Ectoparasitic mites and their Drosophila hosts

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ABSTRACT

Only two parasite interactions are known for Drosophila to date: Allantonematid nematodes associated with mycophagous Drosophilids and the ectoparasitic mite Macrocheles subbadius with the Sonoran Desert endemic Drosophila nigrospiracula. Unlike the nematode-Drosophila association, breadth of mite parasitism on Drosophila species is unknown. As M. subbadius is a generalist, parasitism of additional Drosophilids is expected. We determined the extent and distribution of mite parasitism in nature Drosophilids collected in Mexico and southern California. Thirteen additional species of Drosophilids were infested. Interestingly, 10 belong to the repleta species group of the subgenus Drosophila, despite the fact that the majority of flies collected were of the subgenus Sophophora. In all cases but 2, the associated mites were M. subbadius. Drosophila hexastigma was found to have not only M. subbadius, but another Mesostigmata mite, Paragarmania bakeri, as well. One D. hydei was also found to have a mite from genus Lasioseius attached. In both choice and no-choice experiments, mites were more attracted to repleta group species than to Sophophoran. The extent of mite parasitism clearly is much broader than previously reported and suggests a host bias mediated either by mite preference and/or some mechanism of resistance in particular Drosophilid lineages.

Parasites are ubiquitous and often create a strong force on potential hosts to evolve resistance mechanisms. Generalist parasites, as they use a range of hosts, will have broad host ranges, while specialist will be restricted to a single host or group of hosts. The determinants of host range will reside in the parasite as well as in the host. Dissecting the basis of host range is best accomplished in a group of organisms with defined ecological and genetic properties.

Drosophila species have great potential to disentangle the contributions of both host and parasite to patterns of infestation and resistance. So far, however, the only horizontally transmitted Drosophila macroparasites whose host ranges have been studied in an ecological and evolutionary context are nematodes of the family Allantonematidae associated with mycophagous flies. Of the 10 nematode species (8 species of Howardula and 2 of Parasitylenchus) parasitizing mycophagous Drosophila, the majority are generalists.

Less well studied are ectoparasitic mites. Several Drosophila species routinely encounter mites that are ectoparasites of adults and/or predators of eggs and larvae. The first association between an ectoparasitic mite and a Drosophilid was described in detail by Polak & Markow for the mite Macrocheles subbadius and the Sonoran Desert endemic cactophilic D. nigrospiracula. Drosophila nigrospiracula utilizes necrotic saguaro (Carnegia gigantea) or cardón (Pachycereus pringlei) cactus as its feeding and breeding site. The flies are attracted to the necrotic cactus tissue because of the volatiles released during decomposition of the plant tissue. Adult flies find mates and oviposition sites at the cactus necroses where they feed. Larvae undergo their development in the decaying tissue. Once they begin to decompose, large columnar cacti such as saguaro and cardón serve as breeding sites for multiple arthropod species, including mites. Mites arrive, transported by dispersing flies, to new cactus necroses, where they detach and reproduce along with the other arthropods. When a necrosis finally dries out, mites attach to departing flies to colonize new cactus rots. A mite also was reported on a D. mettleri, which shares the same cactus habitat with D.
nigrospiracula,\textsuperscript{5} and while we assume it was \textit{M. subbadius}, it was not keyed to species.

