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CHARACTERIZATION OF THE STOCHASTIC RELATIONSHIP OF *POECILOCHIRUS* (MITES) TO *NICROPHORUS* (BEETLES) USING STABLE ISOTOPE ANALYSIS

by

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Abstract

Burying beetles (Genus: *Nicrophorus)* are characterized by their unique natural history: bi-parental care, cooperatively burying carcasses of small vertebrates, and rearing young on the carcass. This study focuses on mites that live on and travel with the beetles, using them as a transportation service and gaining access to mates and reproductive resources. The mites (Genus: *Poecilochirus*) have an ambiguous relationship with their beetle hosts. Mites can form a mutualistic relationship with the beetles by feeding on the eggs of flies, whose maggots would otherwise compete with the beetle larvae for food. An alternative hypothesis is that the mites are predatory, and feed on beetle eggs, thereby negatively affecting their host's reproductive success. Previous studies testing these hypotheses have been inconclusive. In this study, I replicated behavioral experiments using *Nicrophorus investigator* and *Nicrophorus guttula,* and used stable isotope mass spectrometry to reconstruct the diet of *Poecilochirus* mites found on *N. guttula*.

Keywords: *Nicrophorus guttula*, *Poecilochirus,* Parasitism, Mutualism, Stable Isotopes

Chapter I: Introduction

Phoresy occurs when one organism, often a mite, uses another for transportation without negatively affecting its life history. In many cases, the host transports the phoretic organism to microclimates that are favorable for survival and reproduction. In the case of hummingbird flower mites, the mites ride in the nostrils of the hummingbird from flower to flower. The microclimate created by each flower is highly variable with regard to temperature, so the mites use hummingbirds to move when their home flower becomes too hot (Dobkin, 1985).

Phoretic species interactions are often not as simple as host organisms providing a taxi service. Houck & Cohen (1995) discovered that mites living on the beetle, *Chilocorus cacti*, were not simply phoretic, but fed on their host's bodily fluids. Their discovery caused the species interaction between *C. cacti* and its mites to be described as parasites. However, Holte et al. (2001) found that the *C. cacti* beetles extract water from their mite passengers, making their species interaction mutualistic or commensal.

In addition to being complex, species interactions are plastic. Plastic species interactions are likely to have evolved in response to unpredictable environmental conditions. If the biotic and abiotic components of a species interaction vary spatially and temporally, it is adaptive for the species in that interaction to evolve phenotypic plasticity (Agrawal, 2001). For example, Hodgkin et al. (2010), found that mites living with bark beetles were both parasitic and mutualistic depending on the life stage of the bark beetle: parasitic with adults, but mutualistic with larvae.

Phoretic mites in the genus: *Poecilochirus* live on burying beetles (Genus*: Nicrophorus*), riding the beetles from carrion source to carrion source, breeding only when the beetles do (Scott 1998). Burying beetles and their phoretic mites have a unique natural history characterized by the beetles locating and burying a small vertebrate carcass, on which both taxa reproduce (Scott 1998). The beetles stop on large carcasses to eat, giving their mites an opportunity to change hosts, which has allowed the mites to evolve host preferences (Schwarz and Koulianos 1998). *Poecilochirus* mites prefer to associate with reproductively active beetles, as they typically reproduce concurrently with their hosts. Grossman and Smith (2008) demonstrated a positive relationship between size of male burying beetles and mite load, indicating that mites select larger male beetles when given the opportunity. Larger beetles often are better competitors, making it easier for them to defend carcasses from smaller beetles and raise their own offspring (Otronen 1988, Scott 1998). Presumably, the more likely the beetle is to reproduce, the more likely its phoretic mites will reproduce too. Schwarz and Muller (1992) also observed that mites leaving the brood chamber tend to congregate on males. They observed that it took approximately ten days for mites to mature to their deuteronymph stage after their mothers molted, which is the stage where their phoretic behavior occurs. Male beetles, in their study, left the brood chamber around eight days, and females left by eleven days. If the mites develop too late, and miss their chance to leave with either parent, they must stay with the larvae while they pupate, which can be a considerable length of time in certain species. Mites also show host species preferences, and while they can reproduce with

any *Nicrophorus* spp. beetle, they have more offspring when reproducing in the brood chambers of their preferred hosts (Brown and Sloan Wilson 1992). Therefore, the relationship of the mites to their host is specialized, leading to potential divergent behavior even within closely related species. Observational evidence suggests that *Poecilochirus* spp. feeds on invertebrate eggs, larvae, nematodes and other mites: either the eggs of their beetle hosts or the eggs of dipterans (Blackman and Evans 1994; Springett 1968; Wise et al. 1988; Geden et al. 1988, Geden et al. 1989). Consequently, the mite's interaction with *Nicrophorus* spp. beetles may be parasitic (if they eat beetle eggs) or mutualistic (if they eat the eggs of the beetle's competitors, flies, mites, and nematodes). Past studies have obtained mixed results, with evidence suggesting that the beetle-mite interaction can be parasitic, mutualistic, or commensal (Blackman 1997; Blackman and Evans 1994; Beninger 1993; Springett 1968; Wilson and Knollenberg 1987, Wilson 1983). Each of these previously cited studies used multiple beetle and mite species (Table 1), potentially explaining their conflicting outcomes regarding the impacts of mites on the reproductive output of the beetles. Mites in the *P. davydovea* and *P. carabi* morphological complexes were present in studies that provided evidence for a parasitic relationship, while mites in the *P. necrophori* complex were mutualists or commensalists. In a study by Schwarz and Walzl (1996) two mite species within the *P. carabi* morphological complex were identified and observed on the same hosts. The heterospecifics could not interbreed, though the mites attempted to copulate, and had different developmental life histories. Their study suggested that there is active niche partitioning even on one host, and speciation actively occurring within a single species

