Effects of Monarch Larval Host Plant Chemistry and Body Size on Polistes Wasp Predation

Linda S. Rayor

INTRODUCTION

Monarch larvae are specialists on milkweed (Asclepias spp.) host plants. Milkweeds produce cardenolides, which are bitter, toxic, and effective deterrents for many herbivores and predators that feed on those herbivores (Brower 1984). Cardenolide concentrations, specific array of cardenolides, and relative emetic potency vary significantly among different Asclepias species (Malcolm 1991; Martin et al. 1992). Monarch larvae sequester and concentrate the cardenolides from their host plants in their tissues, hemolymph, and epidermis. Although monarchs do not sequester cardenolides in direct proportion to the concentrations or array in the plants, there is a monotonic increase in cardenolides in monarchs relative to the concentrations in their host plants (Malcolm and Brower 1989). Larvae that feed on host plants with different cardenolide concentrations are assumed to vary in their palatability to predators (Brower 1984). Because Asclepias species with different concentrations of cardenolides in their leaves overlap in their distribution, predators would be expected to encounter monarch larvae that present a spectrum of palatability (Brower 1984). To test the hypothesis that a palatability spectrum exists, I examined the predatory behavior of paper wasps in response to monarch larvae raised on Asclepias species that varied in their cardenolide concentrations.

Predatory wasps exert a major selective force on lepidopteran larvae (Montllor and Bernays 1993; Raveret Richter 2000). The social paper wasps (Vespidae: Polistes spp.) specialize on lepidopteran larvae, which they capture to feed to their developing carnivorous larvae. The wasps are generalist predators, capturing whatever larval prey is abundant and sufficiently palatable. Because the adults feed solely on nectar, their foraging behavior for prey is dictated by the nutritional requirements of their larvae (Raveret Richter 2000). Acceptance or rejection of prey depends on the palatability of plant chemical defenses sequestered in the tissues of the larva or present in its gut (Stamp and Bowers 1991; Stamp 1992). Wasp larvae need to be fed continuously for a period of several weeks prior to pupation, and as soon as the first generation of adult daughters emerges, each nest has multiple foragers searching continuously for prey to satisfy their larval siblings.

Paper wasps are abundant worldwide in both urban and rural areas (Carpenter 1991; Reeve 1991). Their distribution completely overlaps the breeding ranges of monarch butterflies throughout North America (Ackery and Vane-Wright 1984; Carpenter 1991) and Australia (Richards 1978; James 1993). Fertilized queens overwinter in a state of diapause. Depending on the wasp species and climatic conditions in temperate North America, the queens start
building their nests in the spring or early summer (Reeve 1991). The nests contain larvae from early summer into the early autumn (Reeve 1991) and, depending on the species, may be composed of 20 to over 100 individuals by the end of the summer (Reeve 1991). Polistes nests are found on eaves of buildings, fence posts, mailboxes, or in vegetation—essentially any location sufficiently protected from the elements. Wasp nests are numerous, in close proximity to one another, and most of the available nest sites are occupied in suitable habitats (pers. observ.).

Paper wasps are efficient, intelligent, generalist predators capable of flying over vast areas to search for prey. They search plants for prey while hovering or walking on them (Raveret Richter and Jeanne 1991; Nannoni et al. 2001; pers. observ.) and are attracted to frass and herbivore-damaged plants (Cornelius 1993). Wasp search behavior is influenced by the structure of the larval host plant (Geitzenauer and Bernays 1996). However, paper wasps are large enough that they are rarely deterred by a plant morphology (waxy or hirsute leaves) that can inhibit the movement of smaller insect predators (Eigenbrode et al. 2000).

Polistes predation involves locating prey, attacking it by repeated biting (never stinging), processing it to a manageable size, and flying back to the nest with pieces of prey (Rabb and Lawson 1957; Raveret Richter and Jeanne 1991; pers. observ.). Wasps make orientation flights as they leave the site of a successful prey capture (Raveret Richter 2000). After dropping off the prey on the nest, the forager rapidly returns to the site, where she may have left behind pieces of the prey, or to another site where hunting has been successful. The wasps return again and again to successful predation sites, which may be tens to hundreds of meters from their nests (Rabb and Lawson 1957; pers. observ.). Although wasps do not recruit nest mates to prey, they are highly visual and alert to what other wasps are doing, and are attracted to prey inspection or processing by other individuals (Raveret Richter 1990; Raveret Richter and Tisch 1999).