Effectively nothing is known about the host range of \textit{M. subbadius} with respect to other Drosophilids. Infestation has not been documented for other Sonoran Desert cactophilic \textit{Drosophila} species, such as \textit{D. mojavensis} and \textit{D. pachea}. One explanation is that these other species have not been the target of studies measuring mite infestation in nature. Another could be the particular host resource of the flies. These other \textit{2 Drosophila} species utilize different columnar host cacti, senita (\textit{Lophocereus schottii}) and organ pipe (\textit{Stenocereus thurberi}) respectively, both of which are smaller and chemically different from saguaro or cardón.\textsuperscript{8,10} Larger cacti produce necroses that last much longer than those of smaller cacti, providing a longer period for development of new generations of mites. Senita and organ pipe, for example, are smaller than saguaro and cardón and the necroses do not last as long.\textsuperscript{8} But mites have been found in the necrotic tissues of both of these other cactus species,\textsuperscript{4} arguing against the effect of host resource on the apparent differential distribution of mites on \textit{Drosophila} species. Furthermore, \textit{M. subbadius} is a widespread generalist, associated with other Diptera such as houseflies (\textit{Musca domestica}) and stable flies (\textit{Stomoxys calcitrans}) on other continents\textsuperscript{11} where its immature stages are thought to develop in dung. The generalist ecology of \textit{M. subbadius} thus predicts that multiple additional \textit{Drosophila} species, using substrates other than cacti, would be parasitized.

Originally thought to be strictly phoretic, infestations by \textit{Macrocheles subbadius} were shown to exert profound effects on the fitness of both male and female adult \textit{D. nigrospiracula}.\textsuperscript{5,12} In addition to interfering with mating,\textsuperscript{5} mites extract hemolymph from the flies, severely impacting the reproductive output of infested males and females.\textsuperscript{12} \textit{Macrocheles subbadius} belongs to the Mesostigmata, an order of approximately 8,000 species of mites, many of which are parasitic on other insects.\textsuperscript{13} Given the large number of Mesostigmatid mite species, it would be surprising if parasitism were limited to \textit{M. subbadius} - \textit{D. nigrospiracula} association of the Sonoran Desert. Given the fitness consequences of mite infestation for the flies, understanding the extent and distribution of parasitism among Drosophilids should provide insights into the evolution of host-parasite interactions in general.

We screened flies from a range of habitats and geographic localities in southwestern North America to ask (1) if species of \textit{Drosophila} other than \textit{D. nigrospiracula} are infested with mites; (2) if mite species in addition to \textit{M. subbadius} parasitize \textit{Drosophila}. Indeed, we found many other \textit{Drosophila} species to be infested with mites, but the distribution of parasitism was not random with respect to species. Parasitized flies were mainly species of the repleta group in the subgenus \textit{Drosophila}. We therefore (3) performed additional tests to determine if mite “preference” for particular \textit{Drosophila} taxa could explain the differential distribution of parasitism.

\textbf{Materials and methods}

\textbf{Field collections of Drosophila}

Flies were collected either directly from rotting fruit or from banana baits over a period of several years. Earlier collections were taken from the Anza Borrego desert in southern California (April 2009), La Paz in Baja California Sur, Mexico (January 2012) and Alamos, Sonora, Mexico (February 2010). Collections from central Mexico were from areas either of high cactus density or commercial fruit density. We placed from one to 5 baits in each location. The localities from which the collections were made are shown in Figure 1.

Flies from the earliest collections in Baja California Sur, Anza Borrego, and Alamos were examined under a dissecting scope and those flies with mites were saved and preserved for identification, while the numbers and species of infested vs. uninfested were not tabulated. Because in these earlier collections infested flies appeared to belong primarily of the \textit{repleta} species group of the subgenus \textit{Drosophila}, we subsequently initiated a more quantitative method of addressing bias in infestation. In 2014 we collected in central Mexico, from September through December and in 2015, from April through October. We aspirated all flies from fruits or baits and took them directly to the laboratory to separate them by taxon, sex and whether they had a mite or mites attached. We also recorded the location on the fly at which a mite was attached and the number of mites on each fly.

\textbf{Identification of Drosophilid species}

Flies were initially sorted to subgenus or species group, or in the case of \textit{Zaprionus} indianus, or
genus. There are 2 major subgenera of Drosophila, Sophophora and Drosophila. Within the subgenus Sophophora, the majority of the flies we found either were D. melanogaster or its sister species D. simulans and because females of these 2 are not always reliably distinguished, we grouped the 2 species into a category designated MEL-SIM. In the subgenus Drosophila, the majority of the flies we captured were members of the repleta species group. Because repleta group species are morphologically difficult to distinguish, collected flies were grouped into a category called “repleta.” Only those flies with mites were identified to species, using the 658 bp barcode region of mitochondrial Cytochrome Oxidase 1, mtCO1.

