Investigating the Origins of Niche Shift in Bagheera kiplingi

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***Abstract*— As a predominantly herbivorous forager among a wide range of predators, the jumping spider Bagheera kiplingi's diet of Beltian bodies, a detachable nutrient-filled tip found on certain species of Vachellia trees, is unique among the more than 6,000 members of the family Salticidae. The jumping capabilities of Salticidae spiders is widely accepted to be an evolutionary trait designed to help them capture far-away prey. Therefore, the herbivory of B. kiplingi presents a fascinating area of study as its ability to digest high-fiber, nutrient-poor plant material could provide key insights into the evolutionary processes behind niche shifts. Analysis of the dietary habits of Bagheera prosper, B. kiplingi's closest relative, characterizes this species as an obligate carnivore. Moreover, polymerase chain reaction using nifH primers has resulted in the successful amplification of DNA from surface-sterilized B. kiplingi, but not from B. prosper or Frigga crocuta (another spider species that has been found on Vachellia collinsii plants). These results document the first discovery of nitrogen-fixing activity within an arachnid and support the hypothesis that B. kiplingi benefits from the presence of symbiotic bacteria in its gut to supplement a low-nitrogen diet. Additionally, behavioral analysis of B. kiplingi's diet in controlled settings suggests that they require regular inoculations of ant larvae in order to survive on plant material, supporting the hypothesis that B. kiplingi obtains a portion of its microbiome through consuming ant larvae. Meanwhile, video behavioral analysis of B. kiplingi behavior in comparison to B. prosper and F. crocuta provides evidence for the optimal foraging theory and the locomotor crossover hypothesis.**

I. Introduction

Bagheera kiplingi is a species of jumping spider, also known as a salticid, that resides in Mesoamerica from Mexico to Costa Rica [1]. Past studies have demonstrated that *B. kiplingi* possess a uniquely herbivorous diet consisting of roughly 60-90% plant tissue from the Beltian bodies of *Vachellia* trees with the remainder consisting of mainly larvae of *Pseudomyrmex* ants that live in mutualistic relationships with these trees, in addition to small flies and occasional cannibalism [1]. In contrast, the over 50,000 other species of spiders are carnivores, with only rare exceptions, such as the orb-weaving spider that occasionally consumes pollen from its web as a juvenile [2]. *B. kiplingi* is the only consistent forager to have been described [1], making its primarilly-diet a fascinating area of study.

While no other arachnid is known to be herbivorous, prior research suggests that the vast majority of plant-eating arthropods benefit from symbiotic relationships with bacteria in their digestive tracts: particularly those that can digest cellulose and fix nitrogen [3]. Modifications to the mouth and digestive tracts are also common, including broader and flatter mouthparts to grind down plant material and added length and surface area in the gut that permit populations of specialized bacteria to thrive [4,5].

Microbial transmission has been documented to occur in two main ways: vertical transmission where a parent passes microbes directly to offspring [6], and horizontal transmission where bacteria are usually acquired from members of the same species through infection [7]. In rare instances, interspecies transmissions of bacteria symbionts have been observed, such as between the pika and the yak in Tibet [8]. It has been proposed that *B. kiplingi* accelerated its adoption of a plant-based diet through the consumption of ant larvae, which provide an infusion of bacteria that allows it to temporarily digest cellulose and fix nitrogen [9].

To test this, DNA analysis of the *B. kiplingi* microbiome was used to compare them to their sister species, *Bagheera prosper* [10], and a distantly related species of salticid, *Frigga crocuta,* that has also been observed living on *Vachellia* trees in Panama (where *B. kiplingi* has not been found). Further experiments studying *B. kiplingi's* diet under different controlled environments were also used to supplement the previous findings.

