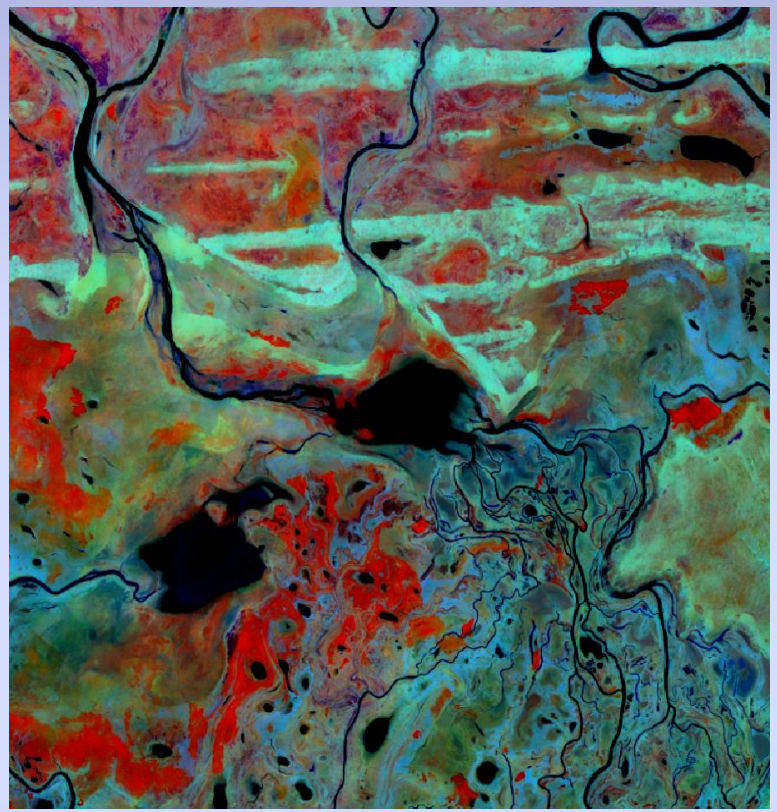
In previous, already published studies, we explored in Mali if *An. gambiae* has any preferences for specific sugar sources

