Novel Insights into Tritrophic Interaction Diversity and Chemical Ecology Using 16 Years of Volunteer-Supported Research.

Lee A. Dyer, David L. Wagner, Harold F. Greeney, Angela M. Smilanich, Tara J. Massad, Moria L. Robinson, Mark S. Fox, Rebecca F. Hazen, Andrea E. Glassmire, Nicholas A. Pardikes, Kirsha B. Fredrickson, Clark V. Pearson, Grant Gentry, and John O. Stireman III

Sixteen years ago, a schoolteacher from New Jersey collected a caterpillar in a Costa Rican rainforest. When a parasitoid emerged several days later, it became the first data point of a long-term volunteer–mediated study on tritrophic interactions across the Americas. The teacher was an Earthwatch Institute scientist and the project an ongoing ecological investigation of caterpillars, host plants, and the wasps and flies (parasitoids) that kill them (Fig. 1). Over the course of 16 years, 1,200 volunteers have contributed to the project, including adult and youth citizen scientists from Earthwatch, teachers, and a number of other volunteers who have offered their time for months or years.

With the help of these citizen scientists, we have created a robust data set on plant-herbivore-enemy interactions in order to test specific hypotheses about how the diversity of trophic interactions varies across major environmental gradients. Well over half the described organisms in the world are involved in plant-insect-enemy interactions, and research on these diverse interactions has provided the basis of our understanding of fundamental issues in ecology and evolutionary biology. Nevertheless, most biodiversity studies still rely heavily on static species lists, and prominent theories of diversity do not yet include quantification or discussion of the interaction diversity that shapes ecosystems. This interaction diversity approach to ecological research contributes to all major theoretical and applied issues in biodiversity research, including the latitudinal diversity gradient, neutral theory, diversity-stability relationships, biodiversity and ecosystem function, specialization, latitudinal and elevational range size, and effects of climate change on biodiversity. In addition to the focus on basic ecology and natural history, the project contributes to systematics of our study organisms and prioritizes work with teachers, students, local communities, and the general public to increase awareness of the importance of biodiversity.

Methods

Study Sites. Collecting in Costa Rica started in 1995 and is based at La Selva and Tirimbina Reserves, Heredia Province, Costa Rica. We collect within an elevational range from 35 - 1500 m. Mean annual precipitation is 4,200 mm with a mild 2-month dry season. In 2000 we added another tropical site, the Yanayacu Biological Station (YBS) at 2,200 m in the Quijos Valley, Napo Province, of the eastern Ecuadorian Andes, facilitating easy access to a diversity of humid habitats ranging from paramo (3,000 m) to lowland rain forest (500 m).

