Predicting peptides from tick salivary glands that suppress host detection

We predicted tick salivary gland peptides that may help the tick evade host detection while feeding. Using phylogenetics and peptide prediction, we identified 12 candidates. However, testing the trait in the lab proved challenging, so we aren't continuing the project.

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Purpose

In this pub, we predict peptides from tick salivary glands that may inhibit the host's ability to detect parasitic activity and try to test for this activity *in vitro*. When ticks bite their hosts, the host often doesn't feel it. This is partly because ticks secrete molecules in their saliva that can interfere with sensory perceptions such as itch, pain, and inflammation. We refer to these systems as "host detection." We're interested in understanding how long-feeding ticks suppress host detection to uncover new therapeutic strategies for skin conditions. Motivated by our previous studies on chelicerate proteins that suppress host detection, we have extended our focus to peptides, given their inherent drug-like properties.

We used a computational framework to predict peptides that contribute to the tick's ability to feed on hosts undetected for long periods of time. We used phylogenetic trait-mapping data to find proteins statistically linked with suppression of host detection and then checked for the presence of signal peptides to select those likely secreted into tick saliva. We then refined our selection by considering additional factors such as expression in tick salivary gland transcriptomes, ease of synthesis, solubility, and similarity to other peptides (which helped us span the diversity of peptide sequences). This multi-tiered filtering process pinpointed the most viable candidates for experimental testing.

We identified 12 peptides that we think are likely to suppress host detection and have properties that make them easier to work with in experimental settings. We were initially excited to test these peptides for their ability to modulate host immunity by looking at mast cell degranulation, since mast cells are one of the first immune cells encountered by ticks [1]. However, common mast cell degranulation assays were unreliable in our hands, leading us to ice this line of research [2]. Given that putative trait host detection suppression is so broad, we have been unsure how to follow up in the lab. We're sharing our results in case others are interested in our approach or further testing these predictions.

- All **data** and **code** to predict peptides from proteins associated with suppression of host detection are available in this GitHub repository.

- All associated **data** and **code** to predict peptides from tick salivary gland transcriptomes are available in this GitHub repository.

We've put this effort on ice!

Background and goals

Ticks have adapted to consume host blood without detection. In particular, female hard-bodied ticks have lengthy "blood meals" spanning over a week. Through specialized molecules in their saliva, these ticks not only facilitate the extraction of blood but also manipulate host sensory perceptions like pain and itch and immune responses like inflammation to evade host detection [3][4]. We define "host detection" as the systems employed by the host to rapidly identify and react to parasites or other sources of danger. By interfering with these systems, ticks remain unnoticed and continue their feeding undisturbed. Some of these molecules involved in host detection suppression could have therapeutic benefits for humans, especially in managing itchiness, pain, and inflammation in the skin [5].

While tick saliva is a cocktail of pharmacologically active molecules, we were interested in whether ticks use peptides to suppress host detection. Peptides are a diverse class of small protein sequences. The exact definition of a peptide varies, but for this pub we'll use short chains of 2–100 amino acids that are genomically encoded or ribosomally synthesized and cleaved from a precursor protein [6][7][8][9][10][11]. We were drawn to peptides as a class because of their appealing therapeutic properties. Peptide drugs typically have a low toxicity and high potency compared to small molecules. They can also be easier to synthesize than larger biologics. Moreover, our interest in peptide discovery dovetails with our focus at Arcadia on proteins and evolutionary tools.

There's evidence that ticks use salivary peptides to modulate many parts of host biology [12][13][14][15][16][17][18][19][20], so we set out to identify novel tick peptides that suppress host inflammation, itch, or pain. After initial computational prediction, we narrowed the list of candidate peptides based on which are easiest to synthesize and work with in the lab. In total, we identified 12 peptides for further testing. We followed up with these peptides in the lab using a mast cell assay, but didn't gain insights into the biology of these peptides because of difficulties with the assay itself [2]. We're unsure how to follow up with these peptides experimentally, but are sharing our results in case they're useful to others.

If you're interested in learning about our methodological details, read on. If you'd like to see how our pipeline performed, skip to our results.

The approach

For a high-level sense of how we performed this work, continue to the "Overview" section below. To jump straight into all the nuts and bolts of each step, jump to the "Detailed approach" section.

