

Natural Enemies and Survival of Monarch Eggs and Larvae

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INTRODUCTION

Natural enemies are important to many Lepidoptera species. They can alter larval foraging behavior (Montllor and Bernays 1994), adult oviposition behavior (Bernays and Chapman 1994), and population dynamics (Dempster 1984; Haukioja 1994). They can also mitigate the effects of lepidopterans on their host plants (Montllor and Bernays 1994) and have been implicated in the evolution of host plant specificity in some species (Bernays and Graham 1988; Montllor and Bernays 1994).

A commonly held view states that monarchs are protected from natural enemies by their sequestration of cardenolide toxins from their milkweed host plants, and much research has utilized this assumption. For example, Rawlins and Lederhouse (1981) suggested that this chemical defense, along with larval warning coloration, allows monarch larvae to spend significant amounts of time basking in direct sunlight. Additional examples include studies of mimicry (e.g., Cohen 1985) and natural history (e.g., Urquhart 1960).

However, monarchs do have natural enemies and their effects are not negligible, at least on the adult stage. In Mexican overwintering sites, avian predation results in mortality rates of at least 9% (Calvert et al. 1979; Brower and Calvert 1985), and mice are estimated to kill about 5% of the monarchs in a given overwintering colony (Brower et al. 1985; Glendinning et al. 1988). Avian predators also consume monarch adults at the Californian overwintering sites (Sakai 1994). Wasps are the only

invertebrate predator so far observed to attack overwintering adult monarchs (Leong et al. 1990). Additional observations have been made of natural enemies of adult monarchs during the breeding season. A few observations of bird predators exist (Smithers 1973; McIsaac 1991), but in most cases it is unknown whether these birds are significant predators of monarchs or naïve birds tasting a monarch for the first time. There are also several records of invertebrate predators feeding on adult monarchs, including spiders, mantids, and dragonflies (Smithers 1973; White and Sexton 1989).

Despite this research focus on predators of adults, natural enemies are likely to have a more significant impact on monarch eggs and larvae. Monarchs suffer significant mortality in these stages, with less than 12% surviving to the fifth instar (Borkin 1982; Oberhauser et al. 2001). Predators and parasitoids are important factors influencing survival and population dynamics in many other insect herbivore systems (Haukioja 1994) and are likely to be important sources of mortality in monarchs as well (Borkin 1982). Only a handful of studies, however, have investigated the specific effects of predators and parasitoids on immature monarchs.

Anecdotal field observations of monarch eggs and larvae cite ants, various Hemiptera species, coccinellids, cockroaches, spiders, wasps, lacewing larvae, and red velvet mites as monarch predators (Smithers 1973; Borkin 1982; Zalucki and Kitching 1982; pers. observ.). However, quantification of the importance of these predators is inconsistent or lacking, with a few exceptions. Calvert (1996, 1999, this volume) describes introduced fire ants (*Solenop-*

sis invicta Buren) as a major predator of monarch eggs and larvae in Texas, and suggests that the presence of fire ants severely limits the monarch population in that area. In a study of variation in monarch densities during the spring in Louisiana and Texas, Lynch and Martin (1993) frequently observed predation of monarch larvae by both crab spiders and ants. They found only weak correlations between the presence of monarchs and the presence of spiders or ants on milkweed, but still suggested that spiders and ants together could be a major mortality factor for monarchs in that region. Finally, Zalucki and Brower (1992) observed no effect of predator exclusions on monarch survival to the second instar, and concluded that host plant characteristics are a larger determinant of monarch survival through the early stages than are natural enemies.

Though generally thought to be more effective on vertebrates (Brower 1984), the sequestered cardenolides may deter some invertebrate predators. Berenbaum and Miliczky (1984) demonstrated that cardenolide-containing milkweed bugs were distasteful to mantid predators. Malcolm (1989) observed that the consumption of cardenolide-containing milkweed aphids by spiders resulted in a disruption of the spiders' web building and a reduction in the capture rate of future prey. Finally, Raylor (this volume) found that wasps that consume monarchs fed on either *Asclepias tuberosa* or *A. curassavica* (high-cardenolide milkweed species) developed more slowly than those that consumed a prey species without cardenolides.