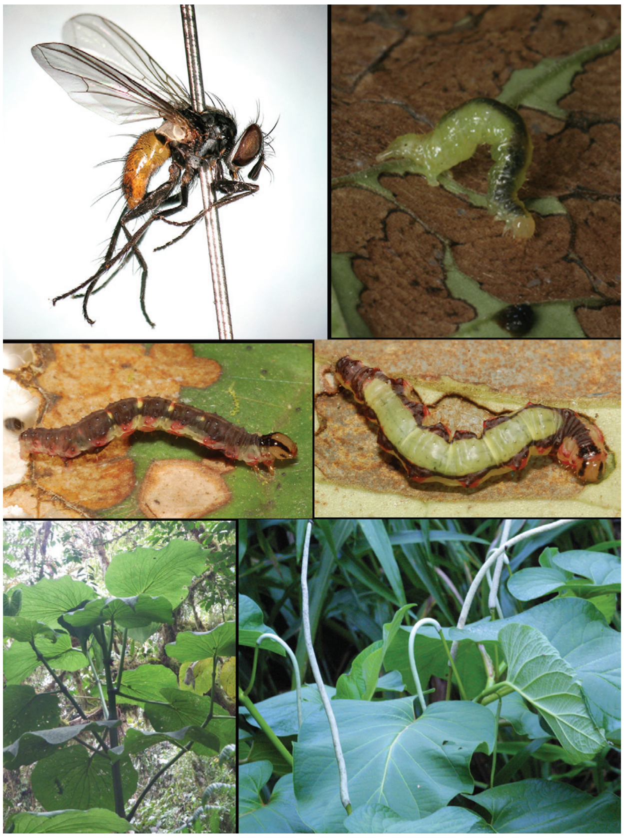

Figure 1. A model tritrophic system studied by our citizen science research program. The host plant (bottom) is in the genus *Piper L.* (Piperaceae) and is attacked by a high diversity of specialist and generalist herbivores, including specialist caterpillars (middle) in the species-rich tropical moth genus, *Eois* Hübner (Lepidoptera: Geometridae: Larentiinae). These, in turn, are attacked by specialist and generalist parasitoids, such as wasps (top – immature inside caterpillar) in the genus *Parapanteles* Ashmead (Hymenoptera: Braconidae: Microgastrinae) and flies in the genus *Erythromolana* (top – pinned adult). There are over 1,000 species of *Piper* in the Neotropics, and as a dominant understory shrub with high diversity of secondary metabolites and arthropod communities, this genus is an ideal model study organism. Citizen volunteers have collected over 100 morphospecies of *Eois* at our Costa Rica and Ecuador sites. There are currently about 250 described species, but Neotropical Eois richness may be as high as 2,000 species (Rodríguez-Castañeda et al., 2010), and many of the species collected by volunteers are new to science. *Parapanteles* and *Erythromolana* are endoparasitoids of lepidopteran larvae and most of what is known about host associations within these genera has been contributed by citizen science collections and rearings.

In 2001 we added two North American sites: 1) Southwestern Research Station, in the Chiricahua Mountains of southern Arizona, which provides access a diversity of life zones spanning 1,200 - 3,000 m elevation; and 2) Pearl River Wildlife Management Area and Tulane University in southern Louisiana, which provides access to thousands of hectares of forests and marshes. Finally, in 2008 we added Sagehen Biological Station and a University of Nevada field station, which provide data from the Great Basin and the Sierra Nevada mountains, with sites ranging from 1,100 - 2,100 m elevation. All of these Earthwatch sites are described in greater detail in publications resulting from our investigations across the Americas (Stireman et al. 2005; Dyer et al. 2007; Dyer et al. 2010).

Collecting, rearing, and curating Lepidoptera, Hymenoptera, and Diptera. All species of externally feeding or shelter-building Lepidoptera (38 families currently included) are collected using two methods: 1) haphazard collection on all host plants encountered along elevational or disturbance gradients at all sites; 2) collection in temporary 5-25 m diameter plots throughout the study areas to allow quantified, standardized estimates of caterpillar-parasitoid abundances and interaction diversity. Once a plot has been searched for eggs, caterpillars, pupae, and parasitoids, leaf abundance and percent herbivory for focal host plants in the plot are estimated. This method provides basic data on caterpillar loads of individual plants, parasitoid loads of caterpillar species, and quantitative data on the number of species per leaf area (Dyer et al. 2010). Caterpillars are reared employing standardized methods, which are described in numerous publications (e.g., Stireman et al. 2005; Dyer et al. 2007). Reared Lepidoptera, Hymenoptera, and Diptera are identified by project staff in collaboration with an established network of taxonomists, and selected specimens are processed for molecular characterization.

Selected Results and Discussion

Natural History and Diversity. The most important contribution of our citizen scientists to ecology and evolutionary biology is the documentation of plant-caterpillar-parasitoid interactions, providing the first biological information known for many genera and even subfamilies. Across the five sites citizen volunteers have contributed significantly to datasets that include over 180,000 individual caterpillars representing ~10,000 species from ~1,750 host plant species, and reared out approximately 11,000 adult parasitoids. More than 20,000 images have been produced by Earthwatch volunteers, and these images are used in numerous products, including a Web database (www.caterpillars.org) and publications such as Wagner et al. (2011). Almost all volunteer-collected caterpillars and associated images yield some level of new natural history, distributional, phenological, or interaction information (Fig. 2), and at least 10% of the reared species are new to science. Collectively these data provide excellent opportunities for ecological, evolutionary, chemical, taxonomic, and climate-change studies. Moreover, the preserved specimens themselves are invaluable for systematic studies and sources of DNA for molecular analyses.