Overview

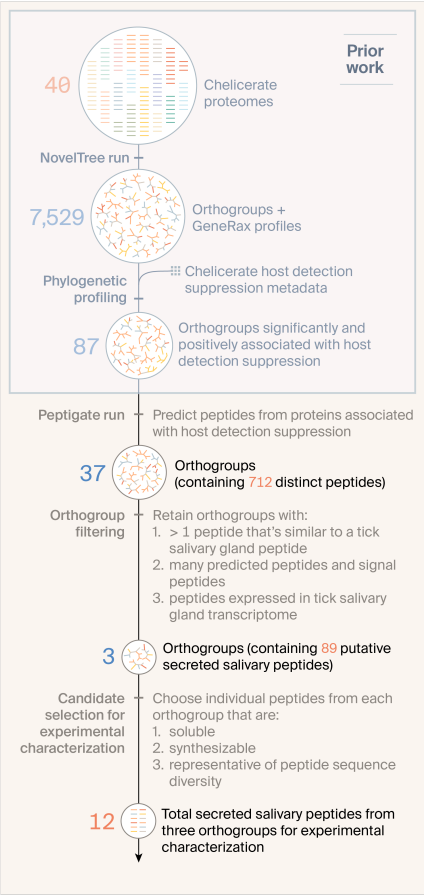

Figure 1. **An overview of our approach to identifying candidate tick salivary gland peptides that suppress host detection and selecting peptides for experimental validation**.

We predicted peptides from groups of protein sequences known as orthogroups (collections of proteins from multiple species descended from a single ancestral protein) that we previously identified as likely to be associated with host detection suppression. We filtered these peptides down to a small number of candidates for experimental follow-up. The first round of filters used biological information to increase the likelihood that a candidate peptide is a genuine bioactive peptide that suppresses host detection. The second round of filters focused on selecting experimentally feasible peptides to work with and represent the sequence diversity of candidate peptides.

Our overarching goal is to discover peptides that suppress host detection by learning from the evolutionary adaptations of ticks, which have developed mechanisms to feed on hosts undetected for long meals. Using an evolutionarily-inspired approach, we recently identified groups of proteins that we believe are associated with suppression of host detection [21]. The bulk of this pub describes how we predicted peptides from these proteins and identified which peptides we thought were genuine bioactive peptides that may suppress host detection and which are feasible to follow up with experimentally (Figure 1).

Our analysis began with proteins from chelicerates, a subphylum containing ticks and other arachnids. We'd recently used a phylogenetic trait-mapping approach to identify "orthogroups" that are statistically associated with host detection suppression [21]. An orthogroup, also known as a gene family, is a set of proteins from multiple species that all evolved from a single protein in the last common ancestor of those species. To determine these orthogroups, we first applied our previously released NovelTree phylogenomic workflow to 40 chelicerate species

with differing propensities to bite humans, blood feed, and cause and suppress itch, inflammation, and pain [21]. NovelTree infers gene families, gene family trees, species trees, and gene family evolutionary history [22]. Using the NovelTree output, we applied a trait-mapping approach to select the orthogroups most strongly associated with host detection suppression [21]. This analysis produced the proteins we analyze in this pub. We reasoned that if any of these proteins encode a peptide, the peptide might be the causative host detection-suppressing molecule.

Our first step in this analysis was to predict peptides from the proteins in orthogroups significantly associated with host detection suppression. To do this, we used our previously released peptigate pipeline in protein-only mode [23]. Peptigate is a workflow that predicts and annotates three types of bioactive peptides from transcriptomes or proteins. We filtered the peptides predicted by peptigate to those we thought had the highest chance of being bona fide peptides present in the saliva using the filters below.

1. **Removed predicted propeptides**: One tool within the peptigate pipeline predicts propeptides, a part of a protein that's cleaved during maturation or activation but usually has no independent function once cleaved. We removed these predictions.
2. **Removed orthogroups where no peptides had similarity to peptides expressed in tick salivary glands**: We removed peptide predictions where there was little evidence that the peptide is present in the salivary gland (and therefore unlikely to be in tick saliva). We expect salivary gland predictions to be incomplete, so we only required one peptide per orthogroup to match a salivary gland peptide.
3. **Kept orthogroups where at least half of the proteins had a predicted peptide**: We wanted to enrich for orthogroups where a peptide was most likely to be involved in the protein's function. We did this by keeping orthogroups where at least half of the proteins had a peptide prediction.
4. **Kept peptides/parent proteins that contain a signal peptide**: In arthropods, a signal peptide decorates most proteins that are exported to the saliva from the salivary gland [24][25][26][27][28].
5. **Expressed in tick salivary gland transcriptomes**: The initial peptide predictions from our candidate host detection-suppression-associated proteins [21] came from whole-genome or transcriptome data, so expression could occur in any tissue. Since host-manipulating peptides are likely produced in the salivary gland, we kept peptides similar to those predicted from salivary gland transcriptomes (see below for how this was determined).

After applying these initial filters, we ended up with peptides in orthogroups where computational evidence most strongly suggested the potential for suppression of host detection. We then applied a second set of filters to refine our selection, focusing on the peptides that seemed most feasible for downstream experimental testing and best showcased the diversity within the orthogroup. Our target was to narrow down to 10–20 peptides. We used the three filters below.