As an additional objective, *B. kiplingi's* unique position in the ecosystem was capitalized upon to contribute to the field of behavioral ecology as their behaviors were compared to that of *B. prosper* and *F. crocuta*. Considering that the two species are documented obligate carnivores and share either a common evolutionary history (*B. prosper*) or a common habitat (*F. crocuta*) with *B. kiplingi* [10], an analysis of the three spiders' behaviors provide a strong platform for analyzing the validity of the optimal foraging theory (OFT) and the locomotor crossover hypothesis (LCH). Under both frameworks, *B. kiplingi*, an herbivore, is expected to move more to access stationary Beltian bodies, its main food source, while reorienting less due to reduced energy demands for food detection [11,12,13].

This potential shift in niche—the trophic and behavioral position of an organism within its ecosystem [14]—could provide new insights into how evolution may proceed, particularly if other spiders with similar habitats and common lineages might still be carnivores.

II. Methods and Materials

DNA Analysis

Sample Collection. For this study, *B. kiplingi, Pseudomyrmex peperi* adults*, P. peperi* larvae, and *Vachellia collinsii* leaves were collected from Akumal, Mexico. This location was chosen because prior studies suggested that Mexican *B. kiplingi* from this region were more herbivorous than *B. kiplingi* found elsewhere [1]. Samples of *B. prosper* were collected from the University of Oklahoma Biological Station (UOBS) at Lake Texoma—the site of prior studies of *B. prosper*— and samples of *F. crocuta, Pseudomyrmex spinicola,* and *V. collinsii* were collected from El Cortezo in central Panama [1]. *P. spinicola* was collected due to prior studies that document them as a part of *B. kiplingi's* diet, however, not enough were collected to conduct DNA extraction and analysis [1]. All collected samples were stored in 95% ethanol to preserve DNA before extraction [15].

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DNA Extraction. DNA was extracted from *P. peperi* adults (n=5), *P. peperi* larvae (n=5), and *V. collinsii* Beltian bodies (n=2) using the Zymo Research Quick-DNA Tissue/Insect Kit and its listed procedures (Catalog: D6016). For the extraction of DNA from the spiders- *B. kiplingi* (n=3), *B. prosper* (n=4), *F. crocuta* (n=4)- samples were surface sterilized to ensure all sequenced DNA was from inside the spider and therefore reflective of its microbiome and not its environment. This was done in a UVC Sterilization Cabinet for 15 minutes on each side. Afterward, each specimen was split into Head/Legs and Abdomen with a flame-sterilized blade. The resulting samples then underwent the same DNA extraction procedures as the other specimens.

Polymerase Chain Reaction (PCR) and Purification. PCR was performed on each DNA sample using the iProof PCR kit (Catalog: 1725331) and its listed procedures with *nifH* primers to target nitrogen-fixing bacteria. After a 30-second denaturation at 98°C, the reaction mixture was run through 35 cycles of denaturation for 10 seconds at 98°C, annealing for 30 seconds at 53°C, and extension for 30 seconds at 72°C, followed by incubation for 10 min at 72°C and then a permanent hold at 10˚C until the samples were retrieved.

After PCR, the DNA samples were purified using the QIAGEN QIAquick PCR Purification Kit (Catalog: 28106) and the procedures listed within.

DNA Sequencing and Analysis. The purified samples were then sent to Genewiz in South Plainfield, NJ for DNA sequencing and analysis.

Controlled Environment Analysis of Diet

Set-up and Analysis. In Akumal, Mexico, *V. collinsii (*with *B. kiplingi* residing on it) were stripped of all ants and netted to prevent *B. kiplingi* from leaving or *Pseudomyrmex* ants from entering. These plants were then separated into two groups: a plant-only (n=15) group where *B. kiplingi* only had access to their usual food source of Beltian bodies, and a flies (n=15) group where flies from the genus *Drosophila* were provided as an additional food source. These flies were chosen for two reasons: their similarity to the non-ant insect food source that *B. kiplingi* has been observed to consume [1], and their similarity to the nectar-stealing flies that are the principal prey of *F. crocuta* [1]. The diet and feeding behavior as well as the duration of survival of *B. kiplingi* from each group were documented.

Behavioral Analysis

Video Collection.