Identification of mites

Mites were identified using morphological and molecular methods. Dr. Gerald Krantz was instrumental in the morphological identification of the mites, which we then verified with the 18s rDNA gene (see below).

PCR conditions and sequencing

Extractions of total genomic DNA from individual flies and mites were performed using the DNeasyTM (QIAGEN Inc., Valencia, CA) protocol. While fly homogenate was incubated for 20 min at 56°C, mite homogenate was incubated for 2 hr at the same temperature. We amplified a 658-bp Barcode fragment of mitochondrial Cytochrome Oxidase 1, mtCO1 using the primer pair LCO1490f (GGTCAACAAATCATAAGATATTGG) and HCO2198r (TAAACTTCAGGGATCATATAAGATATTGG) and HCO2198r (TAAACTTCAGGGT-GACCAAAAAAT), using 5.25 µL DNA as template in a 35 µL reaction. PCR were amplified starting with one cycle of 5 min at 95 °C followed by 35 cycles at the following cycling conditions: 30 s at 95 °C, 45 s at 52°C, 1 min at 72°C, with a final extensión step at 72 °C for 7 min.

For mite identification, a 530-bp fragment of the 18S rDNA was amplified using 5.25 µL DNA as template in a 35 µL reaction. PCR products were amplified starting with one cycle of 3 min at 95 °C followed by 35 cycles at the following cycling conditions: 30 s at
95°C, 30 s at 54°C, 90 s at 72°C, with a final extensión step at 72 °C for 30 min. We utilized forward primer Fw1230 5'- TGAACTTAAAGGAATTGACG-3' and a reverse ConsR18S 5'- ATTCAATCTGTTAGGCGAGG-3' developed in the course of the present study. Sequencing was performed on an ABI XL3730 at the Core Facility of LANGEBIO, CINVESTAV, Irapuato, Guanajuato, México and at GENEWIZ, Inc., South Plainfield, NJ. Sequences were deposited in GenBank under accession numbers KX588173- KX588215.

Assessing the role of mite preferences in fly parasitism

Given the bias in parasitism toward flies of the repleta group, we assessed the roles of mite preference in the fly species they attack. Once brought to the laboratory, mites were harvested from the flies they had been attached to. Under CO2 anesthesia, mites were detached and transferred to 5cm petri dishes with 0.5% agar. We found that mites could be cultured by feeding them nematodes or simply on banana and a few grains of yeast. Before use in choice and no choice tests, mites were maintained individually at 25°C for 3 d on agar before being presented with a choice (Fig. 2A) of an immobilized female D. hydei (repleta group, subgenus Drosophila) and an immobilized female D. simulans (melanogaster group, subgenus Sophophora). Ten day old flies were immobilized by placing their head and thorax in a glass tube so that the only part presented to a mite was the abdomen. We observed each mite for 15 min and recorded which fly they attached to. In the no-choice experiments, we presented mites with either an immobilized female D. hydei or an immobilized female D. simulans and observed them for one hour, recording the number of mites attaching to each species (Fig. 2B).

Results

Mites were found to parasitize 13 Drosophila species in addition to those previously documented (Table 1). Mite parasitism was highly biased toward flies of the subgenus Drosophila despite collection locality (Table 2). From baits located in both in cactus and commercial fruit areas of central Mexico, the majority of flies collected were of the Sophophora subgenus. Of the 39 flies with mites, however, 35 were species in the repleta group. Two D. simulans and one D. melanogaster (subgenus Sophophora) were found with a mite, as well as one other Drosophilid, Zaprionus indians. Baits from fruit areas contained a higher number of D. melanogaster and D. simulans and other non-repleta species, but none were parasitized. Only the repleta group species from fruit areas had mites. Regardless of the abundance of particular fly species or groups of species in any given collection, mite parasitism always was significantly greater on members of the repleta group. In those few cases where a D. melanogaster, D. simulans, D. busckii or Z. indians was infested, the fly had only one mite. Infested flies of the repleta group had mean mite numbers of 1.7 (cactus area) or 2.5 (fruit area) mites per fly (Table 2).