1. **Ease of synthesis**: Some peptides are easier to synthesize than others. The two main factors that impact synthesis are the peptide length (shorter is easier) and the hydrophilicity of the sequence (more hydrophilic is easier). We selected peptides that should be easier to synthesize when choosing between similar candidates.
2. **Solubility**: For our downstream assays, we needed the peptides to be dissolved in solution. Therefore, we selected more soluble peptides when choosing between similar candidates.

3. **Similarity to other peptides**: When multiple peptides in an orthogroup met the above criteria, we selected representatives spanning the sequence diversity. We clustered peptides at 80% identity, and if they fell into the same cluster, we advanced only one representative for experimental testing.

Detailed approach

Predicting peptides from proteins associated with suppression of host detection

We used protein sequences associated with the host detection suppression trait, as determined by previous work [21], as input to peptigate [23] to predict peptides produced by those proteins. The outputs of the trait mapping are protein sequences in orthogroups, a score denoting the strength of each cluster's association with host detection suppression, and a *p*-value denoting the statistical likelihood of the association being observed by chance [21].

To briefly summarize the methodology from that work, we conducted the analysis using a two-step approach to determine the *p*-value of these scores. First, we grouped orthogroups into clusters. We then associated the clusters with the host detection-suppression trait and only kept clusters from speciation that were significantly associated. Then, we applied a post-hoc test to determine which orthogroup within these clusters drove the association. We took this two-step approach to mitigate the issue of *p*-value inflation due to multiple testing. Even with this approach, however, no individual orthogroups were significantly associated with host detection suppression after correcting for multiple testing. We therefore filtered to orthogroups that had a positive association with host detection suppression (removed negative scores) and that had a *p*-value < 0.05 before multiple testing correction [21].

We used these sequences as input to the protein-only mode of peptigate [23]. Peptigate predicts three types of peptides: cleavage, ribosomally synthesized and post-translationally modified (RiPP), and small open reading frame (sORF)-encoded. The protein-only mode predicts cleavage and RiPP peptides but only filters to proteins that are less than 100 amino acids in length to identify sORF-encoded peptides. For more information on these types of peptides and how peptigate works, see the peptigate pub [23].

Determining whether a peptide contains a signal peptide

To determine whether a peptide contains a signal peptide, we used annotations from prior work [21]. This previous project annotated the signal peptides on input proteins using the tool DeepSig [29]. We look for signal peptides in sORF-encoded peptides that contain signal peptides themselves, as well as in the precursor proteins for cleavage peptides, as cleavage peptides aren't likely to contain signal peptides themselves.

Identifying peptides expressed in tick salivary glands

We downloaded tick salivary gland transcriptomes on the NCBI's Transcriptome Shotgun Assembly Database (TSA) to identify peptides expressed in tick salivary glands. We identified 28 transcriptomes from 18 species (Table 1). We also added an *Amblyomma americanum* transcriptome that we generated during a recent genome annotation effort [30]. While this transcriptome derives from salivary glands and other tick tissues, we recorded the tissue in which each transcript originated. This enabled us to zero in on just the subset of proteins that originated from salivary glands.

We then ran the peptigate peptide prediction pipeline on each transcriptome [23].

We used DIAMOND `blastp` (v2.1.9) to determine the sequence similarity between peptides predicted from host detection suppression-associated proteins from our prior work [21] and those from tick salivary gland transcriptomes [31]. We considered any BLAST hit as a match, in part because filtering short BLAST matches is difficult.

Species	Transcriptomes	Contigs	BioProject
Amblyomma americanum	1	3,139	PRJNA218793
Amblyomma cajennense	1	5,770	PRJNA241272
Amblyomma maculatum	1	2,9571	PRJNA703041
Amblyomma parvum	1	2,838	PRJNA241271
Amblyomma triste	1	8,098	PRJNA241269
Amblyomma tuberculatum	1	1,812	PRJNA595760
Hyalomma dromedarii	1	142,391	PRJNA358517
Hyalomma excavatum	1	5,337	PRJNA311286
Ixodes ricinus	4	51,452	PRJNA177622, PRJNA217984, PRJNA312361, PRJNA589581
Ixodes scapularis	1	5,950	PRJNA905811
Ornithodoros brasiliensis	2	15,946	PRJNA318033, PRJNA719007
Ornithodoros turicata	1	7,560	PRJNA446065
Rhipicephalus annulatus	2	63,419	PRJNA255770, PRJNA255770
Rhipicephalus appendiculatus	3	171,611	PRJNA297811, PRJNA309182, PRJNA309182
Rhipicephalus bursa	3	79,955	PRJNA348674, PRJNA348674, PRJNA348674
Rhipicephalus microplus	1	8,179	PRJNA329522
Rhipicephalus pulchellus	1	11,227	PRJNA170743
Rhipicephalus sanguineus	1	11,312	PRJNA606595
Rhipicephalus zambeziensis	2	25,336	PRJNA381085, PRJNA905810

Table 1. **Publicly available tick salivary gland transcriptomes**.

