Development and superparasitism of the wasp *Philolema latrodecti* in a native and an invasive widow spider egg sac

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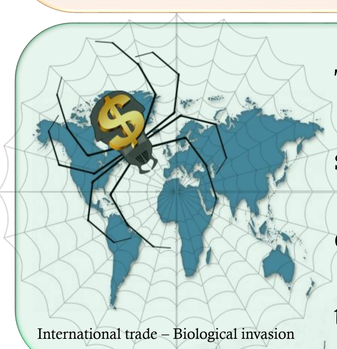
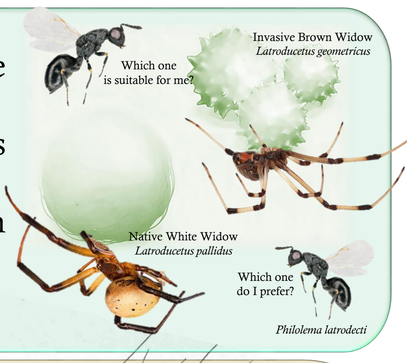
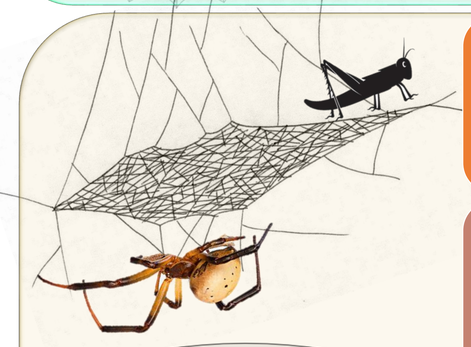


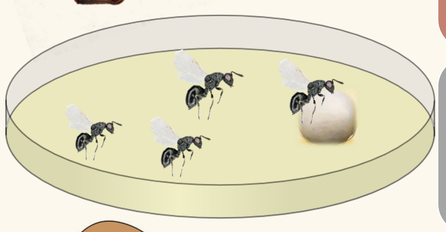
The egg sac parasitoid wasp, *Philolema latrodecti*, oviposits inside widow spider egg sacs and its offspring develops by feeding on the spider eggs. However, little is known about the development of this wasp. We investigated the effect of parasitoid density on its development, while exposing the spider egg sacs to one, and up to four parasitoids. We compared the outcome of parasitism between the white widow, spider *Latrodectus pallidus*, which is native to Israel, and the invasive brown widow spider, *Latrodectus geometricus.*





Spider and wasp rearing

- Field-collected adult *L. geometricus* and *L. pallidus* female spiders were fed weekly with one grasshopper nymph.
- Wasps for the experiments emerged from widow spider egg sacs collected from the field.
- We fed wasps with honey solution immediately after emergence.

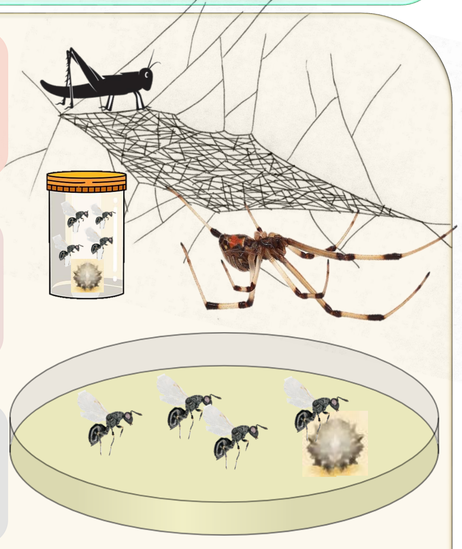


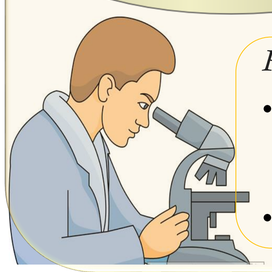
Development of *P. latrodecti* in *L. geometricus*

- We exposed egg sacs (n = 12) of *L. geometricus* each to a single *P. latrodecti* parasitoid female.
- On days 1, 3, 5, 10, 15, and 20 post-parasitism, we dissected two parasitized egg sacs and recorded parasitoid development

Wasp Density

- We exposed *L. geometricus* and *L. pallidus* egg sacs to one (*Lg:* N = 69, *Lp:* N = 26), two (*Lg:* N = 33, *Lp:* N = 30), three (*Lg:* N = 37, *Lp:* N = 17), or four (*Lg:* N = 35, *Lp:* N = 17) females.
- We incubated the egg sacs till the emergence of wasps or spiderlings.





Predictions:

- Parasitism success and brood size will increase with the number of parasitizing wasps up to a threshold, after which they may level off or even decrease due to competition.
- Developmental success of the parasitoid will be lower on the egg sacs of the invasive brown widow spider compared to the native white widow.

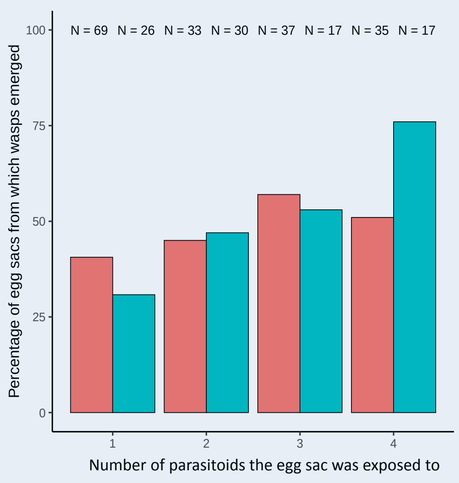


Fig. 1: Parasitism success in egg sacs exposed to 1-4 parasitoid females.

