BUTTERFLY INSECT PARASITOID FAUNA

AT PIERCE CEDAR CREEK INSTITUTE

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November 25, 2011

ABSTRACT

Butterflies (Order Lepidoptera; Superfamily Papilionoidea) at Pierce Cedar Creek Institute were hypothesized to be the hosts for a number of insect parasitoid species that ultimately kill their hosts in the larval or pupal stages. Our research attempted to understand the basic ecology of these native insect parasitoids and their abundance at PCCI. We were able to successfully rear just three parasitoid species (two from Order Hymenoptera; one from Order Diptera) from the following hosts: the monarch butterfly *(Danaus plexippus),* the Spicebush swallowtail *(Papilio troilus)*, and the Baltimore checkerspot *(Euphydryas phaeton).* We discuss these unexpected results and also attempt to operationally define the following terms: parasitoid, parasite, pathogen, and predator. Our intent in this latter regard is to clearly distinguish between insect parasites and insect parasitoids.

A Definition Conundrum: What is a Parasitoid?

In this paper we will first attempt to establish operational (functional) definitions that will distinguish parasitoids from parasites, pathogens, and predators.

The term *parasitoid* was first coined by the German writer O.M. Reuter in 1913 and adopted by the American hymenopterist and insect embryologist William Morton Wheeler in 1937. Only in the last 25 years has this term become universally accepted. Before that, parasitoids were most commonly referred to as insect parasites (van Lenteren, 2004). However, it is common knowledge that *multicellular parasites* such as lice (Order Mallophaga) and *multicellular micropredators* such as fleas (Order Siphonaptera) rarely kill their hosts directly due to their feeding actions. This is in stark contrast to many acellular or cellular *pathogens*, (such as pathogenic viruses, bacteria, and fungi) which may produce mortality levels as high as 100 percent in their host populations. Furthermore, if a multicellular parasite does kill its host, it is usually indirectly through structural damage to the host or by inducing secondary infection via the opportunistic introduction of the above-mentioned pathogens (Hickman *et al*., 2011). We should note here that multicellular parasites also typically do not reduce an organism's reproductive fitness. (In developing countries, for example, humans may harbor a number of multicellular parasites ranging from lice to nematodes, but reproductive success does not seem to be compromised judging by the exponential increase in humanity in those regions.)

In contrast to parasites, *parasitoids* consume and ultimately kill their hosts. For example, Gauld and Bolton (1988) proposed that parasitoids might be best defined as "…insects whose larvae develop by feeding on or within an arthropod host, in which the *host individual* is almost always killed by the developing parasitoid larva." First note that this definition is restricted to *insects* that ultimately *kill* their arthropod host. Therefore it operationally excludes other insect taxa such as members of the Order Strepsiptera, which sometimes are called parasitoids, but should more properly be labeled as parasites because they do not typically kill their hosts. In addition, the first-instar strepsipteran larvae are free-living and planidial. Parasitoid larvae are never free-living and are predacious by nature: they actively consume the host.

We propose that other non-insectan taxa should also be excluded from the parasitoid definition. In our view, mermithid nematodes whose eggs are laid in water or on land and whose subsequent free-living larvae infect a wide range of hosts (Rubzov 1972) should not be classified as parasitoids. Note, however, that Frank and Cody (1990) of the University of Florida propose a slightly broader definition: *A parasitoid is an organism that, during its development, lives in or on the body of a single host individual, eventually killing that host.* In contrast, *a* parasite *can be defined [as any multicellular organism] that has an association with another organism (host), from which it obtains benefits and thereby harms, but does not kill*.

To reiterate, and most important to this discussion, a multicellular parasite typically does not kill its host and a multicellular parasitoid eventually does kill its host. For this reason, parasites and parasitoids must be differentiated and parasitoidy (or parasitoidism) should not be defined as a form of parasitism.

