Gas Chromatographic Analysis of Pheromones from the Femoral Glands of Non-Ophidian Squamates

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Introduction

- *Aspidoscelis gularis* is a polytypic species with a broad geographic distribution. The taxa that make up this species complex are distributed from the south-eastern USA, in parts of Oklahoma and Texas, to central Mexico. There are reportedly 8 subspecies of it named Aspidoscelis gularis colossus, *A. g. gularis*, *A. g. pallidus*, *A. g. rauni*, *A. g. scalaris*, *A. g. semian-nulatus*, *A. g. semifasciatus*, and *A. g. septemvittatus*.

Figure 1: *Aspidoscelis gularis*
[Photo Courtesy: Joshua Rivera, Miles Horne]

- Sexual dimorphism in body size observed in whiptails, like *A. gularis*, often linked to sexual selection and fertility.
- Whiptails, including *A. gularis*, provide a unique opportunity to study chemical signaling development across sexual and asexual branches.
- Unlike other species, femoral glands in whiptails don't show pronounced differences between males and females, making them intriguing for research.
- Parthenogenetic whiptails exhibit pseudocopulatory behavior similar to mating bisexual species, suggesting femoral gland secretions may play a role.
- Femoral gland secretions crucial for intraspecific chemical communication, mate selection, social hierarchies, territorial marking, health status, and environmental adaptations in many lizard species.
- Geographic and ecological variations among populations of *A. gularis* suggest potential chemical signal divergence, motivating investigation across different species and locations.
- Investigating six species from the genus *Aspidoscelis* (e.g., exsanguis, gularis, inornata, marmorata, septumvittatus, tessalatus) from various locations in the DFW area to understand chemical signal diversity.

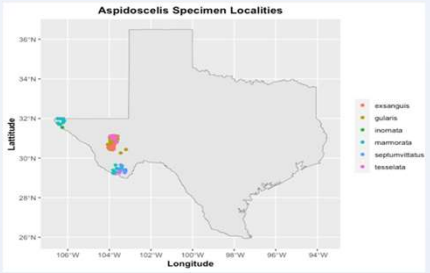

Figure 2: Location from where samples were collected

Objectives

(1) To characterize and compare the chemical composition of the femoral gland secretions of asexually reproducing (parthenogenetic) and sexually reproducing species within the whiptail clade found in different places in Texas.

(2) To examine potential sex-based differences in pheromone secretion, comparing both femoral and epidermal gland secretions in sexually reproducing species.

(3) To compare the genetic composition of these species through transcriptomics (which is being done by Dr. Matthew Fujita and his group).

Materials & Methods

For this study, 6 different species of *Aspidoscelis* lizards (both male, female) were collected from different localities of Texas (shown in the map)

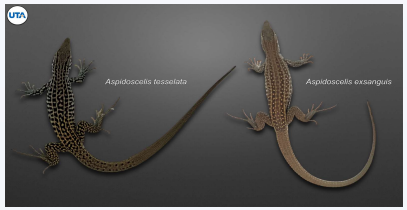

Figure 3: *Aspidoscelis exsanguis* and *Aspidoscelis tessellata* (sample and picture courtesy by Dr. Matthew Fujita and group)

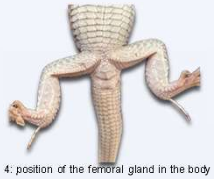

Figure 4: position of the femoral gland in the body of the whiptails

- Femoral gland extracts are under investigation for lipid and small molecule content in Aspidoscelis species.
- Dr. Matthew Fujita, alongside Ph.D. students Joshua Rivera and Joseph Rangel, are examining the genetic makeup through transcriptomics.
- Our study focuses on analyzing the femoral gland secretions' lipid and small molecule composition.
- Quadrupole GC-MS is selected for its ability to separate, vaporize, and ionize volatile and semi-volatile compounds, ideal for complex chemical analyses.

Instrumentation

- ✓ Quadrupole is utilized for separating volatile and semi-volatile organic compounds.
- ✓ Effective because it allows compounds to be vaporized without decomposition for gas chromatography separation.
- ✓ Vaporized compounds are ionized for mass spectrometric analysis.
- ✓ Particularly suitable for analyzing complex mixtures in various chemical applications due to its ability to accurately identify and quantify compounds.