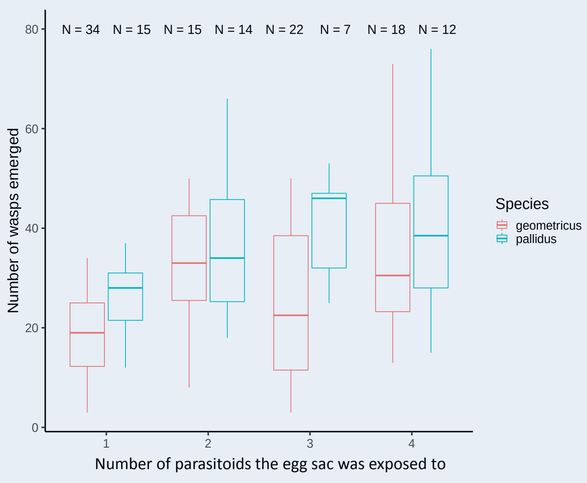
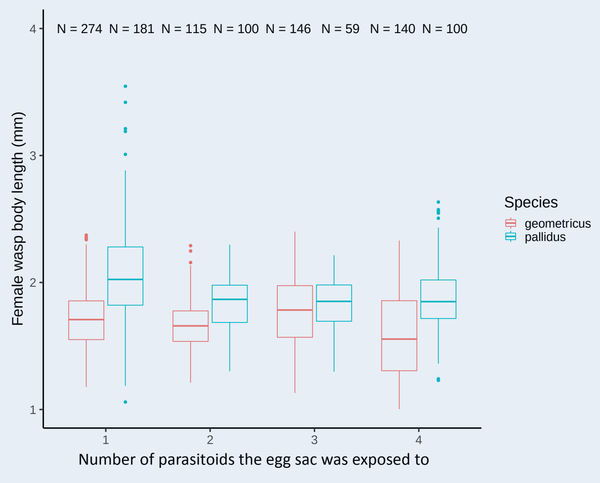


Fig. 2: Number of emerging parasitoids from egg sacs exposed to 1-4 parasitoid females.



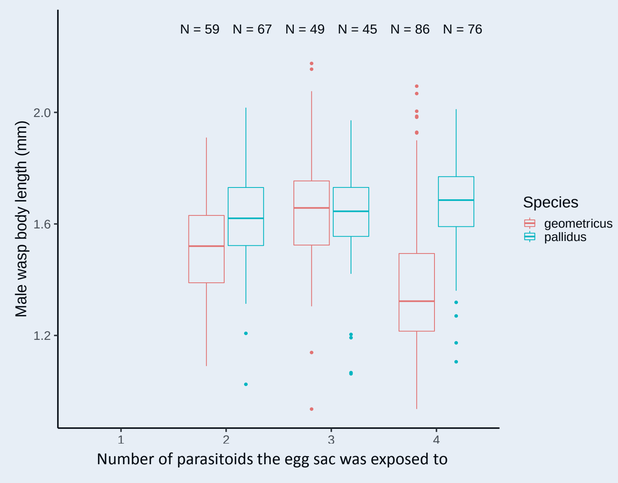


Fig. 3: Body size of parasitoids emerging from egg sacs exposed to 1-4 parasitoid females.

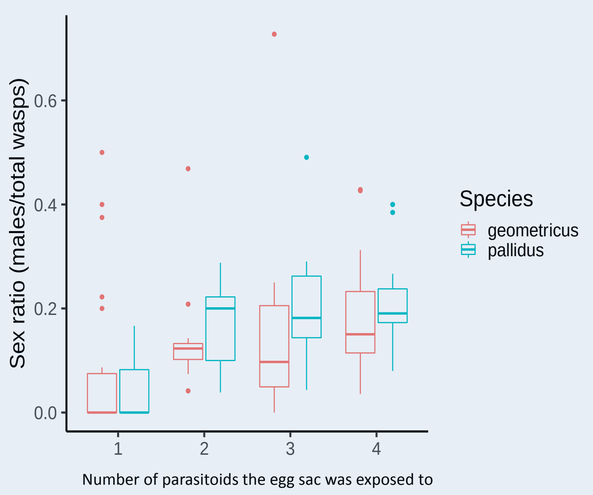


Fig. 4: Sex ratio egg sacs exposed to 1-4 parasitoid females.

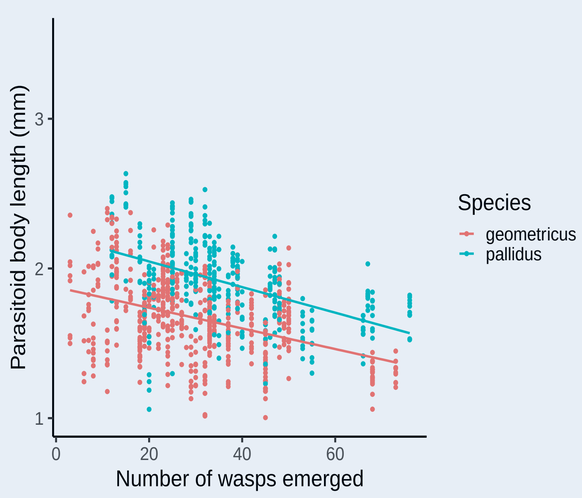


Fig. 5: Body size of emerging parasitoids in relation to brood size.

