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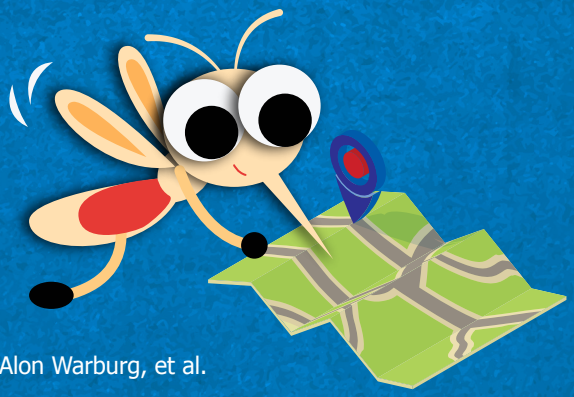
How is *Cannabis* connected to leishmaniasis infections?



Authors:
Ibrahim Abbasi, Artur Trancoso Lopo de Quieroz, Alon Warburg, et al.

Associate Editors:
Lisa Standke and Rachel Watson

Abstract

When you think about dangerous animals, what comes to mind? Spiders? Killer bees? Think smaller — the most deadly insects are mosquitoes and flies! Pathogens transmitted by mosquito bites kill half a million people every year.

Phlebotomine sand flies may not look very scary. They're only 3 millimeters long! But they infect more than a million people with *Leishmania* parasites every year. Early diagnosis and treatment can cure *leishmaniasis* infections. But it can lie undetected for months or even years, causing long-term organ damage or even death. This makes preventing new infections very important.

This is why we wanted to know what exactly sand flies eat. Sand fly females suck blood but both males and females also feed on plant sap. We collected five species of sand flies from five different countries. We were surprised — in all but one site sand flies fed on a plant which is illegal for farmers to cultivate ...*Cannabis sativa*!

Introduction

Imagine you are a gardener. The plant store sells millions of plants, but a few of them attract deadly insects! You don't want any of those, but the store's safety information for each plant is written on individual index cards. They're written in ancient Sumerian, and they're all out of order. Sounds like a pretty hard job, right?

That's the kind of situation an environmental scientist is in. Blood-feeding insects like the phlebotomine sand fly (Fig. 1) live in tropical and subtropical regions of South America, Africa, Europe, the Middle East, and Asia. They mostly eat plant sap, but female flies need blood to develop their eggs.

Sand flies live near one billion people! Insect-killing sprays and fly traps can help, but only if we know where they eat and sleep. Sand flies spend most of their time in cool, damp places under decaying leaves or in animal burrows and caves, but they come out at night to feed. We thought we could help by studying what the sand flies like to eat.

But sand flies are very small — only 1.5 to 3.3 millimeters long! We can't exactly put trackers on them and follow them around to record which plants they like.

Instead, we caught a few and used a tool that lets us examine the sand flies on a molecular level. That means we could see not only what they ate, but also whether they were infected with *Leishmania* parasites.

Figure 1:
A phlebotomine sand fly female taking a blood meal. They have furry wings and abdomens. A photographer has to zoom way in to see this much detail, since these sand flies are only 1.5 to 3.3 millimeters long!
Photo credit: James Gathany, via CDC

Methods

How can we tell plants apart at the molecular level? The answer is in their *DNA*. All DNA is made of four types of nucleotides. The *genes* of every plant *species* are composed of slightly different sequences of nucleotides. For example, while every plant has *chloroplast* genes to help them convert sunlight into energy, the sequence of these genes is a little different between different plants.

This means we can determine every plant species a sand fly ate. We just need to read the full sequence of different chloroplast genes in its stomach!

But sand flies are very small. How can we sequence every plant species and test for parasite DNA with so little material? It's made possible by a powerful genetic tool: *next generation sequencing* (NGS).

How does NGS work?

In short, we make many copies of the gene of interest and determine their nucleotide sequence.

Step 1: We extract all DNA from the sand fly - sand fly, parasite, and plant.
Step 2: We add *primers* for the genes we want to focus on. This makes many copies of the different chloroplast and *Leishmania* genes.
Step 3: We filter away all the other DNA.
Step 4: We read the sequence of the genes in our sample and compare them to a database that includes all chloroplast and *Leishmania* gene sequences.

We captured five species of sand flies from different parts of the world and brought them into our laboratory. There we checked their sex and identified their species. Then we used NGS to look at the sequence of the chloroplast gene *rbcL* as well as a gene marker for *Leishmania* parasites.

Lastly, we checked the GenBank database for sequences similar to our *rbcL* chloroplast gene and the *Leishmania* gene marker. In this way we were able to determine which plant species each fly had fed on and whether it was infected with *Leishmania* parasites.

Results

We discovered many similarities in sand fly diet in different parts of the world (Fig. 2).

- There was no difference in feeding behavior between *Leishmania*-infected & uninfected sand flies.
- There was no difference in feeding behavior between males and females.
- All the sand flies species ate both *native* and *exotic* plants.
- Sand flies preferred exotic plants over native ones.
- *Cannabis sativa* is native only to one of our sites (Bura, Kazakhstan), but it was found in the sand flies' diet at five of the six sites.

In how many study areas did the sand flies eat *C. sativa*?

Figure 2: Plant DNA Analysis of Sand Flies.