Figure 5:GCMS-QP2010 Ultra Gas Chromatograph Mass Spectrometer in SCAAC

After obtaining femoral gland extract, they will be prepared to analyze in GCMS by following steps

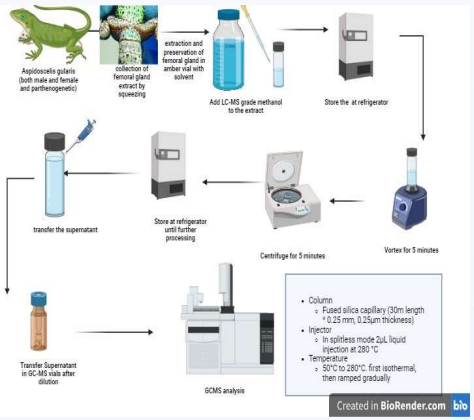

Figure 6: Schematic representation of steps involved in GC-MS analysis of Pheromone

Data

The work of Raya-García et al. in a similar study on *Aspidoscelis lineattissimus* has found the following compounds that might give us an indication of possible compounds:

Table 1 Chemical compounds identified from femoral gland secretions of male lizards *Aspidoscelis lineattissimus* from four distinct populations (Capirio, Maruata, Perula, and Island)

RT (min)	Compound name	R. index	Capirio	Maruata	Perula	Island
Esters						
5.2	Isobutyl acetate	1012	6.89 ± 1.45	8.80 ± 1.89	1.02 ± 1.34	0.44 ± 1.35
6.4	n-Butyl acetate	1074	0.02 ± 0.03	0.14 ± 1.11	0.09 ± 2.91	0.06 ± 1.59
9.1	β-Terpenyl acetate	1622	0.07 ± 8.23	0.89 ± 1.97	4.29 ± 1.93	3.17 ± 2.13
19.3	Methyl laurate	1804	2.89 ± 1.26	1.17 ± 1.73	nd	1.01 ± 0.69
19.8	Isopropyl laurate	1827	1.03 ± 1.10	0.83 ± 0.74	0.37 ± 1.25	nd
22.0	Methyl myristate	2005	19.96 ± 1.50	nd	nd	nd
22.3	Isopropyl myristate	2027	0.09 ± 7.41	0.31 ± 3.28	0.07 ± 8.22	0.07 ± 2.54
23.3	Methyl pentadecanoate	2108	1.28 ± 1.80	0.61 ± 0.86	0.62 ± 1.87	0.59 ± 5.84
24.5	Methyl palmitate	2208	1.20 ± 3.45	0.51 ± 1.03	0.28 ± 1.30	nd
24.7	Methyl palmitoleate	2240	1.10 ± 0.98	1.03 ± 0.65	1.53 ± 4.88	0.20 ± 6.92
24.9	Ethyl palmitate	2251	1.07 ± 1.11	1.04 ± 0.81	1.85 ± 6.53	1.62 ± 1.82
25.6	Methyl margarate	2309	nd	nd	nd	7.54 ± 0.87
26.7	Methyl stearate	2418	3.69 ± 0.47	0.45 ± 0.96	0.21 ± 5.34	0.10 ± 3.25
27.0	Methyl oleate	2434	0.33 ± 3.27	23.48 ± 2.29	8.27 ± 3.67	22.22 ± 2.50
27.5	Ethyl linoleate	2521	0.07 ± 2.34	0.22 ± 2.80	nd	nd
28.8	Methyl arachidate	2639	3.87 ± 9.66	0.05 ± 1.47	nd	nd
30.8	Methyl docosanoate	2750	0.38 ± 0.75	0.59 ± 0.63	0.95 ± 2.46	0.49 ± 1.13
Carboxylic acids						
14.1	Acetic acid	1449	1.50 ± 1.19	1.08 ± 0.62	1.04 ± 1.05	0.62 ± 0.74
31.4	Palmitic acid	2951	21.14 ± 1.69	23.79 ± 2.29	20.88 ± 6.94	23.35 ± 2.54
33.6	Stearic acid	3134	10.06 ± 1.65	9.97 ± 1.00	14.23 ± 1.19	13.49 ± 1.80
Others						
6.78	Undecane	1100	2.06 ± 1.06	1.99 ± 0.72	2.06 ± 2.05	1.10 ± 1.50
11.4	Trimethyl dodecane*	1320	1.01 ± 5.73	1.59 ± 0.59	1.40 ± 0.97	0.69 ± 1.07
25.0	α-Tocopherol*	3149	0.12 ± 4.19	0.19 ± 1.49	0.26 ± 2.68	0.40 ± 3.86
33.1	t-Squalene*	2865	20.16 ± 1.62	21.00 ± 2.31	7.10 ± 2.69	18.00 ± .2.50
41.3	Dihydro-lanosterol*	3823	nd	0.27 ± 2.96	31.02 ± 2.93	7.32 ± 4.95

Relative proportions of 25 compounds (mean ± SE of the percentage of the total peak areas from FID detector). *nd*, not detected compound; *RT*, retention time and retention index are shown. Compounds without standard confirmation (*)

Future Work

- Use GC-MS optimized for sensitivity to detect low-abundance compounds.
- Optimize the chromatographic conditions (column type, carrier gas flow rate, temperature gradient) to achieve the best separation of compounds.
- Validate the method by assessing its sensitivity (limit of detection, limit of quantitation), specificity, precision, and accuracy.
- Use one-way or two-way ANOVA to compare mean differences in pheromone concentrations across species, localities, sexes, and types of sexuality.
- For different species/localities: One-way ANOVA will be used to determine if there are differences among groups.
- For interaction effects (e.g., species vs. locality): Two-way ANOVA will test the main effects and interactions.

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