Numerous anecdotal reports of paper wasp predation on monarch larvae are found on Internet discussion groups, and predatory wasps have been observed to kill substantial numbers of monarch larvae in cornfields (M. R. Berenbaum, pers. comm.). However, there are no published accounts of these behaviors. Monarch larvae have been reported to be (potentially or actually) preyed on by reduvid and pentatomid bugs, coccinellid and lacewing larvae, ants, spiders, and birds (Lynch and Martin 1993; Zalucki and Kitching 1982; Calvert 1996; James 2000; Stimson and Kasuya 2000; Prysby, this volume). Because the Polistes wasps do not remain on the plant with the prey, they may be missed more often than the other predators. Yet these relatively large wasps may be especially important sources of mortality for later instar larvae.

Here, I report studies in which I examined (1) whether Polistes wasps readily prey on monarch larvae, (2) whether cardenolide concentrations in host plants affect the number of monarch larvae that the wasps capture, (3) whether the wasps prefer alternative prey species without cardenolides, and (4) whether wasps preferentially attack and successfully kill monarch larvae of a particular size.

**METHODS**

My students and I collected paper wasps, *P. dominulus* (Vespidae: Polistinae), from the wild around Ithaca, New York, during late May and early June in the years 1995 to 1998, 1999, and 2000. We maintained them in nests composed of 10 to 20 cells with eggs or larvae and a single or double foundress. We enclosed the wasps and their nests in cardboard boxes (approximately 30 × 20 cm) with screened front panels, which we hung in rooms (5.8 × 2.7 m) in the Entomology Insectary Greenhouse on the Cornell University campus. After the wasps had accepted the boxes as new sites for their nests (about 3 days), we removed the screens to allow them free flight throughout their room. Each room contained 4 to 7 nests, depending on the numbers of active foragers. All adults were individually marked with colored enamel paint. Other than the initial foundresses, all subsequent wasps were reared entirely in the greenhouse and had foraging experience only with prey species provided to them.

To evaluate whether the wasp behavior was natural in the controlled greenhouse environment, I investigated prey choice and size preferences in free-living wasps on the Cornell campus in 2000. In the outdoor experiments, the behavior and choices of the wild wasps agreed in all details with what we observed in the controlled greenhouse environment.
I will present only size preference data from the outdoor experiments here.

Monarch colonies were started annually from an inbred colony kept at Cornell University (Hughes et al. 1993) and mixed with wild monarch adults or larvae obtained from Minnesota (most years) or Virginia (in 1999 only). We allowed adults to oviposit on host plants in breeding cages and raised subsequent larvae on the appropriate Asclepias species. For the behavioral choice experiments (below), wasps were given a choice between monarch larvae that were raised on Asclepias species with high (A. curassavica), medium (A. syriaca, A. incarnata), or low (A. tuberosa, A. verticillata) cardenolide concentrations. (Mean cardenolide value for A. curassavica = 1055, A. syriaca = 50, A. incarnata = 14, A. tuberosa = 3, and A. verticillata = 1 μg/0.1 g dry weight of leaf tissue [Malcolm 1991].) Monarchs do not sequester cardenolides in direct proportion to the concentrations found in the Asclepias host plant, but most effectively sequester from species with intermediate cardenolide concentrations (Malcolm and Brower 1989). For example, monarchs raised on A. syriaca can concentrate cardenolides to approximately 350 in μg/0.1 g of dry butterfly, far higher than in the plant (Malcolm and Brower 1989). Monarchs feeding on high-cardenolide plants maintain high levels in their tissues, while monarchs raised on low-cardenolide plants retain low levels in their tissues.