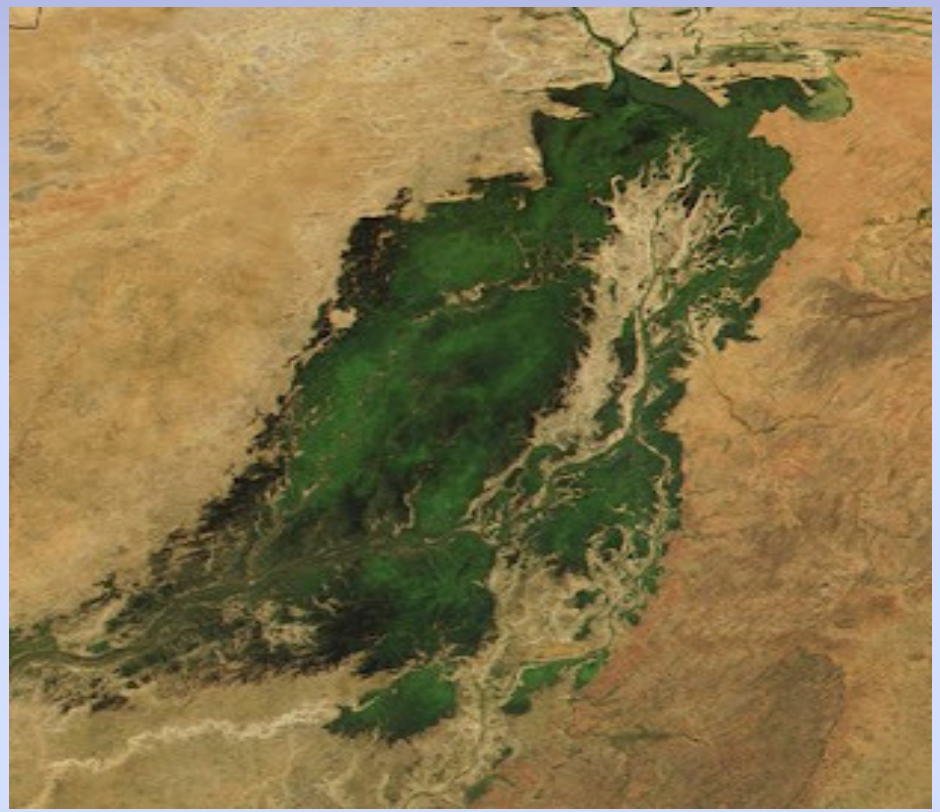





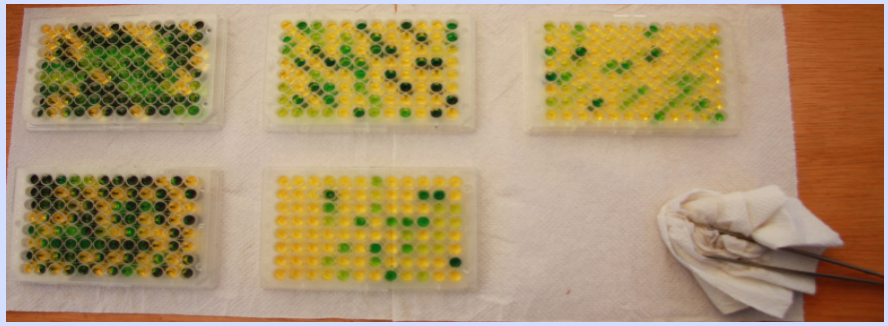

We tested 26 flowering plants and 26 fruits and seedpods. Of the flowers 9 were significantly attractive for females, and 8 were attractive for males while 6 fruits and seed pods were attractive for both female and male *An gambiae.*

In recent, unpublished studies, we explored further aspects of the sugar feeding ecology of *An. gambiae* in the diverse ecosystems of the River Niger Inland Delta in Mali





For this purpose we set up camps in the field with all the necessary laboratory equipment



We wanted to answer the following questions:

- **How early is *An. gambiae* feeding on sugar in the field?**
- **Is sugar feeding influenced by the local environment?**
- **Is there a difference in sugar feeding regarding indoor and outdoor collected mosquitoes?**
- **What is the rate of digestion and detectability of sugar meals over time?**
- **Is there a difference in sugar feeding regarding mosquitoes entering and leaving houses?**
- **How long are mosquitoes staying in houses without feeding on sugar?**

How early is *An. gambiae* feeding on sugar in the field?

To evaluate this we caught overnight, in tent like emergence traps, emerging *An. gambiae* from a shallow lagoon. We exposed these mosquitoes inside the traps in the same night to attractive and sugar rich seedpods. In the following morning the mosquitoes were recovered from the cages and tested for sugar feeding by anthrone

From 230 females
(83.87%, 192/ 230)
and 239 males
(88.7%, 212/ 239)
most were sugar positive.

Is sugar feeding influenced by the environment?

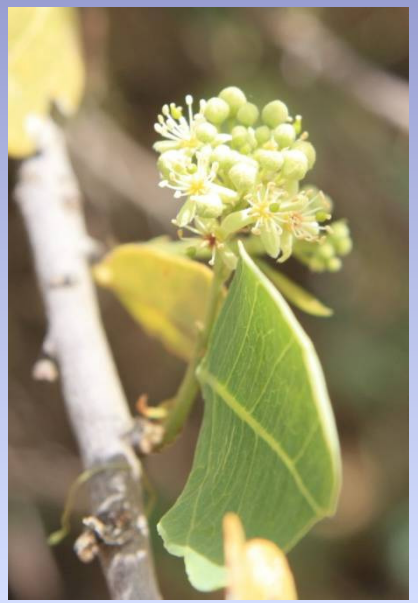
For this question we investigated sugar feeding in 6 types of habitats

Urban environment

in midst of rice fields

Breeding habitats
in sugar rich environment

Breeding habitats
in sugar poor environment



A lagoon in different seasons, first trees with flowers later with fruit

Is sugar feeding influenced by the environment?

