Geosmin attracts *Aedes aegypti* mosquitoes to oviposition sites

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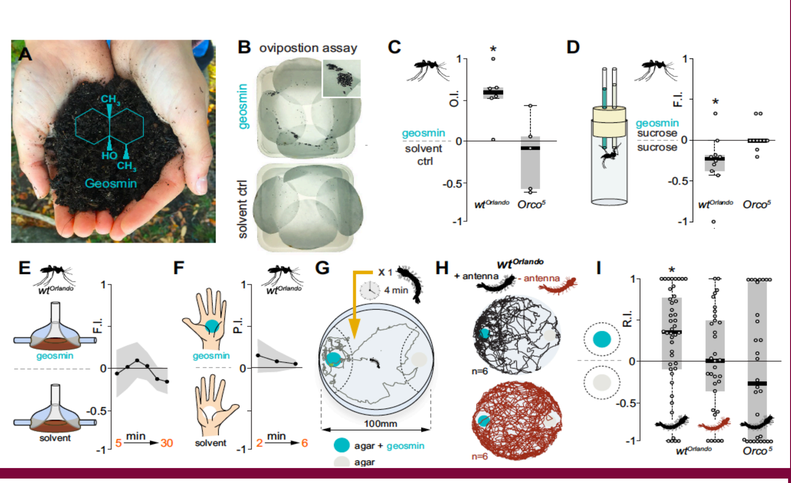
Abstract

Geosmin is one of the most recognizable and common microbial smells on the planet. Some insects, like mosquitoes, require microbial-rich environments for their progeny, whereas for other insects such microbes may prove dangerous. We have here investigated the effect of geosmin on the behavior of the yellow fever mosquito *Aedes aegypti*. We found that geosmin stimulates egg-laying site selection. Female mosquitoes presumably associate geosmin with microbes, including cyanobacteria consumed by larvae, who also find geosmin – as well as geosmin producing cyanobacteria – attractive. Using *in vivo* multiphoton imaging from mosquitoes with pan-neural expression of the calcium reporter GCaMP6s, we show that *Ae. aegypti* code geosmin with extreme sensitivity and with a high degree of selectivity. We further demonstrate that geosmin can be used as bait under field conditions, and finally we show that geosmin, which is both expensive and difficult to obtain, can be substituted by beetroot peel extract - providing a cheap and viable mean of mosquito control and surveillance in developing countries.

Results

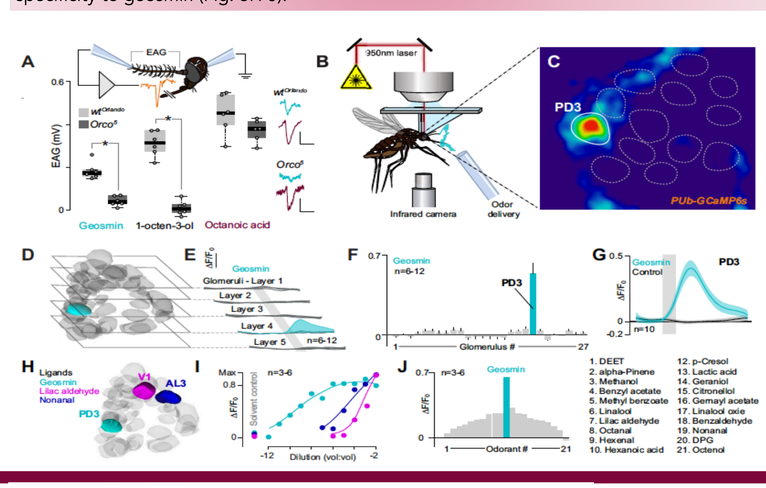
Geosmin confers oviposition site selection and larval attraction

In flies, geosmin signals the presence of harmful microbial contaminants and negatively affects egg-laying preference. Unlike flies, we found that geosmin positively affects egg-laying behavior in *Aedes aegypti* mosquitoes and this is mediated by the olfactory system (Fig. 1B-D). The same attraction was noted in mosquito larvae – and as with adults this behavior is also dependent upon olfaction (Fig. 1G-I).