There are a number of other differences between a parasite and parasitoid. Unlike an insect parasite, an insect parasitoid does not reproduce (except for some species with asexual polyembryony) within the same host after the initial oviposition, nor do parasitoid larvae move from host to host as many parasites may do. Many insect parasitoids also tend to avoid an infected host by recognizing inhibitory factors left by the original parasitoid (this fact was first published by Salt in 1937). It is common knowledge that hosts of parasites may be reinfected over and over again. Perhaps most important, *host quantity* also serves to ecologically separate parasitoids from predators and parasites: parasitoid larvae infect and directly or indirectly kill only *one* host (Eggleton and Gatson, 1990); whereas predators typically kill many hosts over the course of their lifetimes; and many parasites are often associated with multiple, often sequential, obligate hosts required for their life cycle (see also, Godfray, 1994).

For the above reasons, we maintain that *parasitoidism* (the proper etymological term) is really a form of predation in the evolutionary, ecological, and physiological sense: As with true predators, insect parasitoids consume and kill their prey (host). With this understanding, parasitoids can be broadly differentiated into *ectoparasitoids* (which consume the host from the outside-in) and *endoparasitoids*, (which develop as larvae within the host). All parasitoidal larvae then pupate to complete their holometabolic

development either within or outside the prey. A number of authors view parasitoids that prey on other parasitoids (hyperparasitoids) as a fourth trophic level beyond predation (Koul and Dhaliwal, 2003).

In dynamic predator-prey systems, predators are significantly less abundant than their primary prey; the same is true for parasitoid-prey systems. For example, in one study of Monarch butterflies in Kansas, parasitoidism rates by the tachinid fly *Lespesia archippivora*, were under 15 percent (Orley Taylor, personal communication). Other studies by Nancy Stamp show that levels of parasitoid attack in the Baltimore checkerspot (*Euphrydryas phaeton*) have an average value below 6 percent, with some sites having no parasitoidism (Stamp, 1984).

Considering the above discussion, and for the purposes of this paper, we shall more strictly define a **parasitoid** as a predacious [sic] insect whose larva consumes and ultimately kills its prey. By accepting the word parasitoid, the appropriate verb and noun respectively become *parasitoidize* and *parasitoidism* (Frank and McCoy, 1989). As Godfray (1994) humorously points out, "The creature in Alien is immediately recognizable as a parasitoid—specifically a primary, solitary, endoparasitoid with a planidial larva."

Current State of Knowledge for Parasitoid-Prey Systems

Insect parasitoid abundance is currently unknown for most natural ecosystems (Fraser *et al.*, 1997). As such it is conjectured that a large and diverse spectra of parasitoids (perhaps 10% of all metazoan species) exists in a variety of habitats (Hassell 2000), making their classification an enormous and as yet largely incomplete task. Studies designed to classify and identify parasitoids in localized habitats are few. For example, there is only one major study of the tachinid fly biodiversity in North America (Inclan and Stireman, 2011).

Parasitoid and host species' relationships also have been poorly studied and have yielded little data concerning parasitoid natural life history and behavior under field conditions. In fact, most parasitoid-host relationships have been studied only under controlled conditions in the laboratory or as results of parasitoid augmentation programs designed to control the outbreak of alien insect pests (Hassell, 2000). Of the data available, the parasitoid-host relationships do not appear to correlate across different

habitats and thus at this point these data cannot be used to generalize even hypothetically about parasitoid-host systems under natural conditions.

Despite this lack of basic ecological knowledge, there has been an increase in the use of non-native parasitoids as biological controls of pest insects that affect economically important farm and garden plants throughout the world. This increase has posed serious and often unanswered questions relating to the introduction of non-native parasitoid species and their effects on nontarget species in these environments (Barlow *et al.,* 2004). Due to the lack of information of these parasitoids and their host correlation, it is difficult to discern if threats exist to nontarget native species. For example, several species of the egg-parasite genus *Trichogramma* are commonly used in biological control programs (Schmidt *et al.,* 2003). So common is their use that parasitoid suppliers may "guarantee" that their parasitoids will eliminate certain species of "pest" butterflies (such as the cabbage butterfly, *Pieris rapae*, as well as other problematic insects). It should be noted, however, that the success of non-native parasitoids is dependent upon interspecific and intraspecific population selection, mass rearing, distribution to purchasers, and environmental conditions during field release (e.g., factors such as temperature, insolation, and humidity) (Smith, 1996).