Study area	Sand fly species	Number of flies	Proportion eating *C. sativa*	Proportion infected with *Leishmania* parasite
Sheraro, Ethiopia	*Ph. orientalis*	36	11%	no data
Tubas, West Bank	*Ph. sergenti*	24	67%	61%
Kfar Adumim, West Bank	*Ph. sergenti*	107	33%	0
Sde-Eliyahu, Israel	*Ph. papatasi*	30	0	45%
Bura, Kazakhstan	*Ph. mongolensis*	102	34%	10%
Camacari, Brazil (urban)	*Lu. longipalpis*	48	38%	5%
Camacari, Brazil (rural)	*Lu. longipalpis*	19	53%	16%

Discussion

Our results indicate that sand flies like exotic plants such as *C. sativa*. It's not surprising that the sand flies in Bura liked *C. sativa*. It grows there naturally, so it's an easy and familiar meal. But it is surprising that sand flies at four of the other five sites found the plant at all. It's illegal to grow and therefore rare in all of those countries!

That's right, it's the same *Cannabis* species that humans use to get "high." We're not sure why sand flies like *C. sativa*. But it's probably not the same reason people use it — sand flies have different brain structures. They must get some other benefit from the plant. For now we can only guess what that is.

Many countries want to make leishmaniasis infections a thing of the past. That's an important goal, but it is not easy. If sand flies indeed find *C. sativa* appealing, people might develop traps that smell like the plant *C. sativa* to attract and kill sand flies!

Conclusion

Be sure to stay safe if you travel somewhere there are sand flies.

- Wear long-sleeved shirts and pants and use insect repellents on exposed skin to avoid being bitten.
- Stay away from plants and vegetation, especially at night when sand flies are most active.
- Ask your hotel or host if they spray their property with insecticides.

Check your understanding

1. What makes mosquitoes and flies so dangerous?

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2. Is *Leishmania* a A) bacteria B) virus C) parasite D) fungus?

3. What habitat do sand flies prefer and how can people make their gardens less welcoming to them?

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4. How can you protect yourself if you travel to a region with sand flies?

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5. We caught all of our sand flies in the wild. So we only know what the sand flies ate most recently. That means it's possible that the sand flies have other favorite plant meals. Can you think of a study design that would tell us what plant sand flies like to eat the most?

Glossary of Key Terms

Cannabis sativa – a plant grown for fiber used in ropes or clothes as well as for hemp seed oil, but also cultivated for marijuana.

Chloroplasts – organelles that plants use to convert sunlight into sugars for growth and cellular energy. This process uses carbon dioxide and water and releases oxygen.

DNA – the molecule found in each cell of a living organism. It contains all the instructions the cell needs to grow from a single cell into an entire organism and every cell type they have in their body! It is what a cell uses to store the genetic code.

Exotic species – any (plant) species that is not native to the area. Most commonly these plants are brought in by humans for a desirable quality, such as attractive appearance, nutritional value as a crop, or for medicinal or recreational uses.

Gene – a section of the DNA molecule that contains information about a specific task: e.g. producing a certain protein, or producing the pigment which determines eye color. Each gene is encoded (written) in hundreds (or thousands) of base pairs. We can read them in a letter sequence (e.g. ATCCCGTTAAGC).

Host – in biology, the living organism that supports a parasite. In our case, humans and sand flies are hosts to *Leishmania* parasites.

***Leishmania* parasite** – organism that causes leishmaniasis.

Leishmaniasis – a group of diseases caused by *Leishmania* parasites. They manifest in the skin as sores, which can be painful, or on the internal organs, which can be deadly. Early treatment usually cures *Leishmania* infections, but the medicine is not always available.

Native species – (plant) species that naturally occur in a particular area and that were not brought in by human activity.

Next generation sequencing (NGS) – a technique used in molecular biology to determine the sequence of DNA by generating millions of copies of a particular DNA sequence.

Parasite – an organism that lives on or inside a host. The relationship is always one-sided: good for the parasite, bad for the host.

Phlebotomine sand fly – tiny flying insects the *Leishmania* parasites use as hosts. Like mosquitoes, sand flies have piercing mouthparts, which help them suck plant sap. Females also feed on blood to help mature their eggs...and spread leishmaniasis!

Primer – in DNA replication such as NGS, this DNA code is found just before the start of the gene sequence. DNA replication machinery needs a primer to make a copy of any gene. Evolutionarily, the sequences that primers target are mostly conserved between plant species.

REFERENCES

Ibrahim Abbasi, Artur Trancoso Lopo de Queiroz, Oscar David Kirstein, Abdelmajeed Nasereddin, Ben Zion Horwitz, Asrat Hailu, Ikram Salah, Tiago Feitosa Mota, Deborah Bittencourt Mothé Fraga, Patricia Sampaio Tavares Veras, David Poche, Richard Poche, Aidyn Yeszhanov, Cláudia Brodskyn, Zaria Torres-Poche, and Alon Warburg. (2018) *Plant-feeding phlebotomine sand flies, vectors of leishmaniasis, prefer Cannabis sativa*. PNAS.
https://www.pnas.org/content/115/46/11790

World Health Organization: Leishmaniasis
https://www.afro.who.int/health-topics/Leishmaniasis

Centers for Disease Control and Prevention: Parasites - Leishmaniasis
https://www.cdc.gov/parasites/leishmaniasis/index.html

Bite Size Bio: A Beginner's Guide to NGS Technology
https://bitesizebio.com/21193/a-beginners-guide-to-next-generation-sequencing-ngs-technology/