Selecting peptides based on ease of synthesis and solubility

To determine the ease of synthesis and solubility for each peptide sequence, we uploaded all candidate sequences to the GenScript "Peptide Analyzing Tool" (free to use but requires that users create an account to access it). Ease of synthesis is reported categorically as easy, medium, or hard, while solubility is reported categorically as good or poor.

Selecting representative peptides

To determine whether two peptides have similar sequences, we clustered all predicted host detection-suppressing peptides using MMseqs2 `easy-cluster` (v15.6f452) with a minimum sequence identity of 80% [32].

Additional methods

We used ChatGPT and Notion AI to suggest wording ideas and then chose which small phrases or sentence structure ideas to use. We used Grammarly Business to help copy-edit draft text to match Arcadia's style.

The results

We aimed to predict tick peptides that we could test experimentally. Peptide synthesis is expensive, and the downstream assays are low-throughput, so we wanted to end up with 10–20 top peptide predictions for testing.

Narrowing to peptides with the best computational support for host detection suppression

We started our peptide selection journey by working with peptides predicted from 3,690 proteins in 87 orthogroups significantly and positively associated with host detection suppression [21]. We predicted 741 peptides (712 distinct sequences) in 46 orthogroups associated with suppression of host detection. After removing propeptide predictions and orthogroups where no peptides matched those predicted in salivary gland transcriptomes, we ended up with 314 peptides (311 distinct sequences) in 16 orthogroups (Table 2).

Orthogroup	Proteins	Proteins with a predicted peptide	Predicted peptides	sORF & signal peptide	Cleavage & signal peptide	Synthesized
OG0001774	**62**	**45 (0.68)**	**45**	**32**	**4**	**3**
OG0008102	**20**	**18 (0.75)**	**18**	**2**	**11**	**5**
OG0000880	**93**	**68 (0.73)**	**84**	**0**	**40**	**4**
OG0011284	9	1 (0.11)	1	0	0	0
OG0008888	16	1 (0.06)	1	0	0	0
OG0002194	56	2 (0.04)	2	0	0	0
OG0000189	240	21 (0.09)	21	0	0	0
OG0000746	102	5 (0.05)	5	0	0	0
OG0000194	237	23 (0.10)	23	0	0	0
OG0001663	64	6 (0.09)	6	0	0	0
OG0000385	154	9 (0.06)	9	0	0	0
OG0000143	281	10 (0.04)	11	0	2	0
OG0000079	394	55 (0.14)	55	1	0	0
OG0000305	179	26 (0.15)	26	0	0	0
OG0015609	5	3 (0.6)	3	0	0	0
OG0009053	15	4 (0.27)	4	0	0	0

Table 2. **Candidate groups of proteins and peptides that suppress host detection**.

Bold indicates the three orthogroups that met our filtering criteria. “Orthogroup” refers to gene families generated in our prior host detection suppression trait-mapping analysis [21]. “Coefficient” is the host detection suppression score from trait-mapping. “Proteins with a predicted peptide” refers to the number of proteins with at least one predicted peptide from the peptigate pipeline. Fractions appear in parentheses next to the numbers to indicate the fraction of the proteins in the orthogroup that had a peptide prediction. “Predicted peptides” refers to the total number of peptides predicted by the peptigate pipeline for that orthogroup. For some proteins, peptigate predicted more than one peptide. “sORF & signal peptide” is the number of predicted sORF peptides with a predicted signal peptide. “Cleavage & signal peptide” is the number of predicted cleavage peptides that originated from a precursor protein that had a predicted signal peptide. “Synthesized” is the number of peptides we synthesized for experimental validation.

We felt confident that each orthogroup had a high likelihood of suppressing host detection, given the trait-mapping analysis. However, we were less convinced that the proteins in all 16 orthogroups encoded peptides. To optimize for orthogroups most likely to encode peptides, we filtered to orthogroups where we predicted a peptide from at least half of the proteins (Table 2). This left us with four orthogroups and 150 predicted peptides.

Next, we filtered these predictions to those that contain a signal peptide. Chelicerate salivary glands use signal peptides to target proteins to the saliva [24]. Tick saliva contains many of the molecules that ticks use to manipulate host biology [4], so we wanted to optimize for peptides most likely to be in saliva. The three orthogroups that passed our majority-peptide-predictions filter also passed this filter (Table 2). Within these orthogroups, a total of 89 peptides had a predicted peptide sequence as well as a signal peptide. We selected peptides to synthesize from these 89 sequences.