complex of *Poecilochirus*. Variation in the behavior of the *Nicrophorus* hosts may be one driving force behind parasitic, or mutualistic behavior. For example, burying beetles of different species bury carcasses relatively more deeply in the soil, making it harder for flies to lay their eggs (Wilson and Knollenberg 1987). However, there are many other confirmed food sources for *Poecilochirus* spp. in the brood chamber (mites, nematodes, other eggs). Therefore, host behavior cannot be the *only* driving factor behind the behavioral evolution of the mites. Conspecific competition among the mites is inherently very intense. Once a *Poecilochirus* spp. deuteronymph molts it cannot leave the brood chamber, and is doomed to die. If it molts too soon, with too much sexual competition, or when there are no mates available, it will never get another chance to reproduce, and the mites are indeed sensitive to these behavioral conditions (Nehring and Muller 2009).

Beetles demonstrate varying abilities to compete with flies based on species and number of beetles on the carcass. In addition, the act of burying the carcass fundamentally reduces competition with flies by making the carcass inaccessible for oviposition (Suzuki 2000). *Nicrophorus defodiens* experienced an increase in reproductive successes when a pair of beetles were present (51%) rather than a single female (25%) after flies had been allowed to oviposit on the carcass, indicating that the parent beetles behave in a way that reduces fly infestation (Trumbo 1994). However, Satou et al. 2000 found that the proportion of reproductively successful *N. quadrupunctatus* pairs was not significantly different for pairs exposed to flies and pairs without flies: around 75%. The ability of adult beetles to mitigate competition with flies

seems variable, with *N. defodiens* being less efficacious than *N. vespilloides* and *N. quadrupunctatus.* It is important to note that Suzuki (2000) and Satou et al. (2000) removed mites from their beetles prior to experimentation, and Trumbo (1994) did not. It is possible that the *N. defodiens* beetles were being negatively impacted by their mites rather than by competition with flies. Behavioral differences among beetles, mites, and flies make this a more complex evolutionary system to study than previously thought. The system is still appealing, however, because the carcass is the only available resource for competitors to use, and it is relatively easy to measure in laboratory settings. There are few competitive species interactions available for study where each resource available to each species is measurable.

In an evolutionary sense, understanding burying beetles and their mites is useful because the mites' host preferences for specific beetle species can be identified, making it possible to retrace when and why their feeding strategies evolved. It is unclear if *Poecilochirus* mites**,** as a genus**,** engage in species-specific behavior or behavioral plasticity with regard to their diet. Understanding why behavioral plasticity would evolve in one mite species and not another would contribute to our understanding the evolution of resource selection.

In this study, I hypothesized that mites could display behavioral plasticity in response to environmental changes. Behavioral plasticity would be a more adaptive strategy for the mites because good hosts are a potentially limited resource, and the brood chamber can be a variable environment. The mites may not always reproduce on

their preferred host, making a flexible behavioral strategy more advantageous. I speculated that mites in the *P. carabi* and *P*. *subterraneus* complexes found on *N. guttula* and *N. investigator* would change their feeding behavior in the presence of fly eggs. When beetle eggs are the only available food, mites should feed on them, but switch to fly eggs when they are available. Both *N. investigator* and *N. guttula* lay their eggs in the soil surrounding the brood chamber, so the mites would have to expend energy to hunt them. If the mites are eating beetle eggs, beetles with mites should raise fewer larvae than beetles with no mites. If the mites are eating fly eggs, beetles with mites that are exposed to flies should potentially raise more larvae than beetles exposed to flies with no mites, because they will not have to compete as intensely with maggots. To evaluate these hypotheses I conducted a behavioral breeding experiment and collected tissue samples for stable isotope analysis of Carbon and Nitrogen. Stable isotope analysis can provide more detailed data , regarding the tissues mites are feeding on, than relying on behavioral observations alone.

When an animal ingests food, heavier isotopes of carbon and nitrogen fractionate into its tissues creating a stepwise increase in $\delta^{15}N$ (Δ 2-4 ‰), and $\delta^{13}C$ (Δ 0-1‰), depending on the food source and the tissue being tested (DeNiro and Epstein 1981; DeNiro and Epstein 1978). Stable isotope mass spectrometry measures these isotopes in animal tissues, enabling the reconstruction of an organism's diet based on the different isotope ratios found in those tissues. This technique has been used, successfully, to analyze burying beetle diets, but not the diet of their phoretic mites (Hocking et. al. 2009). My study measured $\delta^{13}C$ ‰ and $\delta^{15}N$ ‰ ratios of whole

Poecilochirus mites present on breeding *N. guttula* beetles, and the tissues they might be eating, to determine the components of their diets. I hypothesized that if mites were eating beetle or fly eggs, in my study, their isotopic signatures would reflect stepwise enrichment of δ^{15}N‰ and δ^{13}C‰ compared to the isotopic signature of the eggs

Chapter II: Methods

Study Site, Captive Colony, and Identification

Burying beetles and mites were collected using modified pitfall traps (Merrick and Smith 2004). The traps consisted of coffee cans fitted with mesh lids mounted to trees, and were baited with raw chicken legs. A small hole, approximately 1 cm^2, was cut into each lid allowing beetles to enter, but made escape difficult. Moistened soil was placed in each can so trapped beetles could seek humidity and cover during warm daytime temperatures.