Table 1: Sugar feeding status of *An. gambiae* s.l. in different habitats in Mali in late October, end of rainy season (testing conducted 8:00 in the morning)

Habitat	Proportion of *An. gambiae* s.l. (*An. gambiae* s.s.)	Proportion sugar fed	Percentage sugar fed ✔	Average sugar meal size ✔	Proportion plant tissue fed	Percentage plant tissue fed ✔	Percentage of females with 2-4 gonotrophic cycles ✔
A: urban environment (residential area of Mopti with some flowering and fruited trees)							
females	80 (75)	113/204	53.39	medium	10/205	4.88	13.20
males		89/175	50.85	medium	3/165	1.82	
B: in midst rice fields (without any sugar source in the vicinity)							
females	83 (81)	135/400	33.75	small	60/210	28.57	4.00
males		110/400	27.50	small	46/195	23.58	
C: near ponds surrounded by flowering trees and bushes							
females	60 (55)	326/400	81.50	large	3/250	1.20	22.40
males		338/400	84.50	large	1/250	0.40	
D: near ponds surrounded by vegetation without flowers and fruits							
females	60 (53)	153/400	38.25	small	36/200	18.00	9.20
males		142/400	35.50	small	28/190	14.47	
E: natural lagoon surrounded by flowering trees and bushes							
females	55 (47)	304/400	76.00	large	6/250	2.40	20.80
males		298/400	74.50	large	4/250	1.60	
F: same natural lagoon one month later, now only surrounded by fruited trees and bushes							
females	70 (63)	272/400	68.00	large	25/250	10.00	27.00
males		286/400	71.50	large	19/250	7.60	

In the sugar rich habitats most of the mosquitoes tested sugar positive and the meal sizes were large while in sugar poor sites few were sugar fed and meal sizes were small. The high proportion of plant tissue fed mosquitoes from the sugar poor site suggests that *An. gambiae* resorted to plant tissue feeding in the absence of flowers and fruits. At a natural lagoon *An. gambiae* succeeded to shift in ease from flowers to fruit. **At least in the sites we explored the availability of sugar sources apparently influenced directly longevity and vector competence.**

Is there a difference in sugar feeding regarding indoor and outdoor collected mosquitoes?

For this question we tested mosquitoes collected indoors, in the center of the same village and from 3 types of habitats in the direct vicinity of the village


• Near flowering Acacia
• Near rice paddies
• Near a lagoon without sugar sources

Is there a difference in sugar feeding regarding indoor and outdoor collected mosquitoes?

Table 2: Sugar feeding status of *An. gambiae* s.l. collected indoors and peri-domestic in central Mali
(testing conducted 8:00 in the morning)

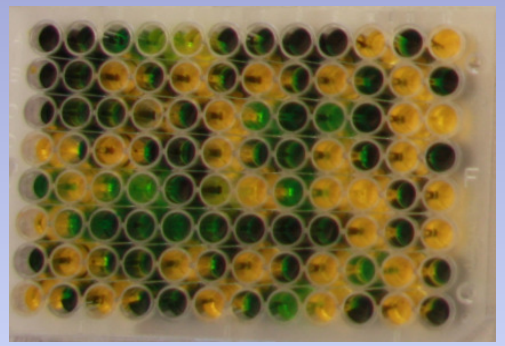
Habitat	Proportion of *An. gambiae* s.l. (*An. gambiae* s.s.)	Average CDC trap catch	Proportion sugar fed	Percentage sugar fed ✔	Average sugar meal size ✔	Proportion plant tissue fed	Percentage plant tissue fed ✔
A: indoors							
females	40 (39)	20.5	37/200	18.5	medium	1/100	1
males		10.7	52/200	26.0	medium	0/100	0
B: in the village center							
females	40 (37)	4.3	172/262	65.7	ND	ND	--
males		3.5	131/210	62.4	ND	ND	--
C: near flowering Acacia trees (100m from village center) sugar rich site							
females	40 (39)	10.3	173/200	86.5	large	0/100	0
males		13.0	163/200	81.5	large	0/100	0
D: near rice paddies, major breeding site (200m from village center)							
females	40 (36)	2.9	159/274	58.0	ND	ND	--
males		2.5	143/235	60.9	ND	ND	--
E: small natural lagoon surrounded by vegetation without flowers and fruit, mayor breeding site (1400m from village center) sugar poor site							
females	40 (37)	6.8	51/200	25.5	small	22/100	22
males		5.0	42/200	21.0	small	18/100	18

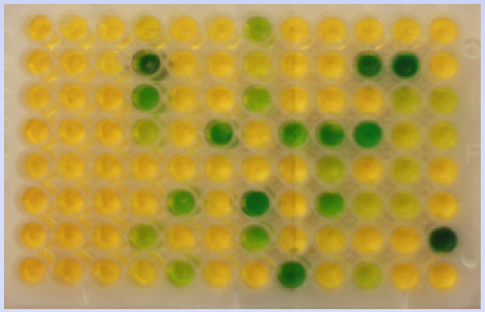
In this context it is important to note that in most (if not all) existing studies sugar feeding of *An. gambiae* was evaluated by mosquitoes collected during day time by PSC in houses.