As an example, this approach allows investigation of latitudinal gradients in diversity and specialization, a major challenge in community ecology and evolutionary biogeography. For herbivorous insects, specialization may be one mechanism affecting species richness at a given locale. We tested this hypothesis by comparing host specialization in lepidopteran larvae for eight different New World forests ranging in latitude from 15°S to 45°N (Dyer et al. 2007) and found that the diets of tropical Lepidoptera larvae are more specialized than their temperate forest counterparts. Four of the sites in this analysis were our Earthwatch sites, and this latitudinal pattern persists when the 4 sites that do not include citizen science data are excluded from analyses (Fig. 3); within these 4 Earthwatch sites, the source of the data (citizen scientists versus project staff) does not significantly affect our measures of specialization (Fig. 3). Furthermore, our standardized plot method yielded data that support the related hypothesis that herbivore specialization and species packing (the number of herbivore species per host plant) have an impact on herbivore diversity that is as great as the effect of host plant diversity along elevational gradients in the tropics (e.g., Rodríguez-Castañeda et al. 2010).

Figure 2. Earthwatch volunteers have collected and reared many hundreds of species for the first time. Life histories of the caterpillars illustrated here, all resulting from our Earthwatch site in southeastern Arizona, were heretofore unknown. Clockwise from upper left corner: *Monostoecha semipectinata* (Geometridae) from *Garrya wrightii* (Garryaceae); *Elasmia* sp. nov. (Notodontidae) from *Sapindus saponaria* (Sapindaceae); *Litodonta wymola* (Notodontidae) from *Fouquieria splendens* (Fouquieriaceae); *Tarache axendra* (Noctuidae: Acontiinae) from *Sphaeralcea laxa* (Malvaceae).

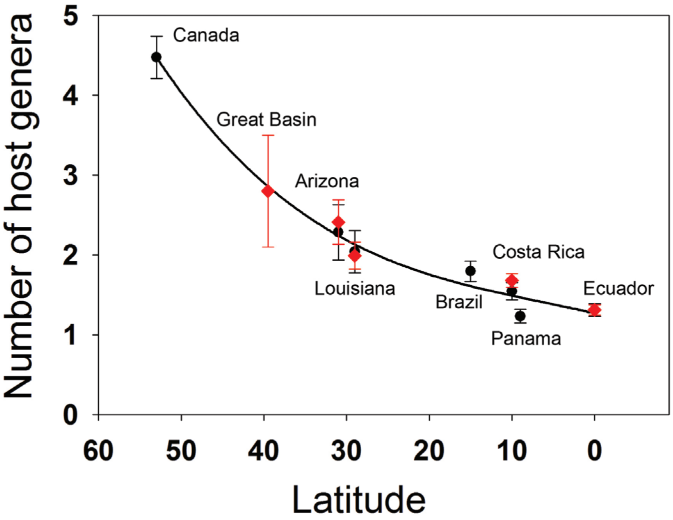

Figure 3. A comparison of results with and without citizen science data. The black points are from Dyer et al. (2007) and are the mean diet breadth (number of plant genera consumed per caterpillar species) for hundreds of species of Lepidoptera, many of which were collected and reared by citizen scientists. The red data points were calculated by excluding data from sites, taxa, or time periods that did not include citizen science data; there was no difference between volunteer-collected data and researcher-collected data (based on 95% confidence intervals). Several sites in this study did not include citizen scientists, and the Great Basin site was added recently so was not included in the previous data analysis (and because of limited data, the standard error is higher).

Chemical Ecology. One important goal of chemical ecology is to understand the relationship between plant secondary compound diversity and the evolutionary history of associated herbivores and their predators. From the beginning, citizen scientists have contributed to the laboratory chemistry needed to understand such chemically-mediated tritrophic interactions. Their work led to the discovery of novel plant compounds with significant ecological roles (e.g., Dodson et al. 2000), as well as the demonstration of synergy between defensive compounds and the discovery that many compounds disrupt caterpillar immune responses. Synergy in plant defenses results when interactions between multiple defensive compounds disrupt caterpillar physiology or biochemistry, and the effects of the compounds in a mixture are greater than additive effects of individual compounds. Such synergy was demonstrated at all five Earthwatch-funded sites, including synergistic mortality caused by alkaloids, furanocoumarins, iridoid glycosides, and saponins (e.g., Richards et al. 2010). These findings suggest that synergy may be the rule rather than the exception and may help explain why it is often difficult to demonstrate biological activity of individual compounds.