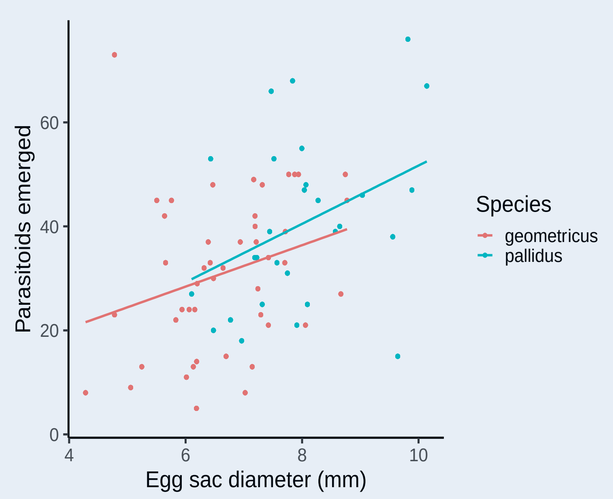


Fig. 6: Brood size of emerging parasitoids in relation to egg sac diameter.

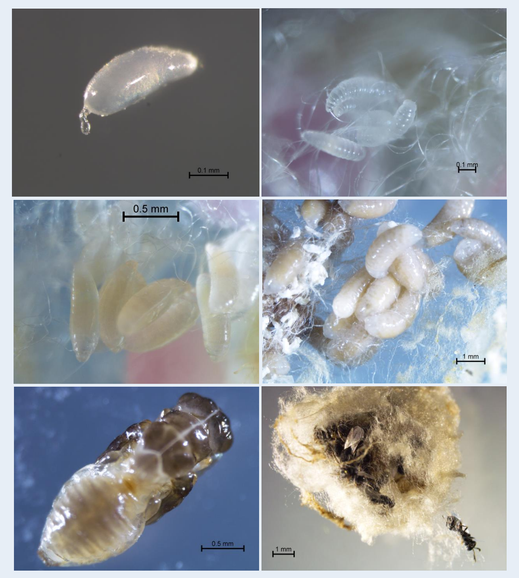


Fig. 7: *P. latrodecti* development inside *L. geometricus* egg sac

- This is one of first descriptions of parasitoid development inside a spider egg sac.
- Our results suggest that the native spider is a better host for this egg sac parasitoid than the invasive spider, especially under high parasitoid densities.
- Another potential advantage of the brown widow spider may be the defensive spike-like structures on the surface of its egg sac.
- This could give a competitive advantage to the less affected, invasive brown widow spider.

Mowery, M.A., Arabesky, V., Lubin, Y. and Segoli, M., 2022. Differential parasitism of native and invasive widow spider egg sacs. *Behavioral Ecology*, *33*(3), pp.565-572.

Van Alphen, J.J. and Visser, M.E., 1990. Superparasitism as an adaptive strategy for insect parasitoids. *Annual Review of Entomology*, *35*(1), pp.59-79.

Identification of species-specific spectral emittance and hydrocarbon composition of the *Chrysobothris femorata* complex

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Abstract/ Introduction

- Flatheaded appletree borers (FAB; *Chrysobothris* spp.), belong to the *C. femorata* complex (Fig.1) that is comprised of 12 distinct species. Individual species are difficult to differentiate and positively identify without molecular analysis or removal of male genitalia.

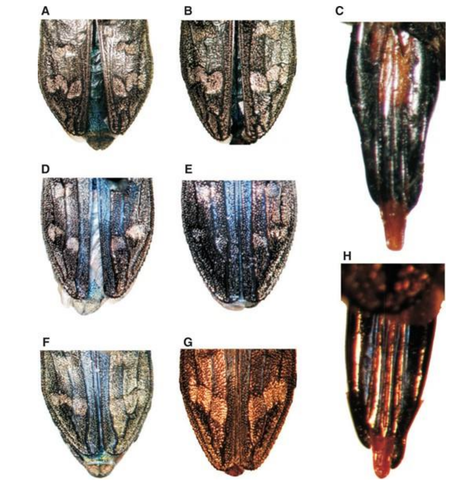

Fig. 1. Image from Hansen et al. (2009) demonstrating overlapping characteristics of closely related *Chrysobothris* species.

- Additionally, FAB are attracted to purple-colored pole traps. However, some evidence indicates an attraction to spectral peaks at opposing ends of the spectrum (i.e. UV and IR), depending on species and sex. Color spectra parameters such as *L*, a*, b*,* and peak reflectance values can be used to quantify differences in visual cues of individuals.
- Related beetles use contact pheromones to recognize conspecifics and potential mates. Analyses of these hydrocarbons may be a possible alternative method for identification of species and sex.

Fig. 2. Purple pole trap used to attract FAB.

Objectives

1. Identify differences in hydrocarbon composition among *Chrysobothris* species
2. Identify differences in hydrocarbon composition between sexes within species
3. Identify possible visual cues for conspecific and mate recognition from spectral emissions

Materials and Methods

- We analyzed UV-VIS spectral emissions and hydrocarbon composition of 3 males and 3 females from 3 species in the *C. femorata* complex. Beetles were hand-captured specimens from Tennessee, USA.

Spectral analysis of beetle elytra

- Spectral emissions were collected from the pronotum, elytra, and the ventral and dorsal abdomen of each beetle using a Konminolta CM2600d UV and VIS spectrophotometer.

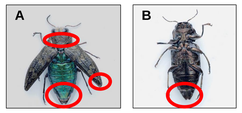

Fig. 3. A) Placement of dorsal abdominal, pronotum, and eltyra spectral readings. B) Placement of ventral abdominal spectral readings.

- ANOVA's were run on spectral parameters and visualized using *'spectralAnalysis'* in R software.