Native parasitoid efficacy is dependent on many factors including location of habitat, ecological factors, host location, quality, and defense, and even immature parasitoid competition (Brodeur and Boivin, 2004). In addition, the availability of nectar/sugar sources is crucial to parasitoid fecundity because habitats with sufficient nectaring resources increase the activity of free-living adult parasitoids that depend on them (Bianchi and Wackers, 2008). Host defenses (i.e., active aggression against parasitoids or escape), are also influential in determining the successful commercial use of parasitoids (Firlej *et al.,* 2009). Finally, change in climate also affects parasitoid physiology, behavior, and reproductive fitness, and therefore success (Denis *et al.,* 2011). As relatively small ectotherms, insect parasitoid metabolic rate will increase with temperature, thereby affecting activity. It can be concluded that these factors (some of which are interwoven with other biophysical aspects of the environment such as relative humidity and insolation), affect parasitoid fitness and thus abundance. Insect parasitoid research is an ambitious task requiring repetition of field work for a number of seasons in

order to produce accurate results due to factors such as climate temperature changes, natural population fluctuations based on prey density, and parasitoid pathogens and predators that undoubtedly affect parasitoid success under natural conditions.

Goals of This Study:

This study was designed to better understand the biodiversity of parasitoids attacking 10 common species of butterflies at Pierce Cedar Creek Institute, with the hope of establishing a knowledge basis (no matter how general) for future studies.

Materials and Methods

Collections of Insect Parasitoids Under Field Conditions

Time Period of Study: From late April to mid-August 2011, we collected insect parasitoids from the larval and pupal stages of the test butterfly species (see complete list and life history characteristics in Table 1) found in the field in an attempt to study the natural abundance of test PCCI butterfly populations as well as to establish the abundance and diversity of insect parasitoids that attack these populations under field conditions.

TABLE 1. Intended butterfly species and their general life history characteristics captured, bred, baited and studied at PCCI.

Scientific Name	Common Name	Family	Food Plant of larva	# of generations	Months Present	Abundance	Special Characteristics
Colias philodice	Clouded Sulpher	Pieridae	Alfalfa, clover, sweet clover, vetch, other legumes	4 or more	May-Early November	Occasional to Abundant	Over winters as chrysalis In the Great Lakes Region
Danaus plexippus	Monarch	Nymphalidae	Milkweeds	3 or more	June-October	Occasional to abundant	Migrate to Mexico for hibernation every year from around North America.
Everes comyntas	Eastern Tailed Blue	Lycaenidae	herbaceous legumes like bush-clover, clover, lupine, pea vine, sweet clover, tick-trefoil, and vetch	multiple	Mid May - October	Occasional to Common	Last instar overwinters
Limenitis archippus	Viceroy	Nymphalidae	Willow, aspen, poplar	2	June-October	Occasional to common; locally abundant	Mullerian mimic of the Monarch; Third-stage caterpillars make a shelter from a rolled leaf tip in which to spend the winter.
Limenitis arthemis astyanax	Red Spotted Purple	Nymphalidae	Cherry, willow, poplar	2	Mid June-September	Occasional to Common	Third-stage caterpillars hibernate as with congener
Lycaena phlaeas	American Copper	Lycaenidae	Sheep sorrel, curled dock (other docks)	3	Mid May to Mid October	Occasional to Common	Overwinters in pupal stage
Papilio glaucus	Tiger Swallowtail	Papilionidae	Tulip tree, cherry, ash	2 or more	Late May-October	Occasional to Common	Chrysalids overwinter
Papilio polyxenes	Black Swallowtail	Papilionidae	Anne's Lace, (other members of the carrot family, Apiaceae)	2	May-September	Occasional to common	Hibernates as a chrysalis.
Papilio troilus	Spicebush Swallowtail	Papilionidae	sassafras, spicebush	2	June-September	Occasional to Common	Overwinters in pupal stage
Phyciodes tharos	Pearl Crescent	Nymphalidae	Asters	2 or more	June-October	Occasional to Abundant	Hibernation is by third-instar caterpillar or pupa.
Pieris rapae	Cabbage Butterfly	Pieridae	wild and cultivated crucifers or mustard	multiple	Mid April-Early November	Occasional to Abundant	Overwinters in pupal stage
Vanessa atalanta	Red Admiral	Nymphalidae	Nettle, false nettle, wood nettle family, all in family Urticaceae	2 or more	Mid May to Early November	Occasional to common; locally abundant	Adults may hibernate. More likely Migrate
Vanessa cardui	Painted Lady	Nymphalidae	Burdock, thistle, hollyhock and other mallows, lupines and other legumes, non-woody and low-growing plants	2 or more	June to October	Uncommon to Common	Adults hibernate only in the South and in mild winters. In Great Lakes region adults migrate.
Vanessa virginiensis	American Painted Lady (Painted Beauty)	Nymphalidae	Cudweed, everlasting, pussytoes, rabbit-tobacco, other asters	2 or more	Early May to October	Occasional to Common	Adults hibernate?
			Primary and secondary	Resources	assembeled	by Julia and	Alica