What is the rate of digestion and detectability of sugar meals over time?

Table 3: Digestion and detectability of sugar meals at 8:00 in the morning, 8,14 and 24 hrs. later

Habitat	Percentage tested sugar positive/n			
	At 8:00	8 hours later	14 hours later	24 hours later
A: indoors				
females	18.5%/200	2.5%/200	0%/150	not done
males	26.0%/200	3.5%/200	0%/150	not done
C: near flowering Acacia trees (sugar rich site)				
females	86.5%/200	40.5%/200	11.3%/150	1.3%/150
males	81.5%/200	30.5%/200	4.0%/150	0%/150
E: small natural lagoon (sugar poor site)				
females	25.5%/200	1.5%/200	0%/150	not done
males	21.0%/200	2.0%/200	0%/150	not done





In the morning significant amount of mosquitoes proofed to be sugar fed from all locations while 8 hours later almost no more sugar meals could be detected from mosquitoes collected indoors and at a sugar poor sites while from the sugar rich site only about half of the mosquitoes were still positive. This shows how important it is to standardize testing for sound comparisons of spatial and seasonal sugar feeding.

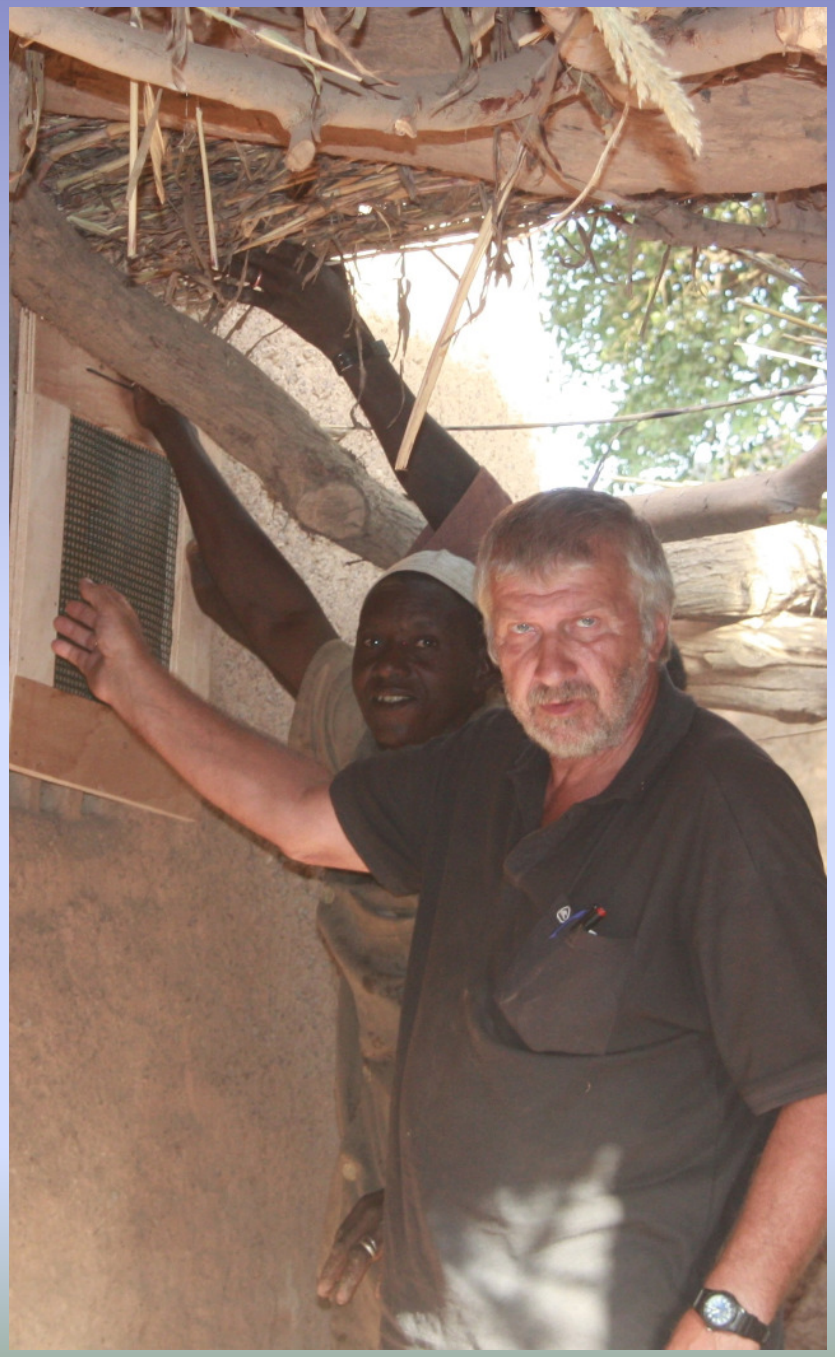
Is there a difference in sugar feeding regarding mosquitoes entering and leaving houses?