Selecting peptides for synthesis

We next worked to narrow down the 89 peptides to approximately 10. To do this, we applied four filters within each orthogroup. First, we looked for peptides in

each orthogroup that were “easy” or “medium” to synthesize according to GenScript’s “Peptide Analyzing Tool” (this tool is free to use but requires that users create an account to access it). We also looked for peptides with “good” solubility. These criteria ensure that the peptide is economical to synthesize and easy to handle in the lab. For example, peptides with poor solubility might not mix into a saline solution, which is the vehicle for most injections. Synthesis difficulty and solubility are influenced by factors such as peptide length, the presence of hydrophobic or charged residues, and sequence complexity. When no peptides in an orthogroup matched these criteria, we selected from all peptides (including “difficult” to synthesize and “poor” solubility).

From this subset of peptides, we then picked peptides that matched peptides predicted from tick salivary glands. Our earlier trait-mapping effort [21] analyzed whole genomes or transcriptomes. Selecting peptides similar to those expressed in the salivary glands increases our likelihood of identifying peptides used in tick saliva to manipulate the host.

We also examined whether peptides within the orthogroup were similar to each other. We clustered the peptides at 80% sequence identity and assigned a representative sequence. When multiple peptides clustered, we selected either the peptide with the best synthesis and solubility profile or the representative sequence.

Predicted peptide	Orthogroup	Solubility	Synthesis	# of similar peptides	Salivary gland peptide match	Sequence
Rhipicephalus-microplus_XP-037271377.1_start70_end114	OG0008102	Poor	Medium	1	GIKN01002979.1.p1_start91_end134	IHPVVATV
Amblyomma-sculptum_GEEX01004552.1.p1	OG0008102	Good	Easy	1	GINV01009842.1	ATISSPKK
Amblyomma-americanum_evm.model.contig-245149-1.2	OG0008102	Good	Easy	1	Transcript_929497.p2_start21_end72	AAMNSPK
Dermacentor-andersoni_XP-054924338.1_start87_end106	OG0008102	Poor	Medium	1	None	NGAISGA
Rhipicephalus-microplus_XP-037271378.1_start78_end115	OG0008102	Poor	Medium	1	GIKN01002127.1.p1_start100_end137	VVVSVSKK
Dermacentor-silvarum_XP-049518196.1	OG0001774	Good	Easy	2	GBBK01002034.1	SEEHGGD
Ixodes-scapularis_tr	B7P452	B7P452-IXOSC	OG0001774	Good	Easy	1
Ixodes-scapularis_tr	B7Q4Z2	B7Q4Z2-IXOSC	OG0001774	Good	Easy	1
Hyalomma-asiaticum_KAH6923445.1_start29_end58	OG0000880	Poor	Medium	14	GFGI01047205.1.p1_start29_end60	GGLLGAG
Rhipicephalus-microplus_XP-037269427.1_start34_end70	OG0000880	Poor	Medium	1	GBJS01000632.1.p1_start34_end70	GGVLGGL
Dermacentor-silvarum_XP-037559871.1_start39_end87	OG0000880	Poor	Medium	5	GINV01004785.1.p1_start33_end81	GGVLGGL
Dermacentor-andersoni_XP-050051547.1_start39_end77	OG0000880	Poor	Medium	6	GBJS01005204.1.p1_start39_end77	GGVLGGL

Table 3. **Peptide metadata and characteristics we used to select the 12 peptides flagged for experimental validation**.

In addition to the information here, all peptides had signal peptides.

In the “Salivary gland peptide match” column, names correspond to transcript names in the Transcriptome Shotgun Assembly database with peptide coordinates appended, while “None” indicates that the peptide didn't match against tick salivary gland peptides.

We identified 12 peptides that best matched our criteria and had diverse sequences (Table 3). These peptides belong to three orthogroups.

The first group of peptides is predicted from OG0001774. We selected three peptides from this orthogroup. Two are annotated as the peptides defensin and drosomycin (Table 3). Defensin and drosomycin are both host defense peptides. Defensin, in particular, can act as an antimicrobial peptide or participate in immune signaling [33]. We find it encouraging that both sequences were annotated as peptides and are interested in whether they interact with the immune system.

The second group of peptides is from OG0000880. These sequences are glycine-rich (Table 3). Glycine-rich peptides have antimicrobial activity across diverse organisms, from plants to chelicerates [34][35][36][37][38][39]. Since we aren't interested in antimicrobial activity in our use case, we were excited to learn that some glycine-rich peptides have other functions in vertebrates [40][41]. The ability of glycine-rich peptides to elicit cellular and organismal phenotypes is promising for the potential function of our candidate peptides.