The effects of plant chemistry do not stop at the second trophic level, and our results demonstrating the negative effects of caterpillar-sequestered plant toxins on their immune defenses (Smilanich et al. 2009) are exciting because they provide unique insight into chemically mediated tritrophic interactions. One of our first publications utilizing multiple years of Earthwatch data showed that levels of parasitism were higher on chemically protected specialist caterpillars compared to generalist species that are often unable to sequester toxins from host plants (Gentry and Dyer 2002). This has been referred to as the "safe haven" hypothesis (for parasitoids) since predators avoid toxic caterpillars, providing protection to the parasitoids within toxic hosts. The negative effects of toxins on caterpillar immune responses add another dimension to the safe haven hypothesis, impairing caterpillars' ability to combat parasites and further providing a particularly safe place for parasitoid larval development.

Systematics. Most specimens available to systematists working with herbivores and parasitoids are divorced from ecological data beyond basic biogeographical and phenological information provided by their collection labels. Rearing specimens from caterpillars preserves much of their ecological, environmental, and behavioral context. Miller's (2009) recent work on prominent moths (Notodontidae) in Ecuador was greatly augmented by material reared by citizen scientists. Miller compared reared specimens to type collections and found that, of the 67 Notodontidae recorded at Yanayacu, 54% were new to science. Miller and Thiaucourt (2011) went on to describe 27 new species of notodontids collected and reared at Yanayacu, emphasizing the high levels of undocumented insect diversity in Andean cloud forests.

Citizen science has also helped uncover phylogenetic patterns across three trophic levels associated with plants in the genus *Piper* (Fig. 1) in the tropics. Volunteer collections contributed significantly to multigene phylogenies for *Piper*, *Piper*-specialized *Eois* caterpillars, and their associated *Parapantales* wasp parasitoids. Host-parasite associations were then mapped onto phylogenies of all three taxa to assess levels of co-speciation, revealing that moth-plant associations are characterized by numerous radiations of closely related *Eois* species (Wilson et al. unpublished manuscript). Volunteer efforts have also recently enabled taxonomic revision and description of 11 new species of *Erythromelana*, a genus of tachinid parasitoid flies largely restricted to attacking *Eois* caterpillars on *Piper* (Inclan and Stireman, unpublished manuscript). Nothing was known of the biology of this wide-ranging Neotropical genus before this work.

Basic ecology. Citizen scientists have contributed to many of our long-term experiments that are designed to test prominent ecological hypotheses. Tritrophic interactions can have large effects on biodiversity through mechanisms such as diversity cascades, where changes in density or diversity at one trophic level (e.g., predators) can have indirect effects on diversity at nonadjacent trophic levels (e.g., plants). Data demonstrating diversity cascades were collected by volunteers from ecosystems as different as alfalfa fields (Pearson et al. 2008) and tropical forests (Dyer et al. 2010). The diversity cascades concept is a broad ecological view that relies on trophic interactions such as those proposed by the well-known Janzen-Connell hypothesis, which posits that high plant diversity is maintained by density-dependent herbivory on seeds and seedlings close to the parent tree or close to some other source of herbivores. For example, generalist caterpillars may move from source plants and kill seedlings of multiple additional species, potentially leading to an increase in species richness if dominant plant species are normally thinned by these herbivores. Thus we hypothesized that plant seedling richness would be lower in plots from which generalist caterpillars were removed. Volunteers quickly learned to recognize generalist caterpillars on *Piper*, making the labor-intensive process of caterpillar removal quite feasible. After 15 months of caterpillar removals, the experimental plots had over 40% fewer individual seedlings, about 40% fewer seedling species and 40% greater seedling evenness, on average, than control plots with generalist caterpillars left feeding on *Piper* and surrounding seedlings (Dyer et al. 2010). One interpretation of these associations between herbivores on *Piper* and local seedling richness is that high caterpillar-induced seedling mortality in dominant families allowed the colonization or survival of less common species.

Climate Change and Tritrophic Interactions. Our citizen science project has also tested a variety of hypotheses related to climate change. Most general climate change scenarios predict that insect outbreaks will increase in frequency and intensity as a result of increases in CO_2 and mean global temperatures, based on direct positive effects of temperature on insect populations as well as through complex effects on tritrophic interactions. Models of climate change have predicted greater frequency and duration of droughts and floods and a widespread increase in the frequency of extreme weather events (reviewed by Stireman et al. 2005). Such increased unpredictability and variability in regional climates, particularly with regard to precipitation, will likely impact interaction diversity, yet the potential effects are largely unknown. We used our databases to compare tritrophic interactions across a precipitation gradient in the Americas and found that four of our Earthwatch sites, combined with 11 other geographically dispersed databases, exhibited striking reductions in parasitism as local year-to-year variation in total precipitation increased (Fig. 4). We hypothesized that this decline is caused by phenological asynchrony between parasitoids and their hosts when weather events such as floods and droughts create temporal shifts in caterpillar populations making them unavailable to specialized parasitoids (e.g., Hymenoptera) during their host-searching phase (Fig. 4).