Behavior of *B. kiplingi* (n=31) in Akumal recorded by Christopher Meehan in 2007 was compared to the recorded behavior of *B. prosper* (n=14) at UOBS, and *F. crocuta* (n=12) in El Cortezo. Videos capturing the spider for longer than 5 minutes were kept and used in the research. For data analysis, videos were split into 5-minute segments as the analysis units.

Video Analysis. 3 major behaviors were documented at 10-second intervals of the 5-minute videos: stationary (quiet), rotation without changing location (reorient), or changing location at a distance equal to or greater than one body length (move) was documented. These procedures were adapted from a previous study on the behavior of a different salticid, *Phidippus audax* [16]. The data was then analyzed using a Tukey Honestly Significant Difference (HSD) test and standard error.

III. Results

DNA Analysis

DNA sequencing returned clear sequences for *P. peperi* and *B. kiplingi,* confirming the effectiveness of the extraction, amplification and purification procedures. In addition, *nifH* was successfully amplified in all samples of *B. kiplingi* and *P. peperi* but was not detected in any samples of *B. prosper* or *F. crocuta*.

Controlled Environment Analysis of Diet

In the plants-only group, *B. kiplingi* were not observed to consume any Beltian bodies and all died within two weeks after the beginning of the experiment. In the flies group, *B. kiplingi* were also not observed to consume any Beltian bodies but were able to survive on a fly-only diet for up to 6 months.

Behavioral Analysis

No difference was found in the occurrence of quiet behavior over the span of 5 minutes between *B. kiplingi* ($\bar{x}$=6.6±0.98), *B. prosper* ($\bar{x}$=6.9±1.57), and *F. crocuta* ($\bar{x}$=7.1±1.57) (Fig. 2). In terms of reorientation, *B. kiplingi* ($\bar{x}$=13.7±0.87) was found to reorient more than *B. prosper* ($\bar{x}$=8.3±1.44) but less than *F. crocuta* ($\bar{x}$=18.1±1.4) over 5 minutes (Fig. 2). The opposite was true for movement, with *B. kiplingi* ($\bar{x}$=9.4±1.17) moving more than *F. crocuta* ($\bar{x}$=4.9±1.88) but less than *B. prosper* ($\bar{x}$=15±1.78) over 5 minutes (Fig. 2).

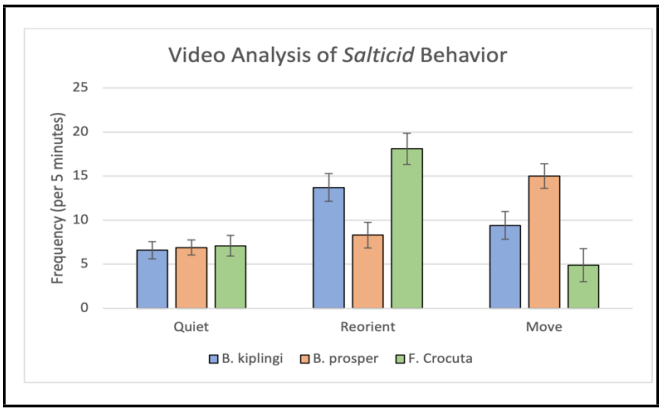

Figure 2. Comparison of the frequency of quiet, reorient, and movement behavior between *B. kiplingi, B. prosper,* and *F. crocuta* sampled at 10-second intervals over 5 minutes.

IV. Discussion

The amplification of *nifH* genes in *B. kiplingi* reveals the first discovery of nitrogen-fixing activity within an arachnid. While past studies on spiders have found strains of *Burkholderia* bacteria—some of which have nitrogen-fixing abilities—they were unable to determine the specific species

of bacteria present, leaving ambiguity as to whether the detected bacteria were actually nitrogen-fixing or merely closely related to nitrogen-fixing bacteria [17]. Meanwhile, *nifH*'s specificity to only nitrogen-fixing bacteria confirms that *B. kiplingi* does have nitrogen-fixing bacteria within its body [18]. Furthermore, the lack of *nifH* amplification in *B. prosper* and *F. crocuta* also supports the results, ruling out the possibility of contamination for *nifH* amplification in *B. kiplingi*. The absence of *nifH* in both *B. kiplingi's* sister species and a species of spider that resides in similar environments also suggests that *B. kiplingi* is unique in its niche.