The last group of peptides is from OG0008102 and comprises five sequences. These peptides are annotated as a mixture of sORF and cleavage peptides. However, the initial proteins in this group ranged from 99 to 114 amino acids in length. The proteins that were 100 amino acids or less are sORF peptides, while those that were greater than 100 amino acids are cleavage peptides. None of the peptides were annotated or had matches against known peptides in the Peptipedia metadatabase [42]. This makes it difficult to predict the potential function of these peptides.

Working with putative host detection suppression-associated proteins from NovelTree [21] was advantageous because the proteins that generated the peptides were already organized into orthogroups. Although some peptides within an orthogroup shared sequence homology, many didn't. These orthogroups established connections between peptides that we couldn't have inferred from their sequences alone.

Testing peptides experimentally for host detection suppression traits

Our goal was to identify peptides that suppress host detection. After predicting these peptides computationally, we next wanted to test for these traits experimentally. In hindsight, we could have done a better job of thinking through which assays would be most informative at the outset of the project. We chose to test whether our peptides could block a form of immune activation involved in host detection [2] using a mast cell degranulation assay. Mast cells are involved in the skin's response to ectoparasites like ticks, and this assay measures β-hexosaminidase release into the supernatant as a marker of degranulation. While developing the assay, we found that compound 48/80, a common mast cell activator, caused substantial cell lysis at the concentrations typically used [2]. This effect raised concerns about the reliability of the assay. Further, even if the assay had worked well, it would have covered only a small portion of the broader pain, itch, and inflammation space. For these reasons, the chance that our peptides would show an effect in this specific context was low. We didn't find a suitable assay that captures a wider range of host detection pathways, so we were unable to move forward with experimental testing of our peptide predictions.

Key takeaways

- **Development of a specific peptide prediction strategy**: We developed an approach to predict peptides that can suppress detection in host organisms during tick feeding. Our approach first used trait-mapping data and signal peptide predictions to identify candidate peptides and then used synthesis, solubility, sequence similarity, and expression profiles in tick salivary glands to select the best candidates for experimental follow-up.
- **Selection of peptides for experimental testing**: We identified 12 peptides from three orthogroups to test in downstream experimental assays. Peptides from two orthogroups have characteristics or annotations similar to known bioactive peptides from other chelicerates.
- **Difficulty in experimental follow-up**: We attempted to test our predicted peptides using a mast cell degranulation assay but found the assay unreliable, technically challenging, and too limited in scope to capture the full range of host detection traits. Without a better-suited assay, we couldn't experimentally evaluate the peptides *in vitro*.

Next steps

Given our difficulty in experimental follow-up, we've decided to put this effort on ice. We're hopeful that our peptide prediction approach may be helpful to others or that someone may be interested in the predicted peptide sequences. We'd also be curious to hear if anyone has thoughts on a general experimental assay or several very simple ones that can quickly test for activity across host detection suppression traits like inflammation, pain, and itch.

Contributors (A–Z)

- **Audrey Bell**: Visualization
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- **Taylor Reiter**: Conceptualization, Formal Analysis, Methodology, Software, Visualization, Writing
- **Emily C.P. Weiss**: Conceptualization

References

1. Nuttall PA. (2019). Wonders of tick saliva. https://doi.org/10.1016/j.ttbdis.2018.11.005
2. Borges AL, Donnelly J, Morazan E, Rollins M. (2025). Compound 48/80 is toxic in HMC1.2 and RBL-2H3 cells. https://doi.org/10.57844/arcadia-3207-4695
3. Šimo L, Kazimirova M, Richardson J, Bonnet SI. (2017). The Essential Role of Tick Salivary Glands and Saliva in Tick Feeding and Pathogen Transmission. https://doi.org/10.3389/fcimb.2017.00281
4. Kazimírová M, Štibrániová I. (2013). Tick salivary compounds: their role in modulation of host defences and pathogen transmission. https://doi.org/10.3389/fcimb.2013.00043
5. Aounallah H, Bensaoud C, M'ghirbi Y, Faria F, Chmelař J, Kotsyfakis M. (2020). Tick Salivary Compounds for Targeted Immunomodulatory Therapy. https://doi.org/10.3389/fimmu.2020.583845
6. Schlesinger D, Elsässer SJ. (2021). Revisiting sORFs: overcoming challenges to identify and characterize functional microproteins. https://doi.org/10.1111/febs.15769
7. Kim Y-G, Lone AM, Nolte WM, Saghatelian A. (2012). Peptidomics approach to elucidate the proteolytic regulation of bioactive peptides. https://doi.org/10.1073/pnas.1203195109
8. Fitzgerald K. (2020). Furin Protease: From SARS CoV-2 to Anthrax, Diabetes, and Hypertension. https://doi.org/10.7812/tpp/20.187
9. Arnison PG, Bibb MJ, Bierbaum G, Bowers AA, Bugni TS, Bulaj G, Camarero JA, Campopiano DJ, Challis GL, Clardy J, Cotter PD, Craik DJ, Dawson M, Dittmann E, Donadio S, Dorrestein PC, Entian K-D, Fischbach MA, Garavelli JS, Göransson U, Gruber CW, Haft DH, Hemscheidt TK, Hertweck C, Hill C, Horswill AR, Jaspars M, Kelly WL, Klinman JP, Kuipers OP, Link AJ, Liu W, Marahiel MA, Mitchell DA, Moll GN, Moore BS, Müller R, Nair SK, Nes IF, Norris GE, Olivera BM, Onaka H, Patchett ML,