Source of information: "Butterflies of the Great Lakes Region", Matthew M. Douglas and Jonathon M. Douglas.

Production of Butterfly Stock

Throughout the summer we also collected both larval and adult butterflies (the latter of which were allowed to mate in order to create breeding stock). Gravid females produced under these conditions (as well as wild-caught gravid females) produced larval offspring used to fill parasitoid "attraction bins" that served as bait boxes for field exposure experiments (see description below). When we found that our larval bait boxes were giving us less than satisfactory results, we constructed larger, more open bait boxes and secured them on elevated platforms (Figure 1) during July and August. We also conducted field sweeps to sample prairie and adjacent field-forest interfaces in order to

record parasitoid species that may attack butterflies but were not attracted to the bait boxes with a sweep method mentioned later.

Figure 1. Platforms for bait boxes to be secured on top of. **Figure 2.** Constructed bait box within breeding tent at site 1.

Breeding of Butterflies to Produce Larval "Bait Stock"

To breed butterflies for the bait boxes (using the field-captured and later, laboratory-reared butterflies), we purchased ten, 18.93-liter plastic waste bins ("terraria") and daily placed appropriate adult nectaring plants as well as fresh larval host plants for oviposition within these containers. The open tops of the terraria were covered with pet mesh (0.48 cm x 0.48 cm) secured by rubber bands and illuminated with either two 40 W bulbs or one 60 W bulb. The inside temperature of the terraria was maintained at approximately 26.7° C, high enough to stimulate mating and ovipositing of the butterflies kept in the terraria. Butterflies that were too large to mate or oviposit in the terraria were kept in larger, closed-top, net hampers with two 60 W bulbs to light and warm them. Photoperiod was regulated on a 12-hour cycle by a plug-in timer from 7:00 in the morning to 19:00 in the evening.

Once the larvae hatched from their eggs on their foodplant, they were relocated to a shoebox-sized plastic storage bin with ventilation screen in the lid and fresh larval foodplant within. These storage bins were cleaned daily to remove old foodplant and frass (this prevented the growth of mold and produced larvae healthy enough to attract

parasitoids). Once the larvae had grown to their 3rd or 4th instar, we placed them in the 10 large parasitoid attraction bins placed around the PCCI property (Figure 3).

Figure 3. Initial setup of bait boxes prior to mounting.