Monarch parasitoids include 12 species of tachinid flies and at least 1 species of brachonid wasp (Arnaud 1978). Records of tachinid fly parasitism in North American monarch populations cite varying rates of parasitism, ranging from 1% in southern Ontario (Urquhart 1960) to 11.5% in Wisconsin (Borkin 1982) to 43% in Texas and Louisiana (Lynch and Martin 1993). The rate is much higher for Australian monarchs, reaching up to 100%, particularly in the fall (Smithers 1973; Zalucki 1981). Similarly high rates (70% to 98%) of parasitism exist in breeding populations of monarchs in central Mexico (E. Montesinos, pers. comm.). Parasitism rates of up to 42% were observed in Hawaiian monarchs. In the case of the Hawaiian monarchs, the parasitoid was *Lespesia archippivora*, a biocontrol agent introduced to control armyworms (Etcheagaray and Nishida 1975a, 1975b).

Given that the studies on invertebrate predators of immature monarchs are few, largely qualitative, and conflicting, the goal of my research was to investigate the overall importance of invertebrate predators and parasitoids on monarch eggs and larvae. I sought to answer three specific research questions: (1) Are natural enemies in general, and invertebrate predators specifically, a significant source of mortality in natural monarch populations? (2) What are the specific effects of ant predators on natural monarch populations? (3) What is the extent of tachinid fly parasitism in natural monarch populations, and how does it vary geographically and temporally? I used two field experiments to study predators and estimated parasitism rates. I gathered additional data on parasitism rates as part of the Monarch Larva Monitoring Project (Prysby and Oberhauser, this volume).

METHODS

Effects of aerial and terrestrial predators on monarch survival

In June of 1998, I compared the survival of monarchs exposed to and those protected from predators. I focused on the egg and early instar stages. In an old field habitat in west-central Wisconsin, I chose 20 triplets of milkweed ramets, with the 3 plants within 2 m of each other. I made an effort to choose triplets of similar size and condition; otherwise choices were made haphazardly. I cut back the surrounding vegetation to a distance of approximately 0.25 m to ensure that no other plants were touching the ramets, and removed all invertebrates from them. I randomly assigned each ramet in a triplet to one of three treatments: exclusion of aerial and terrestrial predators, exclusion of terrestrial predators only, or control (no exclusion). Aerial predators included those likely to approach a ramet from the air, such as wasps, while terrestrial predators were those that crawled up the stem, such as ants. Exclusion treatment ramets had Insect Trap Coating paste (Tanglefoot Company, Grand Rapids, Michigan) applied to an 8 to 10-cm area of the stem, above the ground but below the lowest set of leaves. The "aerial and terrestrial" treatment ramets also had tomato cages covered in bridal veil placed over them. The cages were approximately 1 m tall and cone-shaped. I placed heavy chain over the

bottom of the bridal veil to hold it down to the ground.

On 24 June, I collected eggs from lab-reared monarch butterflies. The eggs were laid on potted *A. curassavica* plants (tropical milkweed). Following the methods of Zalucki and Brower (1992), on 25 June I punched eggs out of the *A. curassavica* leaves using standard-sized hole punches, creating small leaf disks, each with 1 egg attached. Four eggs on leaf disks were glued to each milkweed ramet using milkweed latex from nonexperimental ramets. One disk was attached to the upper side of each of the four uppermost open leaves on each milkweed ramet.

Each morning for the following 7 mornings, I examined the plants and recorded the status of the monarch as alive, missing (monarch not located), or dead (monarch located but dead or nonviable). I also noted any other invertebrates found inside the cages or on the ramets.

Effects of ant and aphid presence on monarch survival

I conducted a pilot experiment in 1998 to see whether *Formica montana*, the dominant ant species at the field site, would attack monarch eggs. I glued one leaf disk with an egg to leaves on eight milkweed ramets on which *F. montana* individuals were tending aphids and to eight ramets without ants. I observed the ants' behavior and egg status on all ramets for several hours. I returned to the site after 48 h and recorded the status of any eggs that survived the initial observation period.