Piel J, Reaney MJT, Rebuffat S, Ross RP, Sahl H-G, Schmidt EW, Selsted ME, Severinov K, Shen B, Sivonen K, Smith L, Stein T, Süssmuth RD, Tagg JR, Tang G-L, Truman AW, Vederas JC, Walsh CT, Walton JD, Wenzel SC, Willey JM, van der Donk WA. (2013). Ribosomally synthesized and post-translationally modified peptide natural products: overview and recommendations for a universal nomenclature. https://doi.org/10.1039/c2np20085f

10. Luo S, Dong S-H. (2019). Recent Advances in the Discovery and Biosynthetic Study of Eukaryotic RiPP Natural Products. https://doi.org/10.3390/molecules24081541

11. Finking R, Marahiel MA. (2004). Biosynthesis of Nonribosomal Peptides. https://doi.org/10.1146/annurev.micro.58.030603.123615

12. Tian Y, Chen W, Mo G, Chen R, Fang M, Yedid G, Yan X. (2016). An Immunosuppressant Peptide from the Hard Tick Amblyomma variegatum. https://doi.org/10.3390/toxins8050133

13. Wu J, Wang Y, Liu H, Yang H, Ma D, Li J, Li D, Lai R, Yu H. (2010). Two Immunoregulatory Peptides with Antioxidant Activity from Tick Salivary Glands. https://doi.org/10.1074/jbc.m109.094615

14. Tang J, Fang Y, Han Y, Bai X, Yan X, Zhang Y, Lai R, Zhang Z. (2015). YY-39, a tick anti-thrombosis peptide containing RGD domain. https://doi.org/10.1016/j.peptides.2014.08.008

15. Karczewski J, Waxman L, Endris R, Connolly T. (1995). An Inhibitor from the Argasid Tick Ornithodoros moubata of Cell Adhesion to Collagen. https://doi.org/10.1006/bbrc.1995.1371

16. Mans BJ, Louw AI, Neitz AW. (2002). Savignygrin, a Platelet Aggregation Inhibitor from the Soft TickOrnithodoros savignyi, Presents the RGD Integrin Recognition Motif on the Kunitz-BPTI Fold. https://doi.org/10.1074/jbc.m112060200

17. Wang X, Coons LB, Taylor DB, Stevens SE, Gartner TK. (1996). Variabilin, a Novel RGD-containing Antagonist of Glycoprotein IIb-IIIa and Platelet Aggregation Inhibitor from the Hard Tick. https://doi.org/10.1074/jbc.271.30.17785

18. Li X, Yang H, Han Y, Yin S, Shen B, Wu Y, Li W, Cao Z. (2021). Tick peptides evoke itch by activating MrgprC11/MRGPRX1 to sensitize TRPV1 in pruriceptors. https://doi.org/10.1016/j.jaci.2020.12.626

19. Liu Q, Dong X. (2015). The Role of the Mrgpr Receptor Family in Itch. https://doi.org/10.1007/978-3-662-44605-8_5

20. Borges AL, Chou S, Patton AH, Reiter T, Weiss EC, York R. (2025). Comparative phylogenomic analysis of chelicerates points to gene families associated with long-term suppression of host detection. https://doi.org/10.57844/arcadia-4e3b-bbea

21. Celebi FM, Chou S, McGeever E, Patton AH, York R. (2023). NovelTree: Highly parallelized phylogenomic inference. https://doi.org/10.57844/arcadia-z08x-v798

22. Borges AL, Celebi FM, Cheveralls K, Chou S, Reiter T, Weiss EC. (2024). Predicting bioactive peptides from transcriptome assemblies with the peptigate workflow. https://doi.org/10.57844/arcadia-6500-9be8

23. Garcia GR, Chaves Ribeiro JM, Maruyama SR, Gardinassi LG, Nelson K, Ferreira BR, Andrade TG, de Miranda Santos IKF. (2020). A transcriptome and proteome of the tick Rhipicephalus microplus shaped by the genetic composition of its hosts and developmental stage. https://doi.org/10.1038/s41598-020-69793-3