Extraction and identification of hydrocarbons on beetle elytra

- Hydrocarbons were collected from beetle elytra using solid phase microextraction (SPME) with a polydimethylsiloxane fiber. Fibers were brushed 10 times across the dorsal side of one elytra from each beetle.
- Hydrocarbons were analyzed from the SPME fiber collections using gas chromatography- mass spectrometry (GC-MS) using the method by Silk et al. (2009).

Results

- The pronotum, elytra, and the ventral and dorsal abdomen showed significant differences of the color space parameters *a** and *b** across sex and species interactions (all $P < 0.05$).

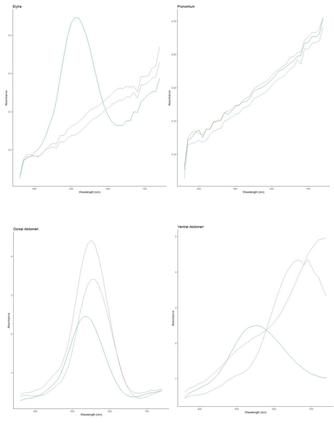

Fig. 4. A) Elytra spectra; B) dorsal abdominal spectra; C) pronotum spectra; D) ventral abdominal spectra. Red = *C. quadriimpressa;* Green = *C. sexignata,* Blue = *C. viridiceps.*

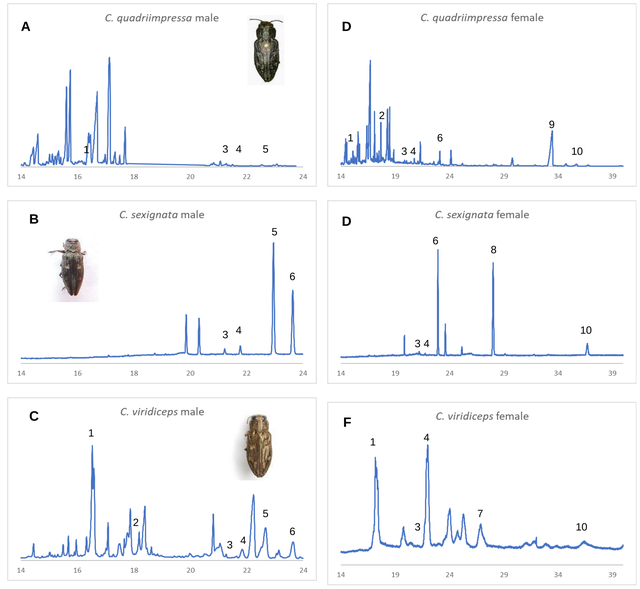

Fig. 3. SPME analysis of cuticular hydrocarbons from the elytra of A) male *C. quadriimpressa*; B) male *C. sexignata*; C) male *C. viridiceps*; D) female *C. quadriimpressa*; E) female *C. sexignata*; F) female *C. viridiceps*. Not all peaks have been identified, but numbers on peaks refer to compounds in Table 1 that are of interest from previous studies on emerald ash borer (*Agrilus planipennis*).

Peak number	Retention time	Suspected hydrocarbon
1	16.53	*n*-Hexadecanoic acid
2	18.19	oleic acid
3	21.06	9-Me-C_{23}
4	21.84	*n*-C_{24}
5	22.67	*n*-C_{25}
6	23.89	unknown
7	27.63	*n*-C_{27}
8	28.94	13-Me-C_{27}
9	33.13	squalene
10	34.84	*n*-C_{29}

*Compounds of interest were identified by cross-referencing hydrocarbons found in previous studies. This is not an exhaustive list and is only a starting place to identify potential compounds for differentiating conspecifics and sex.

Discussion

- There is still much work that needs to be done to understand chemical and visual recognition of conspecifics and potential mates within the *C. femorata* complex. However, preliminary analysis of three species suggests promising alternatives for identification using either spectral or hydrocarbon analyses. The dorsal abdomen of the three species all peak at a similar wavelength. However, there are differences in the peaks' magnitude.
- Several alkanes, including *n*-C_{24}, *n*-C_{25}, and 9-Me-C_{23}have been identified in previous studies as possible volatile oils that are used in beetle mate recognition.
- Our findings so far suggest that the presence/ absence of hydrocarbons or differences in abundance may be possible mechanisms for identification but further isolation of individual compounds and *in vitro* trials are needed to confirm.
- Also from our initial results, some compounds are only found in males, *n*-C_{25} for example. Creating methods for quick determination of sexually dimorphic compounds may offer researchers quick and non-invasive identification results.
- However, the answer to recognition may not be from a single cue, but ratios of hydrocarbons in relation another or several other compounds.

Future Directions

- Changes in spectral emissions occur in other species of beetles due to small pits on the cuticles of the elytra. These pits often contain contact sex pheromones (species-specific), as well as the morphological structure of the pits. Elytra are currently being analyzed for physical properties using scanning electron microscopy and 3D imaging.
- We are further working to understand ecological implications of visual differences. This includes visual perception as a possible general mechanism of conspecifics and contact sex pheromones as specialized cues for mate recognition. Understanding the complexity of color emissions and the textures of the elytra will help elucidate mate seeking behaviors and the general ecology of these closely related beetles.