Experimental design: Cardenolide concentrations

I developed behavioral choice tests to evaluate whether the wasps discriminate among monarch larvae that vary in their cardenolide concentration. My students and I presented freely foraging wasps with a choice of live monarch larvae on plants, with the two types of larvae used in each test based on what they were raised on: A. curassavica versus A. incarnata, 21 hours, 1995; A. curassavica versus Pieris napi or Trichoplusia ni, 13 hours, 1996; A. curassavica versus A. tuberosa, 55 hours, and A. curassavica versus A. verticillata, 3 hours, 1998; A. curassavica versus A. syriaca, 29 hours, and A. curassavica versus Junonia coenia, 2 hours, 1999. We observed foragers in one or two 1-hour sessions daily and recorded detailed behavioral data using the Noldus Observer 3.0 behavioral software on Psion handheld computers. We made focal observations on 5 to 10 individually marked wasps simultaneously. Prey was available only during recording sessions. With this experimental design, it was possible to record how many prey each wasp killed, along with details of her prey handling for her entire foraging life. The data presented here reflect the total number of prey items returned to the nests by the wasps ([initial attacks + prey remains + finds (remains left by other wasps) + stolen prey] – [rejected + dropped prey] = total prey items returned to nest, sensu Rayor and Munson 2002). Only wasps that had 5 or more total prey items were included in the analyses.

At the beginning of each choice test, we placed 6 to 8 size-matched larvae of each type on separate, large, minimally herbivore-damaged Asclepias “presentation” plants. As they were killed, larvae of the appropriate type were replaced on plants to maintain equal numbers of each prey type during the test. Care was taken not to attract the attention of foraging wasps as the larvae were replaced, and larvae were placed somewhere on the plants other than where the previous larva had been killed. Groups of presentation plants were less than 1 m from each other. Presentation plants were exchanged and leaves rinsed between sessions to minimize visual or olfactory cues from larval hemolymph. We moved presentation plants once during and between sessions so wasps could not simply return to the same location where they had experienced previous foraging success. The larvae were presented on the same Asclepias host plant species on which they had developed unless noted otherwise.

Experimental design: Effect of monarch larva size

Palatability is not the only factor that may affect predation by wasps. To determine whether there is a size at which monarch larvae are at particular risk of predation, I compared predation on three size classes of larvae in outdoor experiments for 10 h. My students and I placed live large (late fourth through fifth instar), medium (late third through early fourth instar), and small (second through early third instar), monarch larvae on two large A. curassavica plants. Each plant contained three individuals of each size (9 larvae total), all raised on A. curassavica. We recorded the number of times a wasp encountered or killed larvae of each size class. In addition, for each size class we calculated a “risk factor” (= probability of encounter × probability of mortality ×
100 = [number of encounters within the size-class / total encounters] × [number of times killed / number of encounters within the size-class] × 100).

**RESULTS AND DISCUSSION**

Do wasps prey on monarch larvae?

Paper wasps consumed substantial numbers of monarch larvae in the controlled indoor experiments, and free-living wasps outdoors flew far from their nests to forage on plants containing the larvae. Well over 5000 monarch larvae were killed and eaten by wasps over the course of this study. Although there were statistical differences in how the wasps responded to monarchs raised on the five different *Asclepias* species, the wasps readily consumed substantial numbers of all of these monarchs (see below).

Do wasps prefer larvae raised on milkweed plants with lower cardenolide concentrations?

The tendency of wasps to prefer monarchs reared on different *Asclepias* species was dependent on which two plants were compared. Figures 5.1 to 5.3 illustrate wasp preferences; in all cases the prey type predicted to be more palatable is on the x-axis, and the less palatable on the y-axis. Individual wasps captured significantly more *A. syriaca*–reared larvae than *A. curassavica*–reared larvae in behavioral choice tests (Wilcoxon-signed rank test [WSRT], \( T = 1800, p < 0.0001, \text{ wasp } n = 65; \) figure 5.1), and significantly more *A. incarnata*– than *A. curassavica*–reared monarchs (WSRT, \( T = 87.5, p < 0.002, \text{ wasp } n = 13, \) figure 5.2). Additionally, *A. verticillata*–reared monarchs were preferred over *A. curassavica*–reared monarchs (data not shown due to small total sample size for individual wasps: 52 *A. verticillata* prey, 33 *A. curassavica* prey, wasp \( n = 17, \chi^2 = 4.24, p < 0.05 \)). For choices among monarch larvae raised on these four host plant species, individual wasps were significantly more likely to capture the lower-cardenolide, presumably more palatable larvae than the higher-cardenolide, less palatable larvae.