24. Huang H-J, Lu J-B, Li Q, Bao Y-Y, Zhang C-X. (2018). Combined transcriptomic/proteomic analysis of salivary gland and secreted saliva in three planthopper species. https://doi.org/10.1016/j.jprot.2017.11.003

25. Wang X-J, Li Q, Ye Z-X, Huang H-J. (2024). A pipeline contributes to efficient identification of salivary proteins in short-headed planthopper, Epeurysa nawaii. https://doi.org/10.1038/s41598-024-56896-4

26. Huang H-J, Liu C-W, Huang X-H, Zhou X, Zhuo J-C, Zhang C-X, Bao Y-Y. (2016). Screening and Functional Analyses of Nilaparvata lugens Salivary Proteome. https://doi.org/10.1021/acs.jproteome.6b00086

27. Huang H-J, Yan X-T, Wei Z-Y, Wang Y-Z, Chen J-P, Li J-M, Sun Z-T, Zhang C-X. (2021). Identification of Riptortus pedestris Salivary Proteins and Their Roles in Inducing Plant Defenses. https://doi.org/10.3390/biology10080753

28. Savojardo C, Martelli PL, Fariselli P, Casadio R. (2017). DeepSig: deep learning improves signal peptide detection in proteins. https://doi.org/10.1093/bioinformatics/btx818

29. Celebi FM, Chou S, McDaniel EA, Reiter T, Weiss EC. (2024). Predicted genes from the Amblyomma americanum draft genome assembly. https://doi.org/10.57844/arcadia-9602-3351

30. Buchfink B, Xie C, Huson DH. (2014). Fast and sensitive protein alignment using DIAMOND. https://doi.org/10.1038/nmeth.3176

31. Steinegger M, Söding J. (2017). MMseqs2 enables sensitive protein sequence searching for the analysis of massive data sets. https://doi.org/10.1038/nbt.3988

32. Ganz T. (2003). Defensins: antimicrobial peptides of innate immunity. https://doi.org/10.1038/nri1180

33. Tavares LS, Rettore JV, Freitas RM, Porto WF, Duque APdN, Singulani JdL, Silva ON, Detoni MdL, Vasconcelos EG, Dias SC, Franco OL, Santos MdO. (2012). Antimicrobial activity of recombinant Pg-AMP1, a glycine-rich peptide from guava seeds. https://doi.org/10.1016/j.peptides.2012.07.017

34. Pelegrini PB, Murad AM, Silva LP, dos Santos RC, Costa FT, Tagliari PD, Bloch Jr C, Noronha EF, Miller RN, Franco OL. (2008). Identification of a novel storage glycine-rich peptide from guava (Psidium guajava) seeds with activity against Gram-negative bacteria. https://doi.org/10.1016/j.peptides.2008.03.013

35. Sperstad SV, Haug T, Vasskog T, Stensvåg K. (2009). Hyastatin, a glycine-rich multi-domain antimicrobial peptide isolated from the spider crab (Hyas araneus) hemocytes. https://doi.org/10.1016/j.molimm.2009.05.002

36. Baumann T, Kämpfer U, Schürch S, Schaller J, Largiadèr C, Nentwig W, Kuhn-Nentwig L. (2010). Ctenidins: antimicrobial glycine-rich peptides from the hemocytes of the spider Cupiennius salei. https://doi.org/10.1007/s00018-010-0364-0

37. de Jesus Oliveira T, Oliveira UCd, da Silva Junior PI. (2019). Serrulin: A Glycine-Rich Bioactive Peptide from the Hemolymph of the Yellow Tityus serrulatus Scorpion. https://doi.org/10.3390/toxins11090517

38. Lu J, Chen Z-w. (2010). Isolation, characterization and anti-cancer activity of SK84, a novel glycine-rich antimicrobial peptide from Drosophila virilis. https://doi.org/10.1016/j.peptides.2009.09.028

39. Espino SS, Dilanyan T, Imperial JS, Aguilar MB, Teichert RW, Bandyopadhyay P, Olivera BM. (2016). Glycine-rich conotoxins from the Virgiconus clade. https://doi.org/10.1016/j.toxicon.2016.02.001

40. Cabas-Mora G, Daza A, Soto-García N, Garrido V, Alvarez D, Navarrete M, Sarmiento-Varón L, Sepúlveda Yañez JH, Davari MD, Cadet F, Olivera-Nappa Á, Uribe-Paredes R, Medina-Ortiz D. (2024). Peptipedia v2.0: A peptide sequence database and user-friendly web platform. A major update. https://doi.org/10.1101/2024.07.11.603053