Acknowledgements

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Invasive brown widow (*Latrodectus geometricus*) thwart their parasitoids

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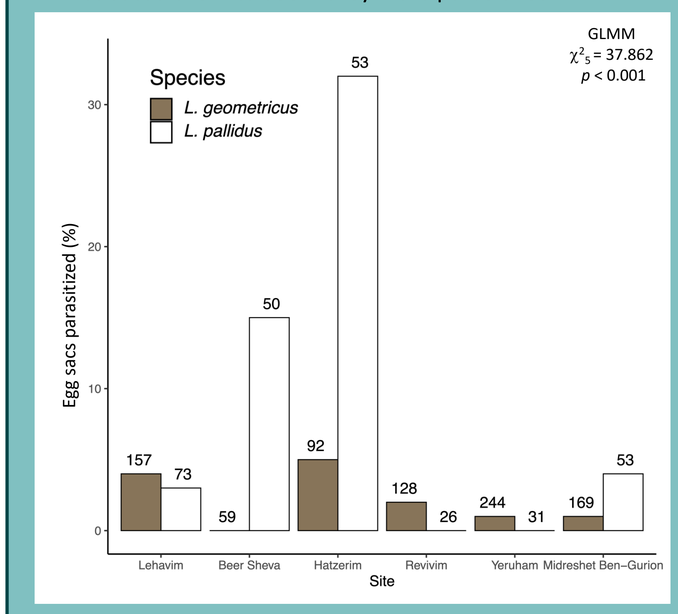
Introduction

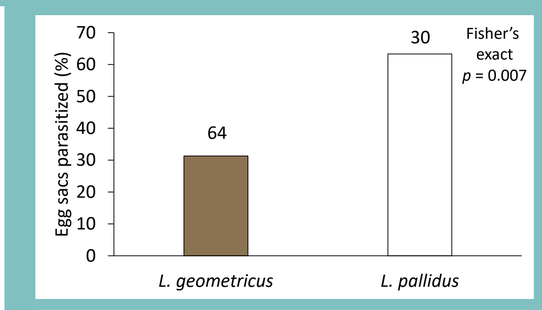
- Invasive species may have advantages over native congeners (Mathakutha et al., 2019)
- Possible advantages include fast reproduction and development, increased dispersal ability, and competitive ability (Alpert, 2006)
- Invasive species also may be better protected against predators and parasites (Ceryngier et al. 2018)
- We investigated the defense mechanisms of the invasive brown widow spider (*Latrodectus geometricus*) against an egg-sac parasitoid wasp compared to the native to Israel white widow spider (*L. pallidus*) (Mowery, Lubin, et al., 2022)

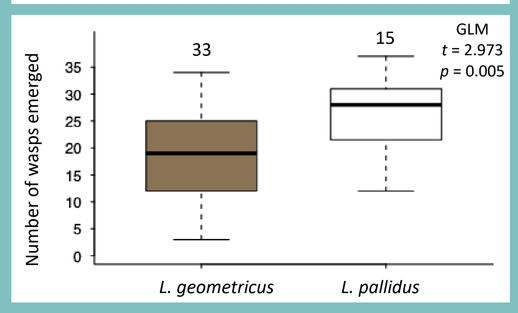
Parasitism rate

In the field:
Collected 286 *L. pallidus* egg sacs and 849 *L. geometricus* egg sacs from 6 sites in the Northern Negev and incubated them to see if they were parasitized



In the lab:
Compared parasitism and number of wasps that emerged from *L. pallidus* egg sacs and *L. geometricus* egg sacs in a Petri dish

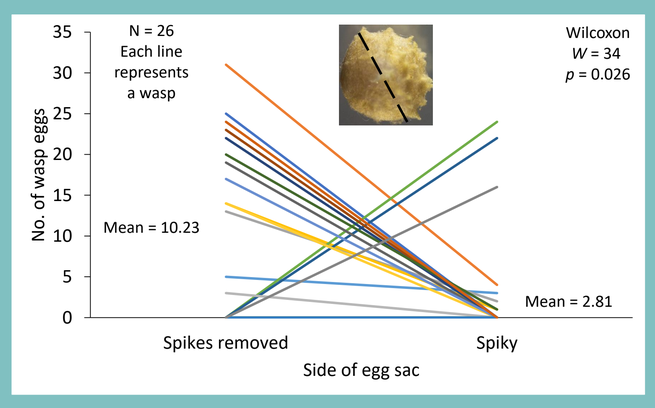




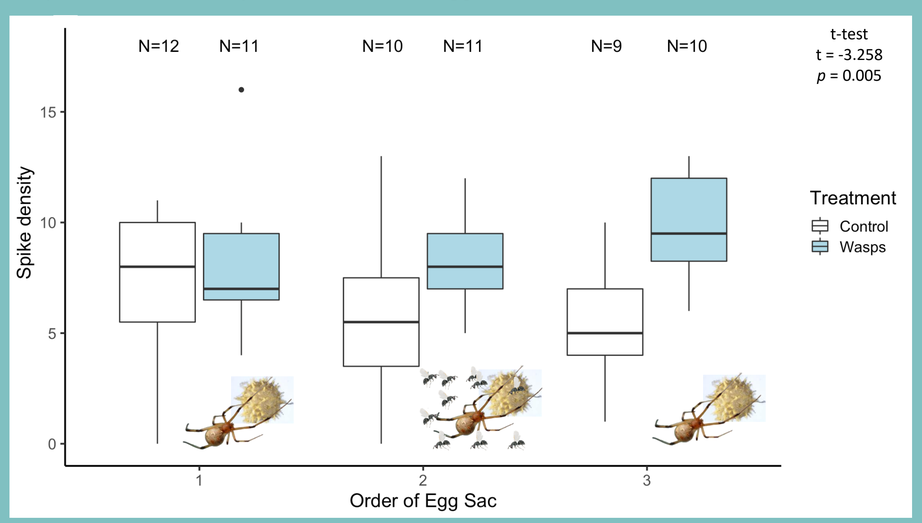
Lower parasitism rate and lower parasitoid reproductive success in *L. geometricus* egg sacs