The methods for breeding *N. investigator* and *N. guttula* vary slightly because of the change in geographic location, and a desire to exert greater control over fly oviposition in the later *N. guttua* experiment.

N. investigator (Colorado Experiment)

Field Site

Trap sites were located within 1 km^2 of the Rocky Mountain Biological Laboratory field station, Gothic, CO, USA, and were checked daily. Gothic, Colorado is located at an elevation of 2891 meters and the local habitat is characterized by spruce-fir forest, aspen groves, and sub-alpine meadows.

Breeding Experiment

Beetles were collected from several pitfall transects (10 cans each, approximately 20 m apart) spread around the field station that were check and maintained daily.

Beetles were maintained in the lab at room temperature in clear plastic containers that snapped closed to prevent escape. They were fed a diet of raw chicken. For breeding experiments, forty large coffee cans were partially buried in soil in raised boxes in an outdoor area, protected from scavengers by a screened box. A shade cloth was used to protect broods from hot temperatures during the day, and they were watered as needed to keep moist. Mice were weighed and placed in each can. Beetles were sexed by visually inspecting the genitals, measured, and placed in pairs. In the "No Mite" conditions mites were removed with a stiff paintbrush prior to placing beetles in their cans. Cans were covered with lids with small holes that allowed fly entry and lids with no holes. The carcasses were observed daily for the presence of maggots, and burial date. Two weeks after burial larvae were dug up, counted and weighed.

***N. guttula* (Idaho Experiment)**

Field Site

Trap sites were placed on Scout Mountain, near Inkom, Idaho, USA. The habitat on Scout Mountain is characterized by sub-alpine forest and sagebrush steppe habitats. Trap sites were placed at three locations: a low elevation site (42.71̊ N 112.37˚W Elevation: 1,769 meters (m), a middle elevation site (42.70̊ N 112.37˚W Elevation: 1,793 m), and a high elevation site (42.69̊ N 112.37˚W Elevation: 1,958 m). Beetles were separated by sex, and species (*N. guttula, N. defodiens, N. investigator*) , and maintained in the lab at room temperature. They were kept in plastic bins, fed raw chicken, and watered using moist sponges and paper towels, and cleaned 2-3 times per week. Mite

samples were collected from each species of beetle and sent to Hans Klompen, PhD Ohio State University, Acarology Laboratory for identification.

Breeding Experiment

Captive breeding consisted of placing a single pair of adult beetles on a mouse carcass (18-26g) within a soil-filled coffee can. Beetles were sexed by visually inspecting the genitals. Mites were manually removed with a stiff bristled paint brush from all beetles. Mites were counted, and placed back on parent beetles in mite treatments while they were waiting in a holding cup to be placed on a carcass. Six to ten mites were added per pair of breeding beetles. A raised box filled with soil and covered with a shade structure housed the breeding containers. Each container consisted of a coffee can filled approximately 3/4 with soil. Soil was a mix of 50% potting soil, and 50% local soil. Petroleum jelly was spread along the rim of each can to discourage mites from climbing out of their cans. Carcasses were thawed, weighed, and exposed to flies for 24-48 hours prior to the beginning of the experiment. Fly eggs or maggots were collected and frozen for stable isotope analysis. The mouse, beetle pair, and mites were combined in plastic cups and transported to the prepared cans. Beetles, mice, and mites were added to each can, and a specific mesh lid was secured to the can with a rubber band. Lids were left intact in the no-fly treatment, and a small hole placed in the middle of the lid in the fly exposed conditions. Each can was randomly assigned a treatment: Flies/Mites, No Flies/Mites, Flies/ No Mites, and No Flies/No Mites. Each day cans were checked to ensure that environmental conditions were appropriate for the beetles. Date of carcass burial, presence of maggots, and presence of adult flies was

recorded during daily checks. After beetles were done breeding (approximately 3 weeks) cans were brought into the lab. Each can was emptied, depth of carcasses burial recorded, and larvae counted and weighed. Larvae were analyzed as a percent of mouse weight because mice varied in size (18g-28g).

Stable Isotope Preparation and Analysis

Tissue samples for stable isotope analysis were collected from the mouse and fly eggs prior to adding the breeding pairs, placed in vials, and frozen. Beetles, mites, and larvae samples were collected at the end of the breeding cycle, placed into scintillation vials, and frozen. Frozen isotope samples were dehydrated in a drying oven for 76 hours at 63˚C. Dried samples were placed into scintillation vials to keep from re-hydrating. Adult beetle carcasses, mouse carcass, and beetle larvae were each mechanically homogenized using a Wig-L-Bug bead homogenizer (Crescent Dental Mfg., Lyons, IL, USA) prior to analysis. Mite and fly egg samples were small enough that entire samples were analyzed and thus, did not require homogenization prior to analysis. The ~ 0.50 µg samples were weighed on an analytical balance and placed in tins. Tins were folded and placed into a loading tray. Each tissue type was analyzed for $\delta^{15}N$ and $\delta^{13}C$ content using a ThermoElectron Corporation Delta Advantage stable isotope ratio mass spectrometer at the Center for Archaeology, Materials, and Applied Spectrometry (CAMAS), Idaho State University. All values are reported relative to the Vienna Pee Dee Belemnite (VPBD) (carbon) and atmospheric air (nitrogen) standards using the following equation (Kendall and Caldwell 1998):

$\delta(‰) = 10^3[R_{sample} / R_{standard} - 1]$

The precision of the $\delta^{15}N$ and $\delta^{13}C$ analyses, based on repeated analyses of several in-house standards, are better than 0.3‰ and 0.2‰, respectively.