To evaluate the coming and going and the physiological state of *An. gambiae* in houses in Mali we designed simple window traps from rigid plastic netting, covered with glue, placed both outside and inside a window. The body of the trapped mosquitoes was in more than 90% of the cases free of glue and could be used for sugar tests and dissections.

We blocked the windows of 28 houses with these traps from in and outside and recovered daily for 5 days mosquitoes.

Almost all females (325/ 349) were exiting the buildings within the first three days (day 1: 148, day 2: 130, day 3: 47, day) while the bulk of the males (265/ 282) were in equal proportions exiting in the first two days.

At day 5 we knocked down the few remaining mosquitoes in the houses (17 females 2 males) with PSC.



Almost all mosquitoes exiting the buildings were sugar negative (91% of the females and 98% of the males) while entering females were in 79% and males in 91% of the cases sugar positive.

The data suggest that at least at the end of the rainy season both males and females leave at least every second day buildings to search for sugar.

Conclusions

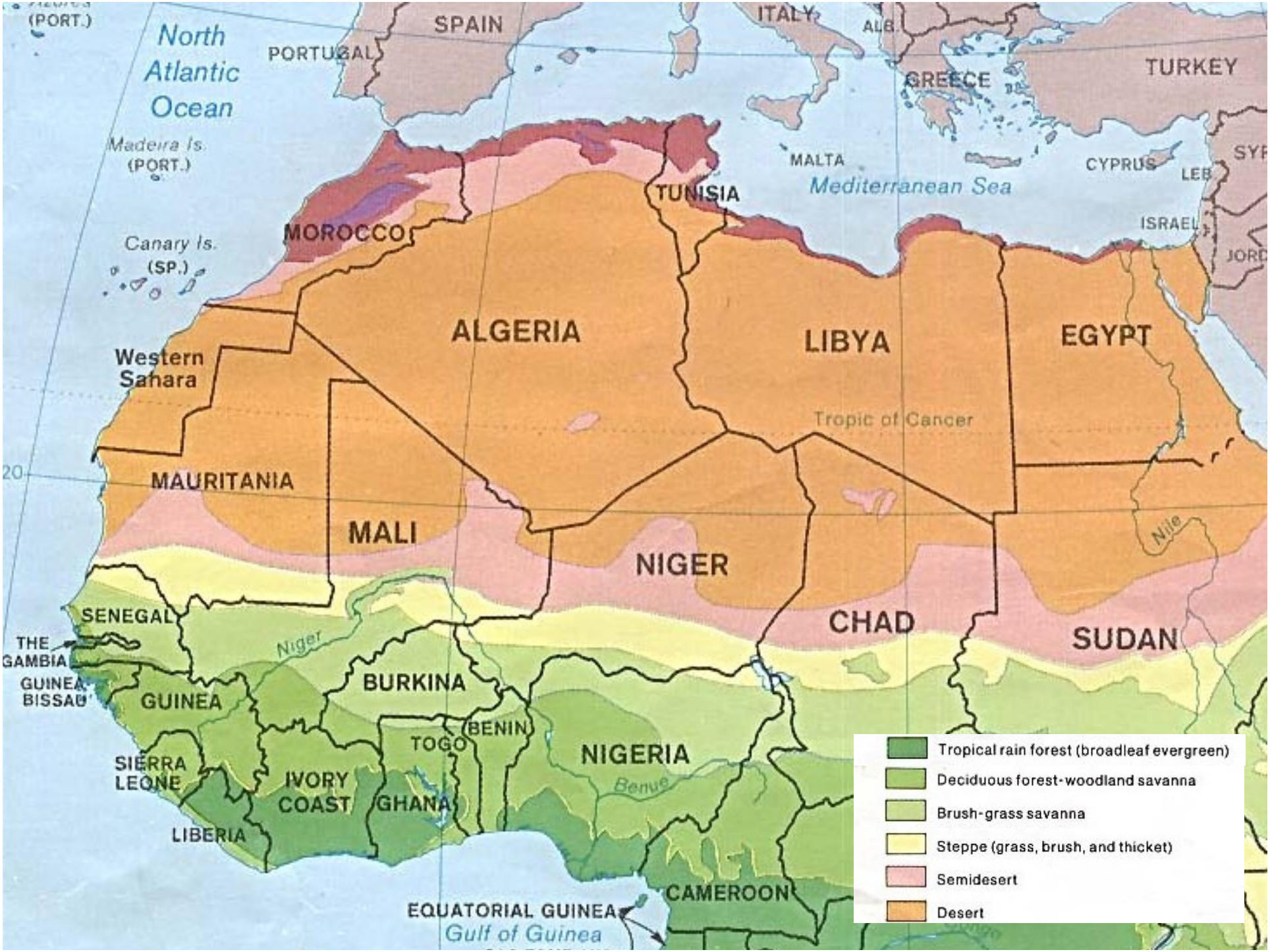
In previous studies mainly indoor resting mosquitoes were collected for sugar testing; testing was often conducted many hours after sampling (oral communication with several colleagues).