On 18 July 1999, I collected eggs from lab-reared female monarch butterflies on potted *A. curassavica* plants. The following day, I walked five belt transects across the same field site as used in the 1998 experiment. For every milkweed occurring along the belt transect, I recorded the presence or absence of ants and aphids. I then glued leaf disks, each with 1 egg, to 20 ramets in each of three categories: ants and aphids present, aphids only present, or clean (no ants or aphids). I assigned ramet categories on the basis of ant and aphid presence at the time when the eggs were first attached; the presence of ants and aphids on a ramet varied over the course of the 5-day experiment. I attached 1 egg/ramet to the underside of a leaf on the first or second open tier of leaves, mimicking the typical natural location of

monarch eggs on *Asclepias syriaca* (common milkweed).

I included the aphids-only category because the presence of *F. montana* on milkweed ramets is highly associated with the presence of aphids (see Results) and because *F. montana* is a known aphid-tending species (Henderson and Jeanne 1992). I predicted that aphid presence would affect monarch survival indirectly, since ramets with aphids would be likely to attract ants.

On each day for the following 5 days, I monitored the ramets and recorded the status of each monarch (alive, dead, or missing), the stage of the monarch if present, the status of the leaf disk (present or absent), and the presence or absence of ants and aphids. On the final day of the experiment, I recorded the actual number of ants on each ramet, and a categorical estimate of the number of aphids (0, 1 to 10, 10 to 100, 100 to 1000, more than 1000).

Tachinid fly parasitism

During each week in the summer of 1999 and 2000, I recorded the presence of monarchs on a large sample of milkweed ramets (more than 100 ramets) (Prysbly and Oberhauser, this volume). All fourth or fifth instar larvae were collected and reared to adults in the laboratory. Each larva was later scored as being a healthy butterfly, parasitized by tachinid flies, infected with the protozoan parasite *Ophryocystis elektroscirrha*, or dead from another cause. The flies were identified to family only, except for three voucher specimens that were sent to the U.S. Department of Agriculture (USDA) Systematic Entomology Laboratory for species identification. In 1999, I collected larvae from the same Wisconsin site where I did the predation experiments. In 2000, larvae were collected at five cornfields, field edges, and nonagricultural sites as part of a larger study of monarch phenology (Oberhauser et al. 2001). In addition, volunteers with the Monarch Larva Monitoring Project (Prysbly and Oberhauser, this volume) collected and reared fourth and fifth instars and recorded outcomes.

Statistical analyses

For both experiments, I used logistic regression (LR) analyses to test the effects of each predictor on the probability of monarch survival. I used back-

ward elimination to select the simplest possible model for explaining the variation in monarch survival (Trexler and Travis 1993; Agresti 1996). I followed a similar procedure to identify differences among treatments by combining two treatments into a single factor and comparing the resulting model to the model with all treatments as separate factors (Agresti 1996; S. Weisberg, pers. comm.). For the predator exclusion experiment, I included a blocking factor for the 20 different triplets of plants. I used the Arc program for all analyses (Cook and Weisberg 1999).

RESULTS

Effects of aerial and terrestrial predators on monarch survival

Overall monarch survival rate over 7 days was 18% (44/240), but it varied significantly among the three predator exclusion treatments (figure 4.1). Most monarchs died in the egg stage. Table 4.1 summarizes the analysis of factors that affected survival. Probability of monarch survival depended both on plant treatment and on plant triplet, the blocking factor. Of the nonsurviving monarchs, 11% were observed dead and 89% were missing. For the analyses, missing monarchs were assumed to be nonsurvivors, since eggs or very small larvae would be unlikely to survive if removed from the milkweed plant (Zalucki and Kitching 1982). Three eggs and one larva recorded as dead were killed accidentally, and these individuals were excluded from the regression analyses. Most surviving monarchs were first instars at the end of the 7 days.