Preliminary mite samples were homogenized, and lipid extracted using chloro-methanol exposure (Post et al. 2007). Samples were saturated in a chloro-methanol solution for 24 hours, and filtered using a Buchner funnel. The remaining organic materials were analyzed for carbon shifts, and displayed very little change. Thus, I presumed that lipids did not drastically affect the carbon signature of mite tissues.

Data were analyzed using R statistical software and managed using Microsoft Excel. The mite data were transformed using standard literature values ($\delta^{15}N$= +3.4‰ and $\delta^{13}C$= +1‰) to account for fractionation (Post 2002), and analyzed in terms of their potential food sources using a concentration-weighted stable isotope mixing model (Phillips and Koch 2002). A two sample t-test assuming unequal variance was conducted on $\delta^{15}N$ and $\delta^{13}C$ values for mites exposed to fly eggs, and mites exposed to beetle eggs only. Mite diet was assessed against potential food sources by comparing their isotope signatures; specifically assuming a stepwise increase in $\delta^{13}C$ (Δ 0-1 ‰) and $\delta^{15}N$ (Δ 2-4 ‰).

Isotope samples were collected for N. guttula only due to availability of tissues, and better opportunity for systematic collection of tissues.

Chapter III: Results

Mite and Fly Identification

Mites were collected from beetle specimens immediately after collection from the field and were placed in vials of 90% ethanol and labeled with location collected, beetle species, and beetle sex. Mites were subjectively sorted by size into "little" and "big" categories with a dissecting microscope. The mites were sent to Dr. Hans Klompen, Associate Professor of Acarology at Ohio State University, for identification. Dr. Klompen identified all mites within the genus *Poecilochirus* to be in the *P. carabi* or *P. subterraneus* morphological complexes. In addition to *Poecilochirus*, mites in the *Uropodina* and *Histiostomatida* genera were also present on the beetles. *P. carabi* was the most abundant species making up 59% of the combined sample. *P. subterraneus* made up 22% of the sample, and mites from other genera made up 18% of the sample. The Steinhaus-Similarity Coefficient (Bray and Curtis 1957) was calculated with relation to host selection for *P. carabi* and *P. subterraneus* (S=.54).

Flies were incidentally collected from Idaho breeding experiments with *N. guttula* beetles and identified as being members of the families *Lucillidea* (Bottle Flies) and *Calliphoridea* (House Flies).

Breeding Experiments

Reproductive Success- N. investigator

N. investigator experienced equal proportions of reproductive success with flies (80%) compared to controls (80%) according to a binomial probability test (χ^2df = 1,

p>=.678). Beetles with mites, however, experienced a significant decrease in the proportion (40%) of successful broods (χ^2df = 1, *p*<0.006).

A logistic generalized linear model (GLM) revealed no significant interaction among the four treatment groups (χ^2df = 1, residual deviance= 47.289, p=.915). A Wilcoxon exact rank sum test revealed that mites had a significant negative effect on the number of offspring produced (*Z*=1.9054, *p*=.029). An identical test was conducted on fly presence and mean larval size, but showed insignificant results (Z=-0.6364, *p*=.7406).

Reproductive Success- N. guttula

N. guttula experienced unequal proportions of reproductive success with flies (42% produced offspring) compared to controls (88% produced offspring) when compared using a binomial test (χ^2 df = 1, *p*<0.001). Beetles with mites also experienced a significant decrease in the proportion of successful broods (36%) compared to controls (p<0.001).

A logistic GLM revealed a significant interaction between flies and mites (χ^2 df=1, residual deviance= 40.870, *p*= 0.002). With the treatment combining mites and flies having a higher probability of successful broods (log odds = 4.536) than either mites (log odds =-3.466) and fly (log odds = -2.927) treatments alone (Figure 1). Pair wise comparisons with Holmes corrected significance values revealed that the probability of a successful brood was significantly worse for broods exposed to mites (table 2).

A Wilcoxon exact rank sum test revealed that mites had a marginally significant effect on number of offspring produced (Z=1.5318, p=.065). A Wilcoxon exact rank sum test was conducted on fly presence and mean larval size, but showed insignificant results (Z=0.4737, p=0.3229)

Stable Isotope Signatures

Poecilochirus spp. samples showed an increase in δ^{13}C VDPB ‰ and δ^{15}N air ‰ relative to their beetle hosts, but their δ^{15}N values were too high to be accounted for by our sampled tissues alone without inclusion of the prepared carcass, assuming that δ^{15}N fractionation from diet to consumer is <4 ‰ (Figure 2). The prepared carcass samples display an increase in δ^{15}N ‰ and a decrease in δ^{13}C ‰ compared to fresh mouse samples. A two-sample t-test assuming unequal variance demonstrated that mites exposed to fly eggs (M=15.11, SE=1.14) had marginally significantly higher δ^{15}N than mites not exposed to flies [M=12.62, SE=.58, t(18)=2.10,p=0.067)]. δ^{13}C did not differ significantly between the groups of mites. Plots displaying the diet sources for fly exposed and fly protected mites show many possible combinations of tissues that could contribute to mite diet (Figure 3 and Figure 4). A concentration weighted stable isotope-mixing model was utilized to confirm those combinations (Phillips and Koch 2002). The model took into account three potential diet sources at a time and plotted them on a concentration dependent mixing triangle. Mathematical solutions were found that could explain the diet for each group, fly exposed (figure 3) and fly protected (figure 4), assuming the correction for trophic level fractionation of diet sources is reasonable.