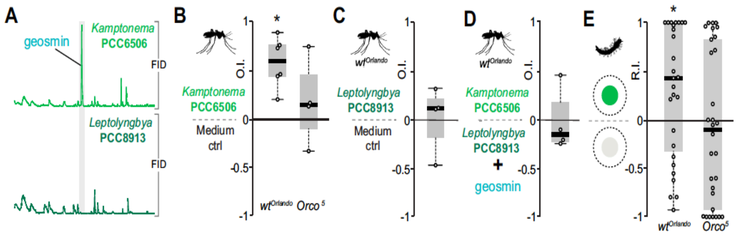
Two-photon imaging reveals sensitive and selective neural coding of geosmin

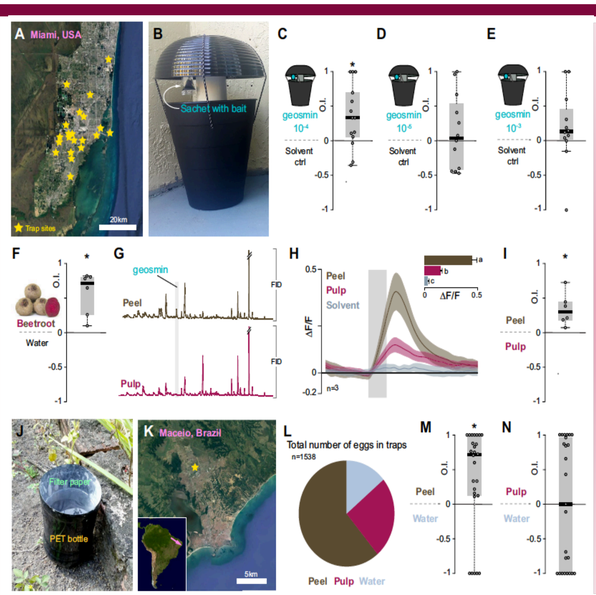
EAG analyses revealed distinct responses to stimulation with geosmin, which was abolished when stimulating *Orco* mutants (Fig. 3A). Imaging across the antennal lobe of *PUb-GCaMP6s* mosquitoes revealed significant response to geosmin in one particular glomeruli – the third posterodorsal glomerulus (PD3)(Fig. 3B-G). PD3 demonstrated an extreme sensitivity and specificity to geosmin (Fig. 3H-J).



Geosmin producing cyanobacteria confer oviposition and larval attraction

Cyanobacteria are a common source of geosmin, we therefore examined how *Ae. aegypti* reacts to two strains: one that produces geosmin (PCC 6506) and one that does not (PCC 8913)(Fig. 2A). A clear oviposition preference was observed to PCC 6506 (Fig. 2B-D). This preference was dependent upon activation of *Orco* expressing neurons (Fig. 2B). Lastly, we examined how larvae reacts to cyanobacteria. An overall preference to cyanobacteria (Fig. 2E) was observed - this behavior was also dependent upon *Orco* neurons (Fig. 2E).





Geosmin as a mosquito control agent

Field studies in Miami showed that geosmin work as an oviposition attractant (Fig. 4A-E).

Oviposition assays showed that females lay eggs in beet juice – chemical analyses and imaging showed that they respond specifically to beetroot peel – where geosmin is present (Fig. 4F-I).

A small-scale study in Brazil showed that beetroot peel is an efficient substitute for geosmin (Fig. 4J-N).

Conclusion

Apart from offering insights into how insects and mosquitoes in particularly decode odors, our findings also provide a novel and sustainable approach for mosquito control. The part of the beetroot that would otherwise have gone to waste, now has its distinct use. Whereas the peel can be used to trap mosquitoes, the pulp can be used to make delicious borscht.

Acknowledgement

DEGENNARO LAB

RIFFELLLAB
Neuroecology and Chemical Communication

Institut Pasteur

LPqRN
Laboratório de Pesquisa em Recursos Naturais

Preprint