Sampling methods and testing protocols for sugar feeding need to be standardized to enable sound comparisons of different studies.

Our data and data of other recent studies suggest that in the past the importance of sugar feeding for *An. gambiae* was under evaluated.

How can the sugar feeding behavior of *Anopheles gambiae* be used for effective but environmentally friendly control?

Attractive Toxic Sugar Baits (ATSB)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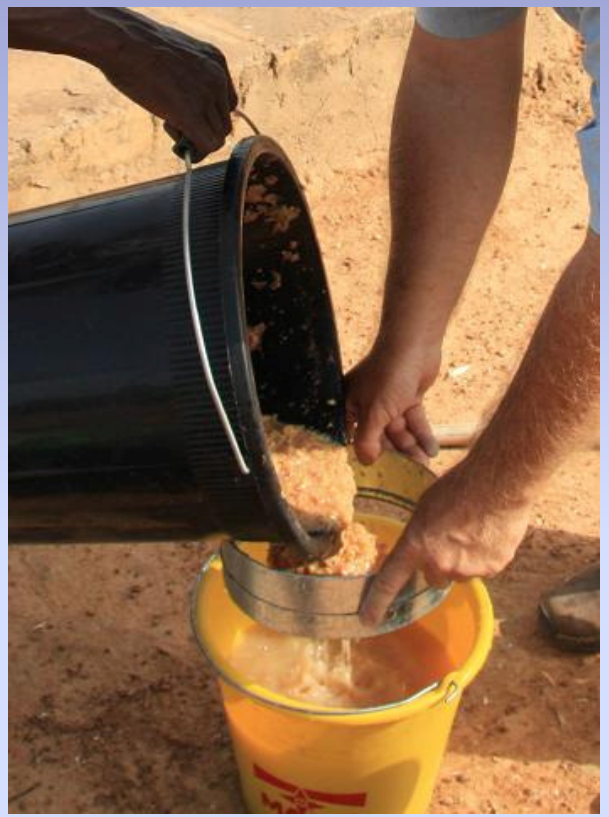
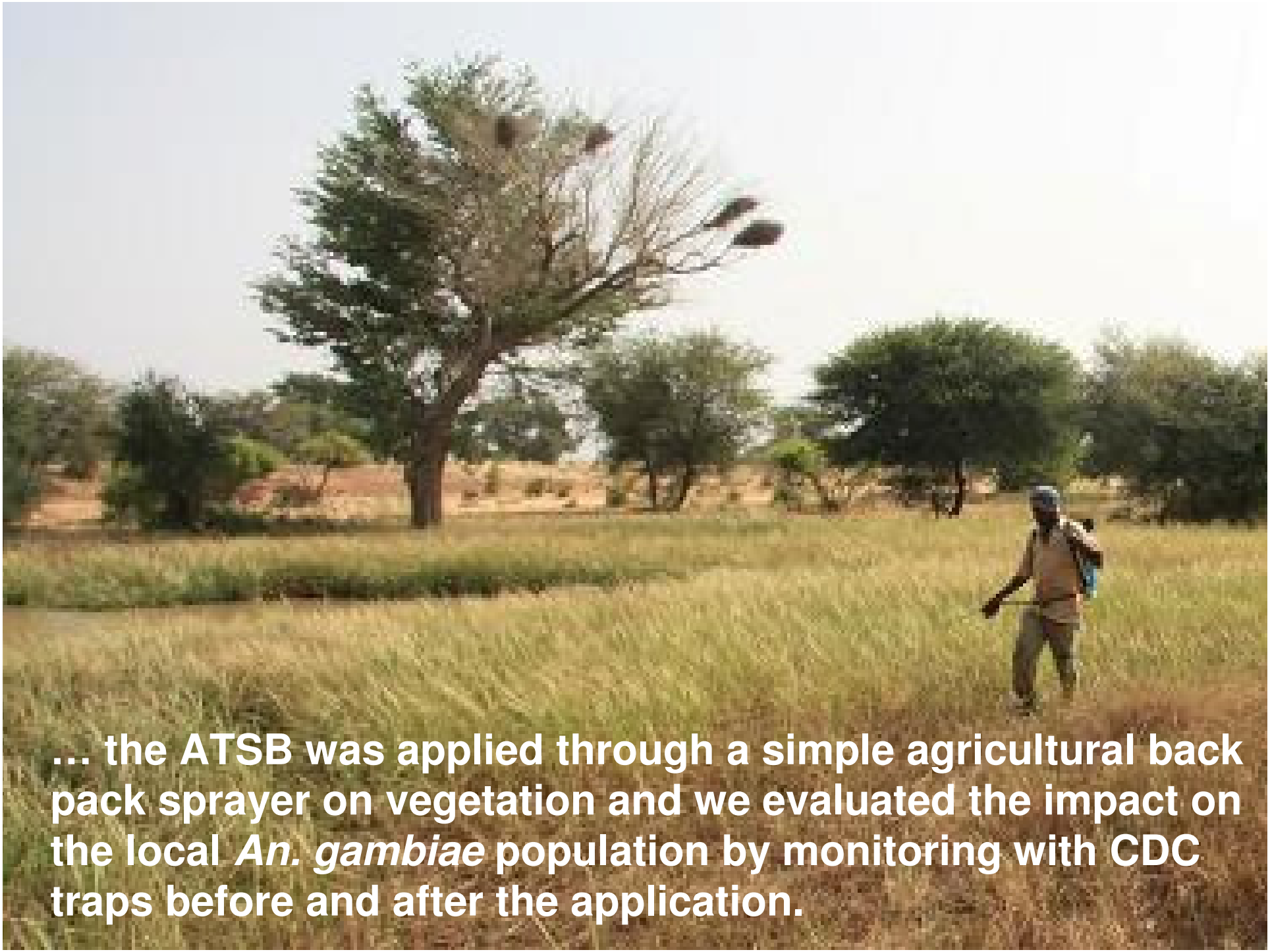
North Atlantic Ocean
PORTUGAL
SPAIN
ITALY
ALB.
GREECE
TURKEY
Madeira Is. (PORT.)
MALTA
Mediterranean Sea
CYPRUS
LEB.
SYR.
TUNISIA
ISRAEL
JORD.
MOROCCO
Canary Is. (SP.)
ALGERIA
LIBYA
EGYPT
Western Sahara
Tropic of Cancer
20
MAURITANIA
MALI
NIGER
Nile
CHAD
SUDAN
SENEGAL
THE GAMBIA
GUINEA BISSAU
Niger
GUINEA
BURKINA
BENIN
TOGO
NIGERIA
Benue
SIERRA LEONE
IVORY COAST
GHANA
LIBERIA
CAMEROON
EQUATORIAL GUINEA
Gulf of Guinea
Tropical rain forest (broadleaf evergreen)
Deciduous forest-woodland savanna
Brush-grass savanna
Steppe (grass, brush, and thicket)
Semidesert
Desert

In early studies we identified attractive fruits and from these we prepared baits ….