Chapter IV: Discussion

Poecilochirus spp. had a negative effect on the probability of reproductive success for both *N. guttula* and *N. investigator,* confirming the hypothesis that mites in the *P. carabi* morphological complex are parasitic (Beninger 1993). The probability of *N. guttula* reproductive success increased when both mites and flies were present on the carcass, but decreased in treatments where broods were exposed to mites or flies only. *N. investigator* displayed the same trend with regard to mites but did not experience a decrease in reproductive success when flies were present, nor a statistically significant increase when both flies and mites were present during the treatment. These results support my hypothesis that mites can opportunistically switch from eating beetle eggs to fly eggs, thereby mediating the negative reproductive consequences to their hosts.

Stable isotope analysis demonstrated that mites exposed to flies had marginally higher δ^{15}N ‰ values, indicating differences in their diets compared to fly protected mites, (e.g. prey switching). The hypothesized diet sources in the fly exposed and fly protected mite groups were sufficient to explain the mites' isotopic signatures, with some subtle differences. Both groups of mites had the potential to feed on both fresh and beetle prepared mouse tissues. Fly exposed mites, however, could only utilize the fresh carcass in two specific mixing model scenarios (fresh carcass, prepared carcass, and adult beetles or fly eggs), making it seem unlikely that the fresh carcass made a large contribution to their diet (Figure 3). The prepared carcass, however, was pivotal to finding mixing model solutions that included any of the other potential diet sources

for the fly exposed mites. Conversely, mixing model solutions for fly protected mite diets relied heavily on the fresh mouse being included in the model (Figure 4). The fresh mouse marginally allowed for a mixing model solution, meaning that any error in the assumed trophic level fractionation for δ^{15}N‰ could lead to different model interpretations with regard to mite or mouse tissues. Therefore, a conservative conclusion would be that mites in the fly protected conditions are eating some tissue similar to the fresh carcass, but that the fresh carcass contributed in a minor way to their diet. A re-analysis using a multi-source mixing model could provide quantitative data of how important each diet source is for explaining mite isotopic signatures in each experimental group.

In past research, mites were observed eating nematodes and other mites (Wise et al. 1988, Gleden et al. 1988). Mites in the genus *Phytoseiida,* another kind of mesostigmatid mite, are cannibals, so it is possible that the mites in this study *Poecilochirus* are also capable of cannibalism. *Phytoseiida*'s level of cannibalism changes with species and life stage, and availability. They prefer to feed on heterospecific tissues rather than on their own kind, if possible (Schausberger 2003). Mites without access to flies are likely altering their predatory behavior to include a source or sources that were not sampled in the study, like conpsecifics, heterospecific mites, or nematodes. If *P. subterraneus,* the less abundant mite, is a mutualistic species, primarily eating fly eggs, they presumably change their diet more drastically when no flies are present compared to a parasitic species. If *P. carabi*, the most abundant mite and most likely to be parasitic, were capable of parasitizing *P. subterraneus*, then their

isotopic signatures would also change in the fly excluded conditions due to reduced food availability. In this study, it was impossible to determine if *P. subterraneus* and *P. carabi* had different prey sources because isotopic samples were combined, possibly confusing their relationship to one another.

While isotopic data and behavioral data in combination support an interaction effect of mites and flies on the carcass, previous behavioral studies have not always had the same results. One possible reason that Blackman (1996) did not observe an interaction between mites and flies could be that he used *N. vespilloides* in his experiments. According to Suzuki (2000) *N. vespilloides* not only buries the carcass completely, but also actively protects the carcass from fly infestation; presumably by eating fly eggs and larvae. In Blackman (1996), the presence of egg deposition by flies on the carcass was not confirmed. *N. guttula* was the only species in my experiments where the interaction of mites of and flies had a significant effect on brood success relative to fly only and mite only treatments, but fly egg deposition was confirmed visually prior to introduction of parent beetles to the carcass. In Colorado (*N. investigator*), fly presence was assumed because fly treatments had lids that would allow flies access to the carcass. The *N. investigator* beetles were added to the breeding can at the same time the carcasses were. *N. investigator* had more time to bury and prepare their carcasses, potentially avoiding competition with flies. In Idaho (*N. guttula*), I confirmed the deposition of fly eggs before the beetles were added to the carcass. Blackman (1996) used an experimental design similar to my *N. investigator* experiments with regard to fly presence, and got similar results. So, it is uncertain that *N. guttula* was worse at

mitigating competition with flies, or if their reproductive failure in the fly treatment was an artifact of the experimental design. Future experiments should control for the extent of fly egg deposition. Fly species should also be controlled for, if possible, as the effect of fly species on beetle/fly competitive interactions is not known.

If beetles were generally efficacious at reducing interspecies competition with flies on their own, why would mites display plasticity in predatory behavior, or bother to behave as mutualists? Evolution of behavioral plasticity with regard to predation is dependent on limited food resources, so there were presumably limited food resources exerting natural selective pressure on *Poecilochirus* during its evolutionary history. Therefore, mutualism between *Poecilochirus* and their beetles may be an adaptation to interspecies competition for food, rather than a direct mutualism that relies on a feedback loop of benefits between beetles and mites.