Field Containment of Bait Larvae and Parasitoid Attraction Methods:

Ten large plastic storage bins were used to hold the reared caterpillars used to attract the adults of insect parasitoids in the field. Infected larvae and chrysalides were to be removed from the bins and placed (in isolation) in plastic shoeboxes. However, all larvae in the attraction bins were reared to the imago stage to ensure that no parasitoids were missed.

To provide adequate adult parasitoid access to the bait larvae, we first cut out sections from the top and sides of attraction bins. These openings were covered with wire mesh with 1cm^2 openings—large enough to permit the entrance of virtually all adult parasitoid known to attack butterflies, but small enough to keep out vertebrate predators (e.g., birds and mammals). Laboratory-reared larvae of the test butterfly species were placed in the attraction bins with fresh foodplant and tended daily for frass removal and replacement of old foodplant with new. No more than three different species of larvae were placed in the

attraction bins at any given time.

Attraction bins were placed along the trails (Figure 2), in different habitats, to permit the encounter of as many different species of insect parasitoid adults as possible. To maximize our efforts, the attraction bins were placed in a number of different environments around the property (e.g., open field, tree-canopy, field-forest interface, etc.); however, we attempted to keep all attraction bins within the preferred environments of the butterfly species within (i.e., larvae of forest-edge species were placed along tree-covered trails, and larvae of field-dwelling species were placed along open trails). All bins were originally protected from rain and falling debris by a tent-quality, one-meter square plastic sheets firmly stationed 1m above each bin. The sheets were later removed at the end of June, however, because we feared that it might be keeping the parasitoids from finding their prey within the attraction bins. We also elevated the attraction bins approximately 1m above the ground by way of a platform of plywood supported by a 1 cm-diameter dowel rod. We checked all attraction bins once or twice daily for infected larvae (we looked for either the entry hole of a large parasitoid's ovipositor, by coagulated hemolymph on the exterior of a caterpillar, or by a dead and discolored larvae and/or pupae).

Butterfly Specimen Collection

Throughout the summer we captured gravid females of most of the butterfly species listed in Table 1. These adults were placed in the terraria with appropriate oviposition food plants and nectaring plants, with the objective of producing future broods throughout the study period.

We did not expect to find many butterfly eggs in the field; however, we found many Monarch (*Danaus plexippus*) eggs as well as Baltimore Checkerspot (*Euphyrdyas phaeton*) eggs that were reared into larvae. Most of the Monarch eggs we reared into adulthood and used to produce more eggs for bait larvae. However, the univoltine Baltimore Checkerspot larvae hibernate through the winter and do not pupate until the following spring. The level of parasitoid attack cannot be known with certainy until then.

Last, we collected all butterfly larvae we encountered in the field and identified them

using *The Princeton Field Guide: Caterpillars of Eastern North America* by David L. Wagner All larvae were kept with their foodplants and placed in containers with larvae of their own species.

Table 2. List of field collected larvae**.**

2011 DATE:	BOX/TRAIL	SPECIES	NO.OF LARVAE
Late June	Yellow Trail (wild caught)	*Euphydryas phaeton*	1
Early June-Early August	Red Trail by Batts Cottage	*Danaus plexippus*	24
August 7th	Ed Building (wild caught)	*Papilio polyxenes*	1
August 20th	Yellow Trail (wild caught)	*Papilio polyxenes*	1

Parasitoid Extraction, Collection, and Preservation:

   We checked the attraction bins in the field once or twice daily for any signs of infected caterpillars or chrysalides. Once parasitoid(s) appeared from the remains of the caterpillars or chrysalides we preserved them (through pinning) to create a synoptic collection for PCCI. Using a 10 step/ 10 swipe (100 swipes total per collecting period) sweeping method we collected as many parasitoids as possible from the field and forest-

field interfaces and euthanized them for later identification by experts at Iowa State University and the Smithsonian (these have been identified only to family or subfamily at time of writing).