The fruits were crushed, mixed with sugar, and left for two days for fermentation. A concentrate of Bait Stab® Westham Ltd. Israel Tel Aviv was added for preservation and bait stabilization, as oral toxin we used boric acid.



… the ATSB was applied through a simple agricultural back pack sprayer on vegetation and we evaluated the impact on the local *An. gambiae* population by monitoring with CDC traps before and after the application.

Populations that were exposed to baits spiked with oral toxins collapsed within days and the few reminding mosquitoes were generally not old enough to transmit malaria.

In additional experiments with non-toxic but color-stained bait daily feeding rate ranged depending on the site
for
females from 48.4 to 73.6%
males from 55.6 to 72.2%.

Site I, northern Sudanian

The Study site near Kenieroba, a small fishing village, at the banks of the River Niger

Belenidanga

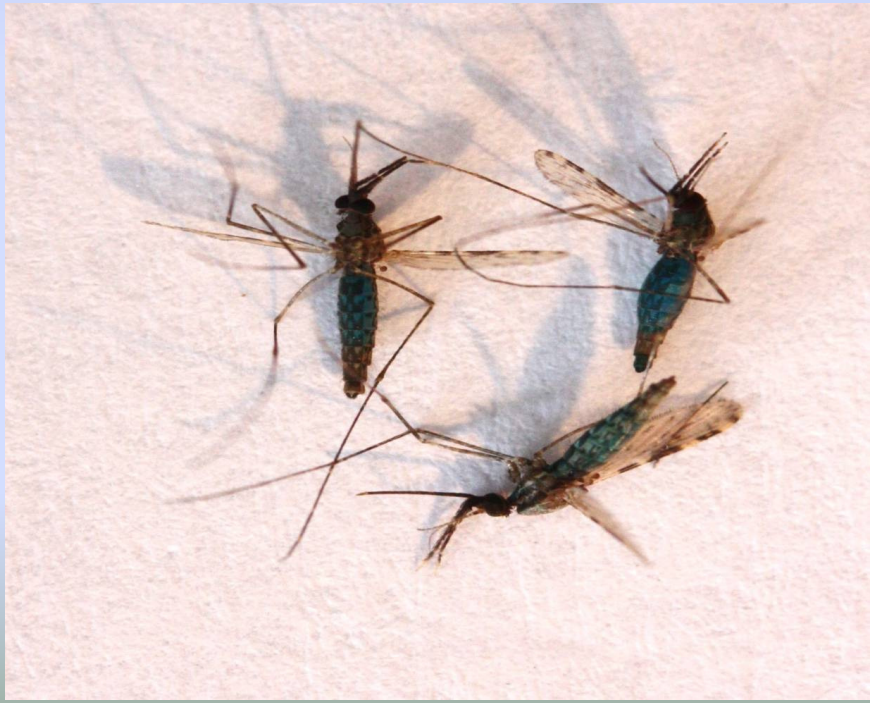
a village with 50 inhabitants, 35 buildings including 20 houses for sleeping

The village was surrounded by 80 bait stations,
40 covered with red stained **bait**
40 with yellow stained **sugar** only as **control**.
The stations were in a distance of 3m from each other, resulting in a distance of 6m for the tested bait.

Within 7 nights we caught with CDC traps
295 *An. gambiae,* 210 females and 85 males.

40.95% females, 52.94% males were stained by the bait
5.24% females, 10.59% males were stained by the control

Of the remaining *An. gambiae* 8.10% of the females and 5.88% of the males were anthrone positive suggesting sugar from other sources than the two stained baits.



Summary and Conclusions

At the experimental site 40.95% of the females and 52.94% of the males were feeding on the ASB on a daily rate. The data suggest that with the present set up sufficient control would have been achieved. Only 8.10% of the females an 5.88% of the males acquired sugar from other sources than the bait stations; ASB attracted and stained 7.82 times more females and 5 times more males than the control.

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