However, wasps were equally likely to capture monarchs raised on *A. tuberosa* (low cardenolide) and *A. curassavica* (high) (WSRT, \( T = 842, p < 0.27, \text{ } n = 48 \text{ wasps, figure 5.3} \)). Female monarchs rarely oviposit on *A. tuberosa* when other host plants are available (pers. observ.), and the growth rate of larvae on *A. tuberosa* is significantly slower than that of monarchs raised on other plants. *Asclepias tuberosa* is low in nitrogen, which may reduce the palatability or nutritional quality of monarchs reared on this plant species (L. P. Brower, pers. comm.).

It is possible that monarchs may entirely escape wasp predation if they are raised on *Asclepias* species with even higher cardenolide levels than those found in *A. curassavica* (1055μg/0.1 g dry weight), such as *A. masonii* or *A. albicans* (7910 and 2845μg/0.1 g dry weight, respectively [Malcolm 1991]), but I did not test monarchs raised on these plants. Wild and captive wasps readily consumed monarch larvae reared on *A. curassavica*.

Although there is evidence that the wasps discriminate between larvae raised on different host plants and that they tend to prefer larvae that have
sequestered lower cardenolide concentrations, the basis for the discrimination is not as tightly associated with cardenolide levels as I had predicted. There are likely to be other morphological, architectural, and nutritional factors associated with different Asclepias species that affect both monarch palatability and relative risk of wasp predation.

Contrary to my expectations, the most productive foragers, individuals with the most experience preying on monarchs, did not capture a greater percentage of palatable larvae than did wasps with less foraging experience. In all linear regressions between “total prey taken” and “percent palatable prey taken” for choice tests between monarchs raised on A. curassavica and on A. tuberosa, A. syriaca, or A. incarnata, less than 2% of the variation (all $R^2$ [adjusted] ≤2.1%, all $F$ = not significant) was explained by greater experience with the prey.

Foraging wasps indicate dissatisfaction with prey by rejecting it entirely, wiping their mandibles frantically, or dropping the prey and then grooming their mouthparts and head persistently. Although some prey are totally rejected and left behind, wasps often resume processing what appears to be somewhat distasteful prey. As a part of the processing sequence with a diversity of lepidopteran larvae, wasps often remove guts that contain plant material. I have shown that Polistes wasps selectively remove the guts of P. napi that contain plant material from the crucifer Erysimum, which contains cardenolides, but do not remove the guts of conspecific larvae that fed on cabbage (pers. observ.). Wasps occasionally removed the guts of monarchs as they maxillated the prey into manageable balls for transport, but the removal of the guts did not appear to be deliberate (as it is for Erysimum-containing pierids). Gut removal was infrequent and not apparently in relation to cardenolide content in the larvae (pers. observ.). Because monarchs sequester chemical defenses throughout their bodies, removing the gut was not a sufficiently successful strategy to increase monarch palatability.

Do the wasps prefer species of larvae without cardenolides to monarch larvae?

When wasps were given a choice between highly palatable prey, such as the apparently succulent cabbage butterflies (Pieridae: P. napi) or cabbage looper moths (Noctuidae: T. ni) that had been raised

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Figure 5.2. Wasp predation on monarch larvae reared on A. incarnata and A. curassavica. Data points represent the total number of prey pieces of each type that 13 individual wasps took back to their nests. Data above the diagonal line indicate a preference for A. curassavica–reared prey; data below the line indicate a preference for A. incarnata–reared prey. More pieces of A. incarnata– ($n = 166$) than A. curassavica– ($n = 90$) reared prey were brought to wasp nests.