Defense mechanism: Egg-sac morphology

Spike removal:
- Removed the spikes of one-half of the surface of 26 *L. geometricus* egg sacs and exposed the egg sacs to the parasitoid
- Counted the number of wasp eggs laid on the spiky and spikes removed sides



Plasticity in spike production:
Compared spike density on the first three egg sacs of *L. geometricus.*
Treatments: Control – not exposed to parasitoids; Wasps - parasitoid exposure to female spider with second egg sac



Fewer parasitoid eggs laid on the spiky side of the egg sac
Parasitoid presence induced production of spikier egg sacs

Study system: widow spiders and parasitoid wasp

Invasive species: *Latrodectus geometricus* (brown widow)
- Likely native to South Africa
- Globally invasive
- Thrives in urban habitats


Photo: Sean McCann


Photo: Monica Mowery
Egg sac of *L. geometricus* with spike-like silk structures

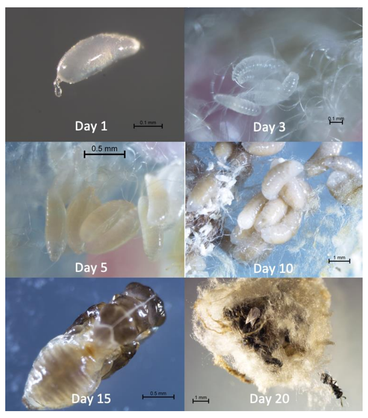
Native species: *Latrodectus pallidus* (white widow)
- Eurasian distribution
- Lives in semi-arid scrub habitat


Photo: Jeff Harvey


Photo: Monica Mowery
L. pallidus egg sac with smooth egg sac surface

Natural enemy: *Philolema latrodecti* (Hymenoptera: Eurytomidae)
- Parasitoid of *Latrodectus* egg sacs
- Widespread globally

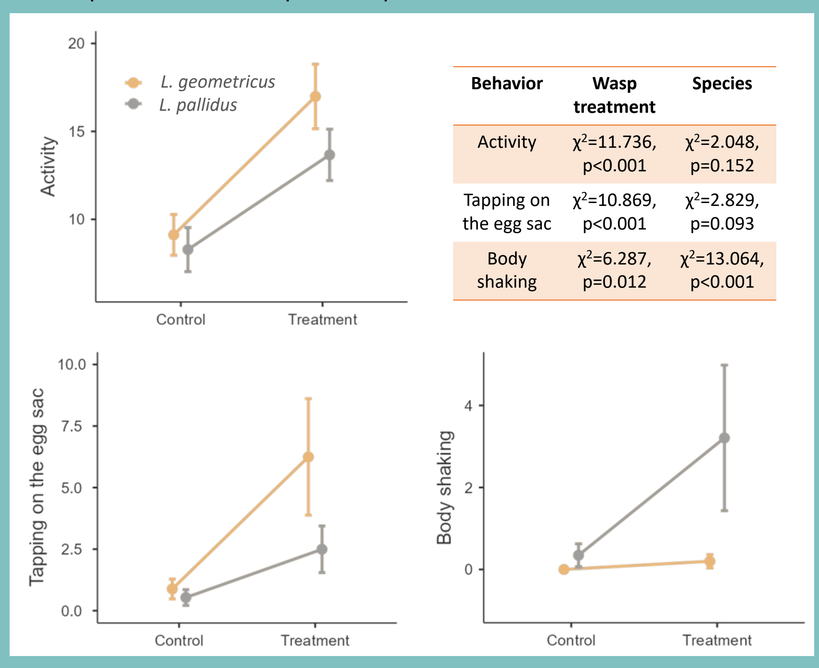

Photo: Monica Mowery



Developmental stages of *P. latrodecti* inside *L. geometricus* egg sac (Daniel et al., 2023)

Defense mechanism: Egg-sac guarding behavior

Compared the behavior of 17 *L. pallidus* and 18 *L. geometricus* females with egg sacs during exposure to 10 parasitoids (GLM, mean ± sd number of 1-hr segments)
Control spiders were not exposed to parasitoids



Behavior	Wasp treatment	Species
Activity	χ^2=11.736, p<0.001	χ^2=2.048, p=0.152
Tapping on the egg sac	χ^2=10.869, p<0.001	χ^2=2.829, p=0.093
Body shaking	χ^2=6.287, p=0.012	χ^2=13.064, p<0.001


Photo: Valeria Arabesky
Experimental setup

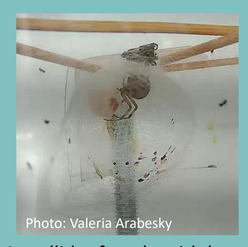
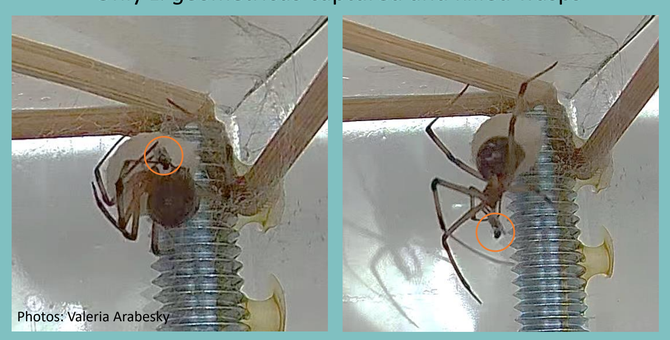