Mites would not get the opportunity to evolve mutualistically with their hosts if they primarily associated with beetles that were already removing all fly eggs and maggots from the carcass. The mites identified in our study belonged primarily to the *P. carabi* morphological *complex,* and there is extensive variation within the complex (Schwatrz and Walzl 1996). The mites found in Idaho and Colorado might be behaviorally distinct from the *P. carabi* mites used in other studies, as they have evolved with their hosts in alpine forest and high desert conditions. As stated previously, mites in the *P. subterraneus* morphological complex were also present on all the beetles. Little is known about the behavior of *P. subterraneus* and their ecological or

evolutionary relationships with their hosts, or even with other mite species. *P. subterraneus* and *P. carabi* mites in my study had a Steinaus-Similarly Coefficient of 0.54, indicating only partial niche overlap in relation to host selection, suggesting some host preference differences. *P. carabi* were the most abundant mite in the study, while *P. subterraneus* were less common and found mostly on wild caught *N. defodiens*. There mere fact that two distinct species of *Peocilochirus* were present on the beetles and that they had host preferences means that there are likely some life history differences between the two species, indicating divergent evolutionary trajectories. Mites have the ability to switch hosts easily, and alleles from one population could spread, or be isolated, plausibly within a few breeding seasons creating the necessary conditions for co-evolutionary hotspots (Thompson 2010). Currently, the geographic mosaic of mite populations is unknown, therefore it is impossible to determine the rate of immigration and migration from one population to another, or predict allele frequencies (Thompson 2010, Thompson 2001). Presumably, mites mirror their beetle hosts geographically with regard to gene flow, so the burying beetles themselves could be used to model mite gene flow, and changes in allelic frequency. This system has the potential to evolve quickly and differently based on geographic location, making behavioral differences hard to quantify in wild populations where the species of mite is not truly known, but lumped into a morphological complex.

Within the literature on burying beetles, little attention is paid to the type of *Poecilochirus* spp mites present in each study, probably because of the previously mentioned identification issues. This is a problem because evidence suggests that

different morphological complexes display different predatory behaviors, and as a result, they have different impacts on beetle reproductive success. Trumbo (1994) measured the likelihood of *N. defodiens'* reproductive success when competing with flies. Before breeding, mites were added to each brood, and reproductive success measured. Without knowing exactly which species of mites were present in his study, there is no way to guarantee his brood failures were due to fly infestation or parasitic mites. Anecdotally, the mites are very good at escaping their containers and special care must be taken to make sure they do not enter beetle containers that they were intended to be excluded from. In addition, isotopic data suggests that mites, in the absence of flies, are feeding on an unknown invertebrate, or invertebrates of a trophic level similar to the fresh carcass. To understand the subtleties of the role of *Poecilochirus* in this system, further research into the multiple components of the diet is required.

Conclusions

Stable isotope analysis provided a new way to identify potential food sources for the mites. Previous observational studies suggested that *P. carabi* fed on fly eggs when associated with *Nicrophorus* spp. beetles, and both behavioral and isotopic data supported that hypothesis (Wise et al. 1988; Geden et al. 1988, Geden et al. 1989). The stable isotope data suggests differences in mite diet depending on the presence of flies. Given my assumptions of tissue and trophic level fractionation, the stable isotope data suggests multiple vs. dominant food sources for the mites. So long as sufficiently sized

samples can be collected, stable isotopes should continue to be an effective tool for piecing apart complex species interactions.

Nicrophorus researchers should not ignore the presence of mites on their research subjects, especially if the mites are in the *P. carabi* morphological complex. The appropriate experimental design needs to be considered for any field or laboratory experiments (i.e. keep or remove mites). In addition, mites should not be lumped together as if they were a single taxa and labeled as mutualistic or parasitic without supporting evidence.

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biology of its carrier *Necrophorus vespilloides* (Coleoptera, Silphidae). *Oecologia.* 89:487-493.

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Figures and Tables

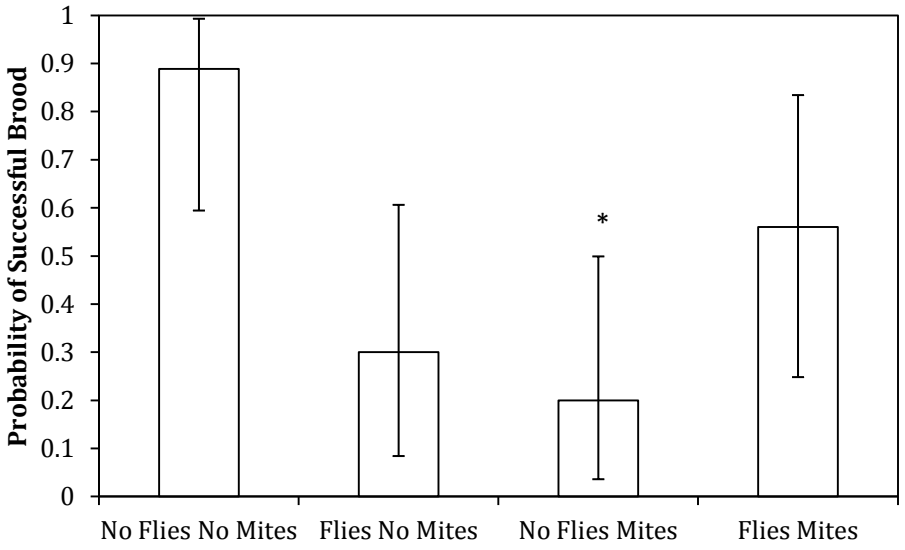

Figure 1: Probability of brood success for *N. guttula* in each treatment. Bars represent the upper and lower ends of 95% likelihood ratio confidence intervals for the true probability. * denotes that the treatment was significantly different from the control group after Holmes correction.