Parasitoid Identification and Comparisons:

We identified all parasitoid adults to the lowest taxon possible using all resources available to us (e.g., Dr. Matthew Douglas and insect taxonomists at Michigan State University). Next, we recorded observations of any parasitoids emerging from all test species of butterfly to determine any possible parasitoid-host butterfly relationships. Finally, using a camera-mounted microscope (provided by the GRCC Biology Department), we took pictures of all of the parasitoids found for future reference. (Note: Identifications of these parasitoids to the lowest taxon possible are not yet available.)

Timetable for Project:

We began our project on May 17th 2011 by collecting gravid female butterflies in order to begin our breeding program. Throughout the summer we collected butterflies as we encountered them and attempted to breed them. By the end of July we were able to successfully and continuously breed 6 species of butterflies, the Common (Clouded) Sulphur (*Colias philodice*), the Monarch (*Danaus plexippus*), the Black Swallowtail (*Papilio polyxenes*), the Spicebush Swallowtail (*Papilio troilus*), the Cabbage Butterfly (*Pieris rapae*), and the Baltimore Checkerspot (*Euphydryas phaeton*).

When enough larvae were available in the middle of June for the experimental portion of our study, we added them to the attraction bins positioned along the trails. Each attraction bin was checked once or twice daily for the duration of the study. This was performed 1) to ensure that no insect parasitoids went unnoticed; 2) to check on the

condition of the attraction bins; 3) to ensure that vertebrate predators had not damaged the attraction bins; and 4) to ensure that the larvae had sufficient fresh food plant.

At the end of the study (Mid to Late August) we counted the number of infected larvae and the number of adult parasitoids collected. We concluded our data collection on August 17th and removed the bait bins from the trails. All larvae remaining in the attraction bins were raised to adulthood and the imagoes were released back to their natural environment at PCCI.

Results

We expected to find a small but significant number of parasitoid species attacking the larvae of multivoltine species because these butterfly species were continuously present at PCCI throughout much of the late spring and summer, whereas we reasoned that univoltine species of butterflies would emerge *en masse* and after a flight period of perhaps several weeks would be gone for the rest of the year. However, only one larva of *Papilio troilus* was attacked by what has been tentatively identified as the ichneumonid *Trogus pennator,* and just three chrysalides of *Danaus plexippus* were attacked by what we have tentatively identified as the tachinid fly *Lespesia archippivora*. The only parasitoidized larva found in the field (not in the attraction boxes) was a *Euphydryas phaeton* larva collected (with a number of nest-dwelling cohorts) along PCCI's Yellow Trail. The parasitoid larvae that emerged from it belonged to *Apanteles (Cotesia) euphydryidis* on July 2nd 2011. Over 20 other larvae found near this larva on plaintain (*Plantago* spp*)* produced perfect imagoes.

Free-living parasitoids obtained via sweeps were identified only to family or subfamily at this point, and are listed specifically in the chart below as the number of obvious types or "phenons" for each sweeping period (consisting of approximately 100 sweeps with a one meter swath each per collection period):

Table 3. Parasitoids collected by field sweeps across PCCI property**.**

DATE:2011	LOCATION	ORDER	FAMILY	PHENON No.
July 8th	Tent in Prairie by Education Building	Hymenoptera	Ichneumonidae	1
		Hymenoptera	Braconidae	1
		Diptera	Tachnidae	1
July 15th	Prairie by Yellow Trail	Hymenoptera	Ichneumonidae	2
		Hymenoptera	Braconidae	1
		Hymenoptera	Pteromalidae	1
		Diptera	Tachnidae	1
July 25th-August 1st	Prairie by Yellow Trail	Hymenoptera	Ichneumonidae	3
		Hymenoptera	Braconidae	2
August 3rd	South Prairie by Yellow Trail	Hymenoptera	Ichneumonidae	2
		Hymenoptera	Braconidae	2
August 4th	Forest-Field Interface by Yellow Trail	Hymenoptera	Ichneumonidae	3
		Hymenoptera	Braconidae	2
		Hymenoptera	Chalcidae	2
		Diptera	Tachnidae	2
August 7th-17th	Forest-Field Interface by Yellow Trail	Hymenoptera	Ichneumonidae	1
		Hymenoptera	Braconidae	2
		Hymenoptera	Chalcidae	1
		Hymenoptera	Trichogrammatidae	1
		Diptera	Tachnidae	2