A number of flies had more than one mite, thus of the 35 infested flies in repleta group, there was total of 64 mites (Fig. 3). While the majority of flies had one mite, 4 flies had 2, 5 flies had 3, one fly had 4 and 2

Figure 2. Apparatus used in mite (A) choice tests and (B) no choice tests. Flies were held in the tips of pipettes to immobilize them.
flies had 7 mites (Fig. 4). The majority of mites were attached to the abdomen near the thorax or at the thorax-head junction (Table 3). Males and females were equally infested.

We found parasitism by 2 mite species in addition to M. subbadius. Paragarmania bakeri, another Mesostigmatid mite, was found on 3 flies of D. hexastigma. While P. bakeri also is a Mesostigmatid mite, it belongs to a different family Blattisociidae, than M. subbadius. Interestingly, one D. hexastigma had a mite of each species. Yet a third mite, Lasioseius sp., also from family Blattisociidae, was found attached to a D. hydei.

We then examined the role of mite preference in the strong bias in parasitism of repleta group species. Out of the 40 choice tests we performed, 19 mites attached to D. hydei while only 2 mites attached to D. simulans (X^2 = 13.76, p<0.01). In the remaining 19 trials, mites didn’t attach to either fly. In the 12 no-choice tests with each species, 4 mites attached to a D. simulans and 8 attached to a D. hydei, and although the difference was not statistically significant (X^2 = 1.33), probably owing to the small numbers, in fact twice as many attached to the D. hydei as to the D. simulans. As in the choice tests, some mites did not appear interested in either species of fly and didn’t attach. In both types of test, flies were immobilized and only the abdomen was in the chamber, precluding a role for active rejection by an attacked fly in the difference between fly species parasitized.

| Drosophila species | Mite species | Mite family | Resource |
|--------------------|--------------|-------------|----------|
| D. arizonae | M. subbadius | Macrophilidae | Cosmopolitan; Cacti |
| D. huichole | M. subbadius | Macrophilidae | Cacti |
| D. hexastigma | P. bakeri | Blattisociidae | Cacti |
| D. spenceri | M. subbadius | Macrophilidae | Cacti |
| D. rita | M. subbadius | Macrophilidae | Cacti |
| D. longicornis | M. subbadius | Macrophilidae | Cacti |
| D. mercatorum | M. subbadius | Macrophilidae | Cosmopolitan, Cacti |
| D. nigrospiracula | M. subbadius | Macrophilidae | Cacti |
| D. hydei | Lasioseius sp | Blattisociidae | Cosmopolitan, Cacti |
| D. eremophila | M. subbadius | Macrophilidae | Soaked soil from columnar cacti |
| D. mettleri | Not identified | Not identified | Soaked soil from columnar cacti |
| D. busckii | M. subbadius | Macrophilidae | Cosmopolitan |
| D. melanogaster | M. subbadius | Macrophilidae | Cosmopolitan |
| D. simulans | M. subbadius | Macrophilidae | Cosmopolitan |
| Z. indianus | M. subbadius | Macrophilidae | Fruit |

Note. 1Present study  
2Polak and Markow, 1995  
3Polak, 1996  
*Mite lost prior to identification  
Cosmopolitan refers to the guild of Drosophila associated with decaying fruits and vegetables in human habitats (Nunney 1996; Markow2015).

### Table 1. Total species of Drosophila found to be parasitized, along with their common resources and species of mite found. Species shaded in gray are those not previously reported to carry mites.