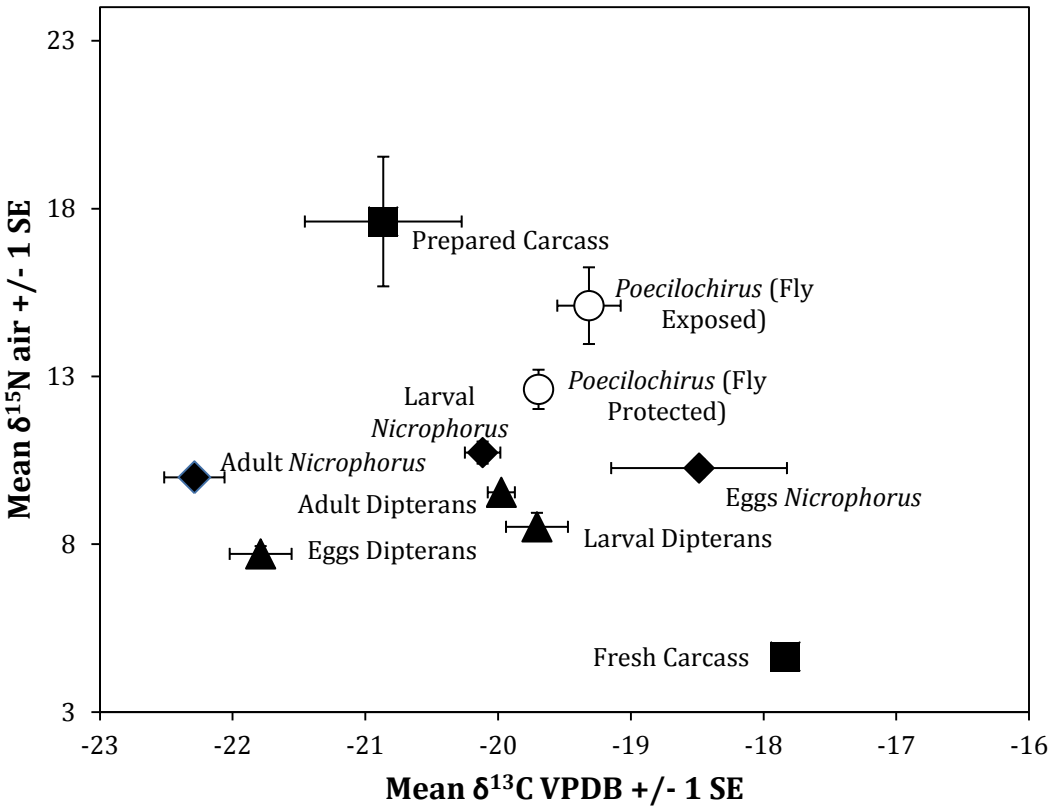

Figure 2: Mean isotopic signatures +/- 1 SE of tissues collected during the *N. guttula* behavioral experiment.

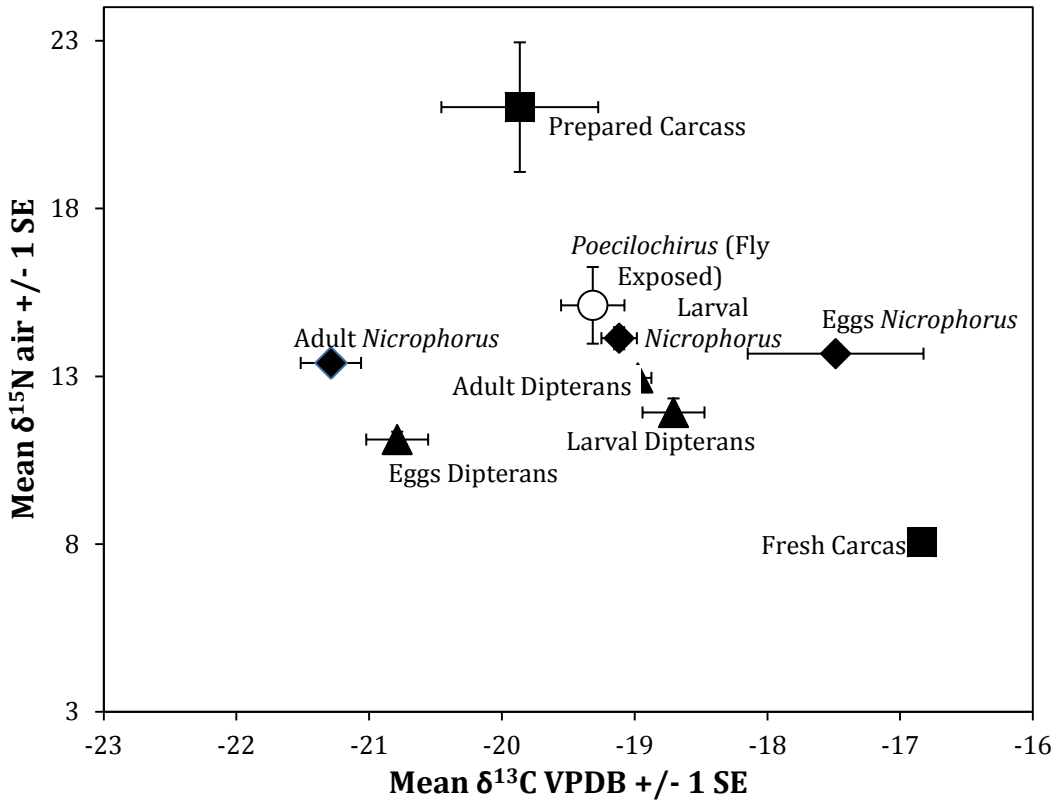

Figure 3: Mean isotopic signatures +/- 1 SE, corrected for fractionation, of mites exposed to flies and all possible diet sources.