To investigate differences among the three treatments, I ran stepwise simplifications of the regression model, setting two treatments equal to each other and checking for significant effects of the simplification using likelihood-ratio tests (table 4.1). All the treatments are significantly different from each other, but the differences between the control and exclusion treatments are much greater than the difference between the two exclusion treatments ($p < 0.001$ vs. $p = 0.032$).

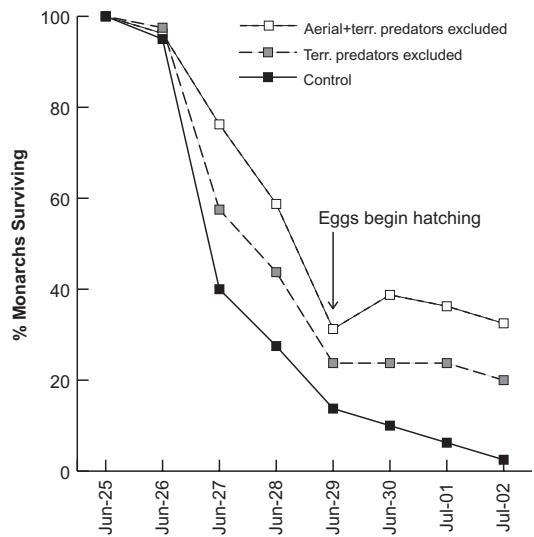


Figure 4.1. Monarch survival rate over time for three predator exclusion treatments. Each treatment group initially had 80 monarch eggs. Survival declined the most during the egg stage and leveled off after the eggs began hatching. The statistical analysis shown in table 4.1 considers only the final survival percentages at the end of the experiment.

Table 4.1. Significant predictors of monarch survival in the predator exclusion experiment

Predictor(s) tested	Deviance	df	ΔDeviance	Δdf	p value
Full model	47.37	38			
Ramet treatment	81.08	40	33.71	2	<0.0001
Block	81.54	57	34.17	19	0.0176
Terrestrial only vs. aerial and terrestrial	51.97	39	4.60	1	0.0320
Control vs. terrestrial only	62.37	39	14.99	1	0.0001
Control vs. aerial and terrestrial	80.90	39	33.52	1	<0.0001

Note: The full analysis of deviance likelihood ratio model has a blocking factor (representing the 20 ramet triplets) and a treatment factor with three categories (control, terrestrial predators excluded, aerial and terrestrial predators excluded). Each row shows the significance of including that predictor in the model or, in the case of the comparisons among treatment categories, the significance of the difference between a pair of categories. In each row, “Deviance” is the model deviance when the predictor is excluded from the model or the categories are combined, and “ΔDeviance” is the change in deviance when compared to the full model that has the predictor included or the categories separated. A significant *p* value indicates that the model simplification causes a significant change in model deviance. Both block and ramet treatment are significant, and all three treatment categories are significantly different from each other.

Neither the cages nor the adhesive were 100% effective. Invertebrates other than the monarchs were found on at least one occasion inside 11 of the 20 cages excluding aerial predators and on 8 of the 20 ramets excluding only terrestrial predators. Nine of these observations were of potential or known monarch predators, mainly ants and mites, and most occurred after two episodes of storms and high winds (on 26 and 27 June) that knocked over the cages and some of the ramets. In comparison, 14 of the 20 control ramets had invertebrates on them during the experimental period; 11 of these had ants.

Effects of ant and aphid presence on monarch survival

Of the 8 monarch eggs attached to milkweed ramets with *F. montana* present, ants removed all 8 within 90 min during the pilot study. Of the 8 eggs attached to ramets without *F. montana*, all survived at least 90 min, but 7 were missing 2 days later.

Table 4.2 shows the number of milkweed ramets observed with ants and aphids in the survey performed on day 1 of the experiment. Ant presence on milkweed ramets is significantly associated with aphid presence ($\chi^2 = 90.1, df = 1, p < 0.0001$). The dominant ant (more than 80% of those observed) was identified as *F. montana* (Emery) (identified by D. R. Smith, Systematic Entomology Laboratory, Agricultural Research Service, USDA).