### Table 2. Distribution of mite parasitism among Drosophila species collected at either cactus or domestic fruit areas.

| HABITAT | Genus | Subgenus | Species | No flies | W/mites | % infestation |
|---------|-------|----------|---------|----------|---------|--------------|
| CACTUS  | Drosophila | Sophophora | D. melanogaster | 2030 | 2 | 0.10 |
|         | D. busckii | D. pseudoobscura | 10 | 0 | 0 |
|         | D. immigrans | repleta group spp | 1680 | 29 | 1.7 |
|         | Dorsilopha | D. busckii | 63 | 0 | 0 |
|         | Z. indianus | 10 | 0 | 0 |
| FRUIT   | Zaprionus | Drosophila | D. melanogaster | 521 | 0 | 0 |
|         | D. busckii | D. pseudoobscura | 2 | 0 | 0 |
|         | D. immigrans | repleta group spp | 2 | 0 | 0 |
|         | Dorsilopha | Z. indianus | 182 | 6 | 3.29 |
|         | 1 | 1 | 100 |
Discussion

Mite parasitism in *Drosophila* is far more extensive in taxonomic scope than previously reported. Mites now have been associated with 15 different Drosophilid species, out of which 13 had never been documented as having ectoparasitic mites (Table 4). Despite collecting large numbers of flies of the subgenus *Sophophora*, the great majority of the infested flies belong to the *repleta* species group of the subgenus *Drosophila*. A large number of the infested *repleta* group flies were *D. hydei*, which in addition to using *Opuntia* cactus belongs to the cosmopolitan guild of *Drosophila*, associated with decaying fruits and vegetables in human habitats.18

*Macrocheles subbadius* is a well-known generalist, occupying other substrates besides necrotic cacti,11,19-21 making it unlikely that cactus breeding is the reason why more *repleta* group species have a higher infestation. Besides collecting infested flies from baits close to rotting cactus, we placed baits around fruit habitats because of the generalist ecology of *M. subbadius*. We predicted that most likely, this same mite would parasitize additional *Drosophila* species, using substrates other than cacti.

Screening the greatest possible diversity of flies for evidence of mites was a primary goal of this project. We therefore employed the baiting approach because banana food baits attract large numbers of different *Drosophila* species. This approach allowed us to screen a greater diversity of *Drosophila* species than possible by searching for and collecting from rotting substrates in the wild. There are reasons to suspect, however, that flies from baits may have lower mite loads than flies found at their breeding sites. Luong et al22 showed that mite load interferes with aerodynamics of *Drosophila* flight and dispersal. Consequently, although we found a large number of species with mites, it likely is an underestimate. While our approach may underestimate the infestation prevalence for a given fly species, we nonetheless discovered a far greater number

Table 3. Attachment sites of the 124 total mites encountered on flies.

| Site of attachment              | No. of mites | %     |
|--------------------------------|--------------|-------|
| Ventral abdomen                 | 108          | 87.09 |
| Side of abdomen                 | 7            | 5.64  |
| Abdomen-thorax junction         | 2            | 1.61  |
| Thorax-head junction            | 4            | 3.22  |
| Back abdomen                    | 2            | 1.61  |
| Back thorax                     | 1            | 0.81  |
| Leg                            | 1            | 0.81  |

Table 4. Total numbers of flies found infested with mites. The total number of infested flies is 90 instead of 124 because some flies carried multiple mites.

| Species           | No. Infested flies |
|-------------------|--------------------|
| D. arizonae       | 2                  |
| D. huichole       | 1                  |
| D. hexastigma     | 4                  |
| D. spenceri       | 16                 |
| D. rita           | 1                  |
| D. longicornis    | 1                  |
| D. mercatorum     | 1                  |
| D. nigrospiracula | 11                 |
| D. hydei          | 46                 |
| D. eremophila     | 1                  |
| D. busckii        | 1                  |
| D. melanogaster   | 1                  |
| D. simulans       | 3                  |
| Z. indianus       | 1                  |
of cases of parasitized Drosophila species and found that those species tend to be phylogenetically related, i.e. members of the repleta species group of the subgenus Drosophila.