Laboratory-based microcosm experiments aided by volunteers over five years have revealed that simple changes in temperature will disrupt plant-caterpillar-parasitoid interactions, causing substantial reductions in plant biomass and complete failure of parasitoid populations (Dyer et al., unpublished manuscript). Our experiments have tested the robustness of different interaction diversities (e.g., alfalfa plants, noctuid caterpillars, and braconid parasitoids) in response to ambient and increased levels of temperature and CO_2. Parasitism rates declined dramatically in response to elevated temperatures and CO_2 due to phenological asynchrony–caterpillar development rates increased enough to allow the caterpillars to pupate before parasitoids could finish their development (Fig. 4). This temporal decoupling of parasitoids from their hosts caused increased caterpillar herbivory and biologically significant reductions in plant biomass.

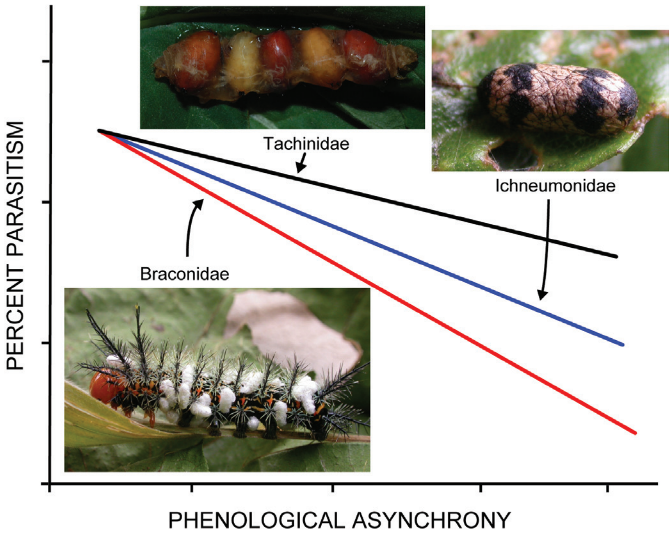

Figure 4. A heuristic graph summarizing results from several citizen science-driven studies on the effects of phenological asynchrony on parasitism. Data from our citizen science sites and laboratory experiments demonstrated that increased temperature, CO^2, and extreme weather events caused parasitic wasps to delink from their caterpillar hosts (phenological asynchrony), resulting in lower levels of parasitism and linear declines in parasitism across gradients (temperature, elevation, extreme weather) that cause asynchrony. Such declines in parasitism will cause increased caterpillar outbreaks and lower interaction diversity in natural and agricultural ecosystems. Different taxa of parasitoids were not affected equally: for example our data suggest that specialist wasps (Ichneumonidae and Braconidae) are more susceptible to phenological asynchrony than generalist flies (Tachinidae).

Scientific and broader impacts of the tritrophic citizen science project. Databases and projects augmented by volunteer contributions have allowed us to:

- Develop a new approach to studying biodiversity that enhances our ability to test prominent and controversial hypotheses requiring long-term and large-scale comparative data sets.
- Coordinate with similar ongoing surveys to provide a more comprehensive data set across the Americas, resulting in important ecological and conservation publications.
- Disseminate results of these efforts via the Internet (www.caterpillars.org).
- Create jobs for locals at many of our sites and increase the involvement of surrounding communities and land managers.
- Provide influential hands-on scientific experiences to high school students and their educators through the Earthwatch scholarship programs.