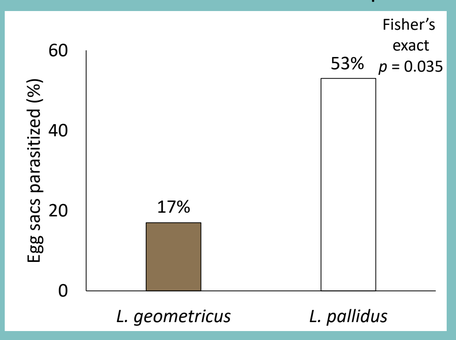
Photo: Valeria Arabesky
L. pallidus female with her egg sac and parasitoid wasps in the web

Scan for videos of widow spider behavior

Only *L. geometricus* captured and killed wasps


Photos: Valeria Arabesky

Parasitism rate at the end of the experiment



L. geometricus has better egg-sac guarding behaviors

Conclusions

- Invasive *L. geometricus* egg sacs had lower parasitism than native *L. pallidus egg* sacs
- *L. geometricus* may escape parasitism due to egg sac morphology and better behavioral defense
- Low parasitism could enhance *L. geometricus* invasion success

Current research

Philolema wasps and *L. geometricus* spiders may have co-invaded, and the parasitoids then shifted to the native widow spider species. We use population genetics tools to learn about the origin of the two species

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STATE OF INDIA'S BIRDS 2023

CERTIFICATE OF APPRECIATION

Alfred Daniel J

The State of India's Birds report 2023 assessed the distribution range, trends in abundance, and conservation status for 942 bird species in India. These assessments were based on checklists uploaded to eBird, among which

6 checklists

of yours made a valuable contribution. Thank you for carefully documenting your birding and enabling this national assessment of our birds.

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A mating pair of black cone-headed grasshoppers *(Poekilocerus bufonius)* in the southern Negev Desert

BLACK CONE-HEADED GRASSHOPPERS,

A BURST WATER PUMP AND A POSSIBLE CASE OF MENTAL TELEPATHY

By Berry Pinshow

There is a peculiar Orthopteran that lives in Israel, primarily in the Rift Valley where it is almost always hot. The black cone-headed grasshopper (*Poekilocerus bufonius*) with bright yellow aposematic markings feeds only on plants of the milkweed family, *Asclepiadaceae* (or *Apocynaceae*). The sap of these plants is poisonous to many animals, containing alkaloids and cardiac glycosides; indeed, the sap of some genera in the family is used to poison arrows in Africa.

Female black cone-heads weigh about 5 grams and spend their time sucking sap from a variety of milkweed species; males weigh about 1 gram and, when not feeding, fly about in search of females. Black cone-headed grasshoppers have an abundant water supply in the plant sap they eat, and can evaporate excess water and maintain their body temperatures below ambient air temperature, even when it is, at least to us, very hot.

This interesting animal is what brought my friend and colleague of many years, Prof. Henry Prange, and his assistant, Chris Oswald, all the way from Indiana University in Bloomington to the Blaustein Institute for Desert Research (BIDR) of Ben Gurion University in the summer of 1992. Henry had received a grant from the National Geographic Society for us to study water balance and temperature regulation in black cone-headed grasshoppers, after he had spent several years studying these phenomena in other grasshopper species. He had asked whether I wished to collaborate in a study.

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After my guests settled in, and we had organized the setup for our experiments in my laboratory, our next order of duty was to find and capture a reasonable sample size of these beasts, and to house and feed them in the lab. So, on a Friday morning, we set out at sunrise for Kibbutz Ein Gedi on the shores of the Dead Sea, about a two-hour drive from the small town of Midreshet Ben-Gurion (the Midrasha), where the BIDR is located and where my family live.

The Midrasha is 450 meters above sea level, whereas the area near Ein Gedi, where we planned to search for cone-heads on Sodom apple bushes (*Calotropis procera*) is almost 400 meters below sea level. We left early because the weather forecast was for a very hot day, and we took with us a more than adequate supply of water and vittles for breakfast and snacks, all in a cold box. It was a bit of a squeeze, the four us in our small family car, a 1300 cc, five-door Opel Kadett with no air conditioner. Needless to say, the windows stayed wide open.

The drive down to the Dead Sea was magnificent. From the Midrasha, we drove north and east, bypassing the towns of Yeroham and Dimona, down the steep incline through the Judean Desert, from where we could see the Dead Sea (it was a little bigger back then), to the junction of road 25 with road 90 that runs from Eilat in the South, via Ein Gedi to Metulla in the very north of Israel. From the intersection we drove up the coast of the Dead Sea looking for appropriate plants. We drove all the way to Ein Gedi, searching for cone-heads on Sodom apple bushes in the wadis along the way.

Alas, by 9 a.m. we were still empty handed, and the temperature was already in the low 30s (°C) and increasing. So we decided to go

home and try somewhere else in the following days.

We drove south and turned west onto road 25 to begin the 800 m climb to Dimona. Somewhere over halfway up the 30 km road, a red light flashed on the dashboard and the car's temperature gauge pegged on the red end. I immediately pulled over onto the, fortunately very wide, verge. When we opened the hood, we found that the water pump had cracked, and we were immobilized; clearly, we needed to make a plan.

I should point out that road 25 was then part of the only traffic artery from Tel Aviv to Eilat and was, for the time, quite heavily trafficked by trucks and, especially on Fridays, tourists driving down to Eilat on the Red Sea. So it wasn't outrageous to think that if Henry and I stayed with the vehicle, Hana and Chris could hitch a ride to the gas station in Dimona, some 20 km up the road, where there was a payphone. From there, they could call the towing company with which our car was insured to take Henry, me, and the car to Beer Sheva, where my service garage was, 30 km west of Dimona. They could also call someone at the Midrasha to come and pick them up. I should also point out that at the time, it was considered perfectly safe to hitch rides in Israel. Unfortunately, that is not so today.