Regarding the bias in phylogenetic distribution, many factors could underlie why mites appear to be more attracted to repleta group species in the lab as well as in the wild. Most likely would be some way that mites recognize fly species prior to attempting to attach. The epicuticular hydrocarbons forming the waxy layer on the surface of flies have a role in water loss but also they can serve as short-range pheromones for species recognition in mating. Interestingly flies of the subgenus Sophophora tend to have shorter chains than those of the subgenus Drosophila. These epicuticular hydrocarbon profile differences among the species could contribute to the bias in mite infestation. With the exception of a few infested D. melanogaster and D. simulans, the flies of the Sophophora subgenus we collected were mite free, despite being collected from the same baits or fruits with parasitized repleta group flies. Macrocheles subbadius is a generalist mite, reproducing in a wide range of substrates from plant material to dung, so it is unlikely that Sophophoran subgenus flies don’t encounter mites. In fact, collecting flies from rotting citrus has yielded infested D. hydei but no infested species of the Sophophoran subgenus.

When placed in the Petri dish for the behavioral tests, all mites performed searching movements characteristic of invertebrate predators. Most of the time the mites performed “edge-walking” in the dish before moving toward a fly. They also performed exploratory walks toward the center of the dish before encountering a fly. When passing close to a fly, they reduced the walking speed, as if they could detect some host-related stimulus. These behaviors are thought to increase the chances of finding a prey. As the choice test results confirm, when given the option to attach to a repleta group fly such as D. hydei despite the availability of D. simulans, the majority attach to D. hydei. We thus conclude, that mites sense a difference between species, a difference making D. hydei a more attractive host. If epicuticular hydrocarbons are involved in host recognition, it is possible that this mechanism of recognition was driven by some yet unknown negative consequence of attaching to the un-preferred species. Why some mites failed to attach to either fly could be a function of mite nutritional condition or age, but our studies were not designed to address this question. Although generalist M. subbadius affects more species than previously known, parasitism is most likely restricted by a preference of mites for certain hosts mediated by characteristics such as cuticular hydrocarbons.

In nature, behaviors involved in host selection involve the recognition of a habitat, and also recognition and acceptance of a host. The actual host range of parasites is determined by a variety of ecological factors as well as by the suitability of potential hosts for parasite infection and reproduction. While M. subbadius is known to be a generalist across broad taxonomic host scales, its host range, at least within the Drosophilids, appears to have some restrictions, which is also the case of a nematode associated with mycophagous flies. Howardula aoronymphium is known to parasitize different Drosophila species in Europe and North America. In North America, however, suitable hosts of this nematode fall within a restricted Drosophila clade. Fly collections from nature demonstrate that within this genus, H. aoronymphium is successful in infecting flies from the quinaria, testacea, and cardini groups (Jaenike & Perlman 2002). Yet while D. tripunctata along with several other species are broadly sympatric with these susceptible species and sometimes even emerge from the same mushrooms, infected flies of these other species have not been found in nature. In general, Drosophila species vary in their susceptibility to nematode parasitism, but the exact mechanism(s) affecting general parasite attractiveness or resistance are still unknown.

Mite distributions are consistent with the findings of Jaenike & Perlman: the suitability of a potential host for a specific parasite may depend on host
phylogeny, with some clades being more suitable as hosts than others because of their physiological, immunological, or biochemical characteristics. In the case of susceptibility to nematodes, even sister species of *Drosophila* can differ greatly in infection levels, which might depend on derived characteristics of individual species, such as evolved resistance to infection. Parasite-host interactions represent an arms race with the host acquiring defenses against parasitism and at the same time, the parasite developing mechanisms to overcome the host’s defenses. The force exerted by a ubiquitous parasite is a driver of the evolution of its host species, hence disentangling the differences among Drosophilid species in regard to parasitism should reveal the factors delimiting host breadth. Being a model organism, empirical studies easily can examine species differences in transcriptomic responses to mite infestation as well as determine the factors making particular species of *Drosophila* more attractive than others to mites.

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

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