Our citizen science project also unites workers from Costa Rica, Ecuador, the United States, Brazil, and other countries to maximize the effectiveness of collaborative databases in the Americas. The education and employment of students and local workers on our projects enhances the credibility of conservation projects and provides research opportunities for local students and paraecologists, generating interest from the popular media. In addition, our approach to interaction diversity helps form a basis for developing a clear understanding of community structure for comparative purposes applicable to conservation (Dyer et al. 2010). The broader educational impacts of including diverse citizen collaborators in basic research are immeasurable and add value to our goals as educators. Teachers volunteering with our project have brought methods and concepts back to their classrooms, students have made informed decisions about pursuing science careers, non-scientists have begun careers in science, and volunteers have contributed personal funds to science or conservation. Finally, the impact of citizen scientists on our funding cannot be overstated. Much of our tritrophic research has benefited from funding from federal agencies and other sources, but our Earthwatch funding allowed us to greatly extend the depth and reach of our studies over the past 16 years. It is increasingly challenging to fund entomological research and natural history with grants from agencies such as National Science Foundation, National Institute of Health, US Department of Agriculture, Department of Interior, Department of Energy, and Department of Defense. What if these funding sources continue to dwindle? There are many creative and non-traditional approaches to supporting basic and applied entomological research, but the citizen science model is particularly attractive in times of both financial exigency and financial excess. Regardless of financial uncertainties, there will always be those who have the time, interest, and motivation to volunteer as hard-working and productive citizen scientists and, with their return to various non-academic spheres, further the cause of continued scientific research and dissemination. At a time when scientific research on important topics such as evolution and climate change is misunderstood and misrepresented, the citizen science research model is one form of academic outreach that can help build public interest and confidence in science and to counteract misconceptions about the process of discovery.

Acknowledgments

This work was supported by Earthwatch Institute, UNR, National Science Foundation grants DEB1020509 and DEB0849361. The authors thank the staff at Earthwatch, the field technicians at all of our sites, and the hundreds of Earthwatch volunteers for their hard work and enthusiasm for the project.

References

Dodson, C. D., L. A. Dyer, J. Searcy, Z. Wright, and D. K. Letourneau. 2000. Cenocladamide, a dihydropyridone alkaloid from *Piper cenocladum*. Phytochemistry 53:51-54.

Dyer, L. A., D. K. Letourneau, G. V. Chavarria, and D. S. Amoretti. 2010a. Herbivores on a dominant understory shrub increase local plant diversity in rain forest communities. Ecology 91:3707-3718.

Dyer, L. A., M. S. Singer, J. T. Lill, J. O. Stireman, G. L. Gentry, R. J. Marquis, R. E. Ricklefs, H. F. Greeney, D. L. Wagner, H. C. Morais, I. R. Diniz, T. A. Kursar, and P. D. Coley. 2007. Host specificity of Lepidoptera in tropical and temperate forests. Nature 448:696-6U9.

Dyer, L. A., T. R. Walla, H. F. Greeney, J. O. Stireman, and R. F. Hazen. 2010b. Diversity of Interactions: A Metric for Studies of Biodiversity. Biotropica 42:281-289.

Gentry, G., and L. A. Dyer. 2002. On the conditional nature of neotropical caterpillar defenses against their natural enemies. Ecology 83:3108-3119.

Miller, J. S. 2009. Generic Revision of the Dioptinae (Lepidoptera: Noctuoidea: Notodontidae) Parts 1 and 2: Josiini. Bulletin of the American Museum of Natural History.

Miller, J. S. and P. Thiaucourt. 2011. Diversity of prominent moths (Lepidoptera: Noctuoidea: Notodontidae) in the cloud forests of northeastern Ecuador, with descriptions of 27 new species. Annals of the Entomological Society of America. In press.

Pearson, C. V., T. J. Massad, and L. A. Dyer. 2008. Diversity cascades in alfalfa fields: From plant quality to agroecosystem diversity. Environmental Entomology 37:947-955.

Richards, L. A., L. A. Dyer, A. M. Smilanich, and C. D. Dodson. 2010. Synergistic effects of amides from two *Piper* species on generalist and specialist herbivores. Journal of Chemical Ecology 36:1105-1113.

Rodríguez-Castañeda, G., L. A. Dyer, G. Brehm, H. Connahs, R. E. Forkner, and T. R. Walla. 2010. Tropical forests are not flat: how mountains affect herbivore diversity. Ecology Letters 13:1348-1357.

Smilanich, A. M., L. A. Dyer, J. Q. Chambers, and M. D. Bowers. 2009. Immunological cost of chemical defence and the evolution of herbivore diet breadth. Ecology Letters 12:612-621.