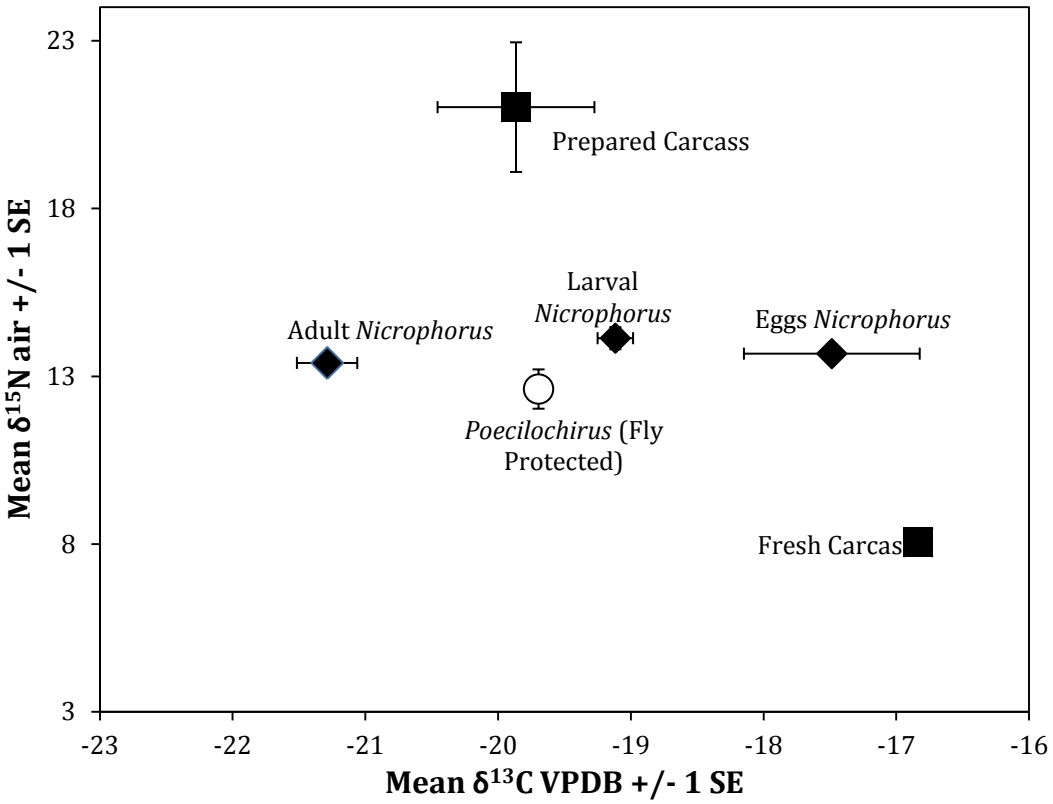

Figure 4: Mean isotopic signatures +/- 1 SE, corrected for fractionation, of mites protected from flies and all possible diet sources.

Table 1: Comparison of studies on *Poecilochirus* spp. categorized by their results. Studies with results supporting parasitism are listed on the left and studies supporting mutualisms, or commensality are on the right.

Parasitic	**Mutualistic/Commensal**
Blackman 1997 (*P. davydovae*)	Sloan Wilson & Knollenberg 1987 (*P. necrophori*)
Blackman & Evans 1994 (*P. davydovae*)	Sloan Wilson 1983 (*P. necrophori*)
Beninger 1993 (*P. carabi*)	Springett 1968 (*P. necrophori*)

Table 2: Results of pair-wise comparisons using a general linear model with residual deviations. * indicates a significant difference between treatments. The symbol π indicates the binomial probability of a successful brood.

	Pair-wise Comparisons						
H_O	Flies	Mites	Flies	Mites	P-value	Residual Deviation	X^2 df
$\pi_{NF:NM}=\pi_{NF:M}$	No	No	No	Yes	0.0528*	18.496	1
$\pi_{NF:NM}=\pi_{F:NM}$	No	No	Yes	No	0.1035	16.287	1
$\pi_{NF:NM}=\pi_{F:M}$	No	No	Yes	Yes	0.48	18.644	1
$\pi_{F:M}=\pi_{NF:M}$	Yes	Yes	No	Yes	0.48	22.373	1
$\pi_{F:M}=\pi_{F:NM}$	Yes	Yes	Yes	No	0.48	24.583	1
$\pi_{F:NM}=\pi_{NF:M}$	Yes	No	No	Yes	0.608	22.225	1

Appendix

Table 1: Stable isotope results, sample sizes, and mean % values for all tissues sampled.

		Mean Values					
Tissue	n	d15N air	d13C VPDB	SE(d15N air)	SE(d13C VPDB)	%N	%C
Adult Nicrophorus	43	9.99	-22.29	0.23	0.23	8.97	51.19
Larval Nicrophorus	14	10.73	-20.12	0.33	0.13	7.07	56.32
Nicrophorus Eggs	3	10.27	-18.49	0.27	0.66	10.39	46.73
Dipterans	4	9.55	-19.98	0.08	0.10	11.89	48.40
Larval Dipterans	5	8.52	-19.71	0.42	0.23	9.15	45.11
Dipteran Eggs	16	7.71	-21.79	0.23	0.36	11.76	49.13
Poecilochirus(Exposed to Flies)	13	15.11	-19.32	1.14	0.24	10.60	47.26
Poecilochirus(Protected from Flies)	12	12.62	-19.70	0.59	0.09	8.79	52.81
Fresh mus muscus	17	4.65	-17.84	0.14	0.02	12.20	47.26
Carcass	5	17.62	-20.87	1.93	0.59	3.88	22.41