Discussion:

PCCI (seems to have) experienced a great abundance and diversity (about 40 species) of butterflies this year in comparison to those of the previous two years. The apparent low rate of parasitoidism (of bait larvae), low sweep counts of parasitoids in the field, and a relatively high concentration of lepidopteran species at PCCI this year suggests that there was indeed low parasitoid diversity and abundance. Interestingly, our data suggest that only 3 species of parasitoids attacking butterflies were actually in residence during this summer. What can explain this apparent anomaly of high butterfly diversity and abundance and very low parasitoid diversity and abundance? As mentioned earlier, many factors affect parasitoid success and thus abundance (Hassell, 2000). Indeed, the rate of parasitoidism in one habitat may not necessarily correlate to that from another nearby. The parasitoidism of the univoltine species *Euphydryas phaeton* is an example of host selection in the previous year (due to its overwintering larvae). In this case, it was serendipitous that we found even one parasitoidized larva, and remarkable that its 20-odd nest cohorts were not attacked.

Although our sweep tests produced perhaps 10 species of insect parasitoids (currently identified as "phenons"), it was difficult to identify them due to lack of current

and complete keys available. Late in this study, it was pointed out to us that parasitoid classification and identification is an enormous task and effective keys to adults of many families simply do not exist (Dr. Douglas Landis and Dr. Gary Parson, Michigan State University, personal communications 2011). These researchers state that identification to family level is possible, but beyond that, because of the virtual death of applied taxonomy and systematics, lack of taxonomic expertise is rapidly growing throughout the world. They go on to say further "…the hosts of many parasitoids are still unknown, and by the same token, we don't even know all the possible parasitoids that attack even the more common Lepidoptera." The only way to remedy this situation is to continue with integrated field and laboratory studies, of which this study is but a small contribution.

Correct identification of the parasitoid wasp and fly species is essential for effective, well-targeted biological control efforts to succeed. To give the reader an idea of the complexity of this level of parasitoid systematics, there are perhaps 85 species of North American *Cotesia*, and there is a need to canvas type specimens to reliably determine conspecific material of the commonly encountered species and prepare interactive keys. To our knowledge, these new keys are still not available.

We concentrated mostly on finding parasitoids attacking multivoltine species of butterflies because the parasitoid life cycle must be completed in each generation as the parasitoids emerge from last-instar larvae or pupae throughout the summer, whereas with univoltine Lepidoptera (such as *E. phaeton*), one would have to wait until the next year to determine the rate of parasitoidism.

The free-living parasitoid adults (both hymenopteran and dipteran) found within our attraction boxes (these clearly did not emerge from our larvae or pupae) were also impossible to identify beyond family level (due to the lack of identification keys) during the time frame of this study.

Parasitoid larvae found emerging from the pupae of *Danaus plexippus* and *Papilio troilus* provide evidence for the existence of parasitoids at PCCI. However, the summer of 2011 was apparently not a "good year" for parasitoid biodiversity and

abundance: Parasitoid abundance level was low, a condition that could be due to numerous factors or combination of factors as stated earlier. A repetition of this experiment every three or four years would produce a greater understanding of the biodiversity and abundance of insect parasitoids attacking butterflies at PCCI.

Conclusion:

We have operationally defined the differences between insect parasites and insect parasitoids. We found several parasitoidized larvae and pupae from host presentation experiments as well as captured a number of as-yet-undetermined species of parasitoid insects through field sweeps. In relation to the butterfly diversity and abundance this year, the parasitoid population at PCCI seemed abnormally low (which could be attributed to many possible causes or combination of causes discussed in this paper). Commercially reared non-native parasitoids are used *en masse* in the biological control of pest insects, but their competitive effects upon native parasitoid populations as well as on non-target butterfly populations remain to be determined.

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