Stireman, J. O., L. A. Dyer, D. H. Janzen, M. S. Singer, J. T. Li, R. J. Marquis, R. E. Ricklefs, G. L. Gentry, W. Hallwachs, P. D. Coley, J. A. Barone, H. F. Greeney, H. Connahs, P. Barbosa, H. C. Morais, and I. R. Diniz. 2005. Climatic unpredictability and parasitism of caterpillars: Implications of global warming. Proceedings of the National Academy of Sciences of the United States of America 102:17384-17387.

Wagner, D. L., D. F. Schweitzer, J. B. Sullivan, and R. C. Reardon. 2011. Owlet Caterpillars of Eastern North America. Princeton University Press. 576 pp. In press.

Lee A. Dyer, Angela M. Smilanich, faculty, Department of Biology, University of Nevada, Reno, NV. **David L. Wagner**, faculty, Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, CT. **Harold F. Greeney**, scientific director, Yanayacu Biological Station & Center for Creative Studies, Cosanga, Napo, Ecuador. **Tara J. Massad**, postdoctoral researcher, Max Planck Institute for Biogeochemistry, Jena, Germany. **Moria L. Robinson**, recent graduate, Department of Biology, Middlebury College, Middlebury, VT. **Mark S. Fox**, and **Rebecca F. Hazen**, Ph.D. students, Department of Ecology and Evolutionary Biology, Tulane University, New Orleans, LA. **Andrea E. Glassmire**, and **Nicholas A. Pardikes**, Ph.D. students, Department of Biology, University of Nevada, Reno, NV. **Kirsha B. Fredrickson**, undergraduate student, Department of Biology, University of Nevada, Reno, NV. **Clark V. Pearson**, Ph.D., Department of Ecology and Evolutionary Biology, Tulane University, New Orleans, LA. **Grant Gentry**, faculty, Department of Biology, Samford University, Birmingham, AL. **John O. Stireman III**, faculty, Department of Biological Sciences, Wright State University, Dayton, OH.

INSTANT SYMPOSIUM

Tachinid Flies and Monarch Butterflies: Citizen Scientists Document Parasitism Patterns over Broad Spatial and Temporal Scales

Karen Oberhauser

The Tachinidae represent the largest family of dipteran parasitoids, with ~10,000 species. Most of their hosts are Lepidoptera, and it is generally assumed that tachinid flies have wide host ranges (i.e., many suitable host species) (Stireman et al. 2006). However, it is likely that tachinid host ranges have been overestimated; in a long-term study of tachinid flies reared from hosts collected in Costa Rica, many species that appeared to be generalists were shown to be host-specific cryptic species (Smith et al. 2007).

Lespesia archippivora (Riley) (Tachinidae) has been reported to parasitize larvae of 25 Lepidoptera species in 14 families, and one species of Hymenoptera (Arnaud 1978). It is widespread throughout North and Central America, has been found in Brazil (Arnaud 1978), and was introduced into Hawaii for biocontrol in 1898 (Etchegaray and Nishida 1975). Except for its occurrence in multi-host species-rearing projects, *L. archippivora* has only been studied in detail in monarchs (*Danaus plexippus* L.) and beet armyworms (*Spodoptera exigua* Huber). It parasitizes monarch larvae in the continental U.S. and Hawaii (Etchegaray and Nishida 1975, Prysby 2004, Oberhauser et al. 2007), with one long-term, broad-scale monitoring project documenting an overall parasitism rate of ~13% (Oberhauser et al. 2007). In the southern U.S., the beet armyworm is reported to be a preferred *L. archippivora* host (Stapel et al. 1997).

North American monarchs complete multiple generations on their milkweed host plants (*Asclepias* spp.) within a summer breeding season, and overwinter in central Mexico (eastern migratory population) or coastal California (western migratory population). They remain in their wintering sites in a state of reproductive diapause from early November through mid-March, before returning to their spring breeding grounds. In the east, returning migrants lay eggs in the southeastern U.S. (Texas to Florida and Oklahoma to North Carolina) and their offspring recolonize the summer breeding range (roughly the northeastern quarter of the U.S. and southern Ontario and Quebec), where they undergo two to three non-migratory generations before the final generation returns to Mexico. There is some fall breeding by southward migrants in the southern U.S., resulting in another generation that presumably migrates to Mexico. Although some monarchs remain throughout the winter in the southern U.S., where at least some of them continue to breed (Prysby & Oberhauser