Figure 5.3. Wasp predation on monarch larvae reared on A. tuberosa and A. curassavica. Data points represent the total number of prey pieces of each type that 48 individual wasps took back to their nests. Data above the diagonal line indicate a preference for A. curassavica–reared prey; data below the line indicate a preference for A. tuberosa–reared prey. As many pieces of A. curassavica– ($n = 465$) as A. tuberosa– ($n = 435$) reared prey were brought to wasp nests.
on cabbage, they clearly preferred to capture the more palatable larvae over monarchs (WSRT, $T = 21$, wasp $n = 6, p = 0.02$). In these choice tests, although the wasps appeared to locate the brightly colored monarchs first, individual foragers rapidly switched to the more cryptic $P. rapae$ and $T. ni$ prey species. In contrast, when given a choice between monarch larvae and those of another unpalatable prey species, such as the buckeye butterfly (Nymphalidae: $J. coenia$), which contains highly unpalatable iridoid glycosides (Bowers 1984), the wasps preferred to take monarchs (WSRT, $T = 32.5$, wasp $n = 8, p < 0.025$). Thus, while monarch larvae are not necessarily the most palatable prey available to wasps, monarchs are highly acceptable to the generalist paper wasps and in some circumstances are considered significantly more palatable than the alternative prey species.

**Do wasps preferentially attack monarch larvae of a particular size?**

Wasps were attracted to large larvae first and tried to attack them (figure 5.4). But large larvae were difficult for most wasps to kill. The large larvae ignored the wasps, jerked their bodies, or dropped from the plant in 78 of 80 encounters. Most wasps simply gave up and hunted elsewhere. Only twice (less than 3%), after a prolonged battle, did wasps kill large monarch larvae. The risk factor for large monarchs is only 1.71.

Medium larvae were often sighted secondarily. But once encountered by the wasps, 67% ($n = 18/27$) were promptly killed (figure 5.4). Medium larvae have a risk factor of 15.38, an order of magnitude higher than that of the large larvae. Larvae in this size range are the preferred size of prey for most wasps (Rabb and Lawson 1957) and are clearly the most vulnerable to this relatively large invertebrate predator.

Small larvae were largely ignored by the wasps. Of the 117 total contacts between wasps and larvae in this experiment, only 10 involved small larvae (figure 5.4). Wasps generally killed small larvae once they were noticed, but such larvae were clearly smaller than the search image of most wasps. Small larvae have a risk factor of 6.84. While small monarchs are likely to be more vulnerable to smaller predators, such as coccinellids, chrysopids, and nabids (J. Losey, pers. comm.), at this size they are not at great risk from *Polistes* wasps. As monarch larvae grow, they bypass the risk of predation by smaller predators, but transiently increase their vulnerability to larger ones.

**CONCLUSION**

*Polistes* wasps are likely to be a significant source of mortality to monarch larvae throughout their range. Regardless of the cardenolide concentrations found in the *Asclepias* species that monarch larvae are raised on, paper wasps find these monarch larvae to be acceptable prey. Overall, wasps prefer to take more larvae raised on species with a lower cardenolide concentration ($A. syriaca$, $A. incarnata$, $A. verticillata$), but cardenolide concentration is not the only factor affecting relative palatability (e.g., monarchs raised on $A. tuberosa$ were less palatable than those raised on milkweed containing higher levels of cardenolide). Larvae that evade small invertebrate predators and reach medium sizes (from the late third through early fourth instar) are at the highest risk from *Polistes* wasps.

To a large extent, the effect of larval mortality due to predators has been underestimated in monarch population biology. Although monarch larval mortality is extremely high in field experiments (Zalucki

![Figure 5.4. Monarch larva size affects both encounter rate and risk of predation by *Polistes* wasps.](image)
and Kitching 1982; Prysby and Oberhauser, this volume; J. Losey, pers. comm.), most reports of predation focus on adults rather than larvae. Observing predation in the field can be difficult, and the predators’ sublethal effects on larval behavior and growth can be subtle (Stamp and Bowers 1991). Future work on monarch larvae should further document the role of predation in monarch population biology.

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References