In the controlled environment experiments, since the spiders in the plant-only group were not observed to consume Beltian bodies, this disputes the possibility of both vertical and horizontal intraspecies microbial transmission in *B. kiplingi,* as both would have continued in this environment; instead, it suggests that the spiders rely on an external source to remain herbivorous.

The lack of herbivory in the fly group demonstrates that *B. kiplingi* is able to survive on a purely carnivorous diet, conflicting with the herbivorous nature of *B. kiplingi* in the wild. This directs us to examine *B. kiplingi's* diet as a whole, where the consumption of ant larvae stands out as the largest carnivorous portion of the diet.

With both the DNA analysis and past studies confirming the presence of nitrogen-fixing bacteria in *Pseudomyrmex* adults and larvae [19], as well as studies that indicate ants pass down their microbiome through vertical transmission (meaning that all larvae no matter age would possess the microbes) [20], the finding that *B. kiplingi* were positive for nitrogen-fixing bacteria suggests that the consumption of ant larvae is connected to its microbiome. This provides a strong case for the hypothesis that microbial transmission occurs between *B. kiplingi* and *Pseudomyrmex* larvae through a predator-prey interaction.

While initially the behavioral comparison between *B. kiplingi* and *B. prosper* appears contradictory to the predictions of OFT and LCH as *B. prosper* reoriented less and moved more than *B. kiplingi*, the frameworks do not consider the behaviors of *B. kiplingi* in response to *Pseudomyrmex* aggression [1]. Crucially, hostility from the ants would likely necessitate higher levels of vigilance in *B. kiplingi* [1], prompting it to reorient more and move less to avoid being detected by the ants. Meanwhile, *B. prosper*, an active predator, would still need to move frequently to search for food. Conversely, the behaviors of *B. kiplingi* compared to *F. crocuta* do remain consistent with predictions from OFT and LCH. Because *F. crocuta* also resides on *Vachellia* plants and likely faces the same behavioral pressures from *Pseudomyrmex* ants as *B. kiplingi*, the fact that their behaviors align with expected behaviors provides strong support for OFT and LCH.

V. Significance and Future Objectives

This research not only contributes to the currently under-researched field of niche evolution, but it also documents the first instance of nitrogen-fixing activity in an arachnid, presenting a significant discovery for the field of microbiology, arachnid behavior, and ecology. Future studies should investigate how the presence of nitrogen-fixing bacteria impacts the diet and behavior of its host.

Furthermore, the diet analysis suggests that *B. kiplingi* relies primarily on their microbiome to consume plants. These findings hint at the possibility that *B. kiplingi* derived this microbiome, which breaks down plant tissue, from *Pseudomyrmex* larvae. This is evidence for the first example of horizontal microbiome transfer from prey to predator between animals, representing a new possible pathway of how microbes may begin to associate with hosts.

This data also represents the possibility of a new class of predator-prey relationships where the primary goal is microbiome acquisitions as opposed to energy gain. The results from this study should be considered in future research on predator-prey interactions and behaviors, rapid evolution into new niches, and microbiome retention.

Since the detailed mechanisms of *B. kiplingi's* herbivory remains a mystery, future research will employ metagenomic sequencing and more in-depth behavioral manipulation experiments to confirm the occurrence of predator-prey microbial transmission and identify the specific species of microorganisms involved.

Lastly, the video behavioral analysis not only provides significant support for OFT and LCH it also advances the understanding of how predator-prey interactions influence changes in an organism's feeding habits. To further test the frameworks on *B. kiplingi,* future experiments should be conducted under controlled conditions without ants to document their baseline behaviors.

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