Hana and Chris put out their thumbs and pretty soon a large truck stopped and the driver offered them a ride to Dimona. So far so good. Henry and I opened the doors of the car, turned on the radio and, sitting in the shade, cooled by the breeze, we enjoyed the magnificent view. We could see a good part of the Dead Sea, from the pans of the saltworks at the south end to what was then Cape Molyneux, which no longer exists because the south end of the Dead Sea is now one big saltpan, mined by the Israelis on the west side and the Jordanians on the east.

After a while, I said to Henry, "You know, the only thing missing from this idyllic scene is cold beer". Henry leaned back with a sigh that said, "wishful thinking". Not two moments later, a car in the east-bound lane (towards the Rift Valley and Eilat) stopped by the side of the road opposite us. The driver rolled down his window and asked me whether I was Berry. I nodded and he put his hand out of the window holding a brown-paper bag. I got out of my car to take the bag and he said, "Your wife stopped me in the Dimona petrol station and asked me to give you this — enjoy" and drove off. In the bag were two cold bottles of Goldstar, a popular local lager. Were we ever surprised (and pleased)!

I popped the caps off in the catch of the door lock and we both leaned back to chill out and enjoy. Then, probably because I never drink beer in the middle of the day, a thought crossed my mind, "Henry, the last time we ate was pretty early this morning; maybe it's not such a good idea to drink on empty stomachs". Henry wasn't bothered one bit, but clearly, I was — and so it turned out was my better half, because no sooner had I voiced my thoughts to Henry, a second car pulled up opposite us.

The driver rolled down his window, stuck out his hand clutching a paper bag, and said, "Berry, your wife said not to drink beer on empty stomachs". In the bag were two large pittas filled with felafel, salad, humus and tehina, and two bottles of Tempo orangeade. For a moment I wasn't far from believing in telepathy. From time to time, I still wonder how Hana anticipates my thoughts. Henry was speechless, at least for a moment, and so was I.

We much enjoyed the felafel and the drink. A short time later, around noon, if my memory serves me right, the tow truck showed up. Hana and Chris had done us proud. The driver gingerly winched my Opel onto the back of the truck and secured it in place. The cabin of the tow truck was comfortable, air-conditioned and had a cold box of drinks between the seats. In relative luxury, off we drove to Beer Sheva.

Convinced as we were that Hana and Chris would manage to find someone at the Midrasha to come and pick them up, we weren't in the least worried that we might not find them at home when we got back. After all, we had to go to Beer Sheva and find a safe place to leave the car

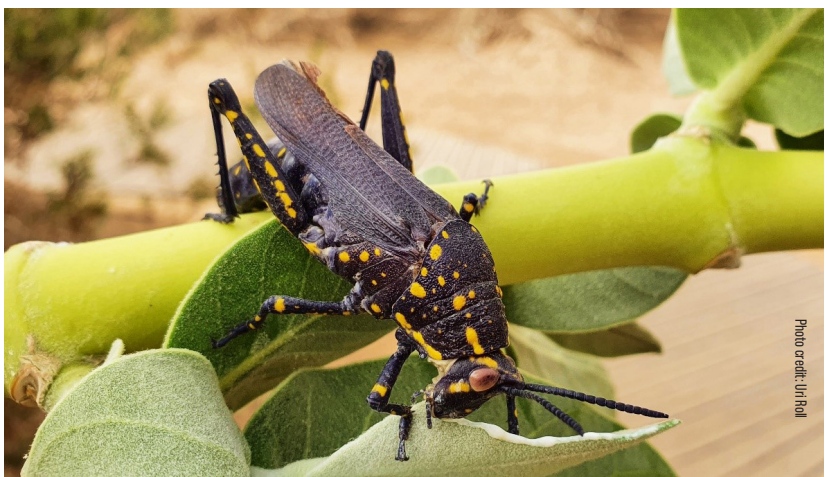

Photo credit: Uri Roll

A female black cone-headed grasshoppers *(Poekilocerus bufonius)*, on a Sodom apple near the Dead Sea

Mentosa, one of the milkweed species on which black cone-headed grasshoppers feed, in its natural Negev Desert Environment

until Sunday. I should explain. At the time all businesses in Israel closed for the weekend at around midday on Friday; the weekend was just a day and a half. That has since changed and the weekend in Israel is Friday-Saturday. The big going-out evening is Thursday. Then back to work on Sunday morning.

So, I formulated a plan to park the car outside the house of my sister and her family who lived in Beer Sheva at the time, borrow their car for the weekend and come back to meet the tow truck early on Sunday morning. From there he would take me to the service garage. All that actually went down as planned. However, Hana and Chris were not

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home when we arrived at about 3 p.m. We had no idea where they were, and had no way to contact them.

Later, Hana told me what happened in Dimona. The truck driver let them off at a petrol station that had both a phone and a restaurant/snack and drink shop. She immediately called the tow company and gave them the details of our predicament. Then Hana set about calling friends at the Midrasha who might be able to help them; all the while thinking about Henry and me "suffering" at the car, and how to send us goodies, which she did with aplomb – twice. No ride was forthcoming.

The problem was that most of our acquaintances had taken their kids to visit grandparents for the short weekend, and whoever remained did not have a car. Luckily, in the end, one of our friends managed to contact the driver of a bus taking high school kitchen staff, who had ended their Friday shift, home from the boarding school at the Midrasha to Dimona. He found Hana and Chris at the petrol station and brought them home, not much worse for wear, but with a story to tell.

A final note: we found the hoppers we needed a few days later on some milkweed bushes of a different species (*Pergularia tomentosa*) at a place much closer to home. We did the proposed study and closure came when our paper was published. ■