Table 4.3 shows the numbers of ramets from the three initial treatment groups according to the five categories of aphid abundance at the end of the experiment. When comparing the aphids-only category to the ants and aphids category, there is an association between category and number of aphids ($r = 0.30, M^2 = 3.54, df = 1, p = 0.06$). Ramets with ants tended to have higher densities of aphids than did

those without ants. In addition, ramets that had only aphids at the beginning of the experiment tended to have fewer ants on the last day than did ramets that had ants and aphids ($T = 1.95, df = 34, p = 0.06$).

The overall monarch survival rate on the 60 ramets over the 6 days was 23% (14/60), with only 5% of the monarchs on ramets with both ants and aphids on day 1 surviving (1/20) (figure 4.2). Most mortality for the ants and aphids category group occurred within 24 h (and thus on the eggs) of the start of the experiment. Probability of monarch survival depended on treatment (LR test = 6.92, $df = 57, p = 0.0314$).

Table 4.3. Ramet totals

Initial treatment group	Aphid abundance at end of experiment				
	0	1-10	10-100	100-1000	>1000
Control (clean)	15	4	0	0	0
Ants and aphids	1	2	6	9	2
Aphids only	3	7	7	3	0

Note: Number of ramets from each initial treatment group in five categories of aphid abundance, as measured on 24 July 1999 (final day of the experiment). There is a weak association between treatment group (ants and aphids or aphids only) and the categorical measurement of aphid number ($r = 0.30, M^2 = 3.54, df = 1, p = 0.06$).

Table 4.2. Milkweed ramets with ants or aphids

	Aphids present	Aphids absent	Totals
Ants present	73	9	82
Ants absent	45	130	175
Totals	118	139	257

Note: Number of ramets with ants or aphids, as measured on 19 July 1999, the first day of the experiment. Ant presence is significantly associated with aphid presence ($\chi^2 = 90.1, df = 1, p < 0.0001$).

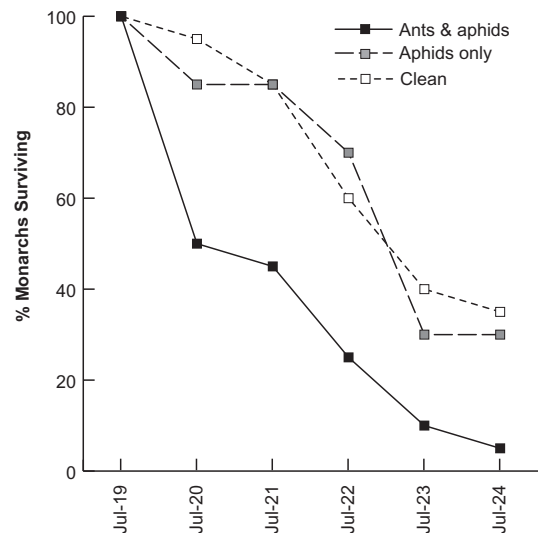


Figure 4.2. Monarch survival rate over time on milkweed ramets with aphids only, aphids and ants, and neither aphids nor ants (clean). Each category initially had 20 monarch eggs. Survival rates on clean ramets and ramets with aphids only are similar, and both are higher than the survival rate of monarchs on ramets with ants and aphids.

Table 4.4. Effects of ant and aphid presence on monarch survival

Model	Deviance	df	Δ Deviance	Δ df	<i>p</i> value
Full model	58.27	57			
Aphids only vs. ants and aphids	63.00	58	4.72	1	0.0298
Clean vs. ants and aphids	64.47	58	6.20	1	0.0128
Clean vs. aphids only	58.39	58	0.11	1	0.7356

Note: The full analysis of deviance likelihood ratio model has one predictor, plant category, with three levels (clean, only aphids present, ants and aphids present). Each row shows the significance of differences among the three categories. In each row, "Deviance" is the model deviance when the two categories are merged, and " Δ Deviance" is the change in deviance when compared to the full model that has the categories separated. A significant *p* value indicates that the model simplification causes a significant change in model deviance. The presence of ants and aphids together significantly affects the probability of monarch survival, while aphid presence alone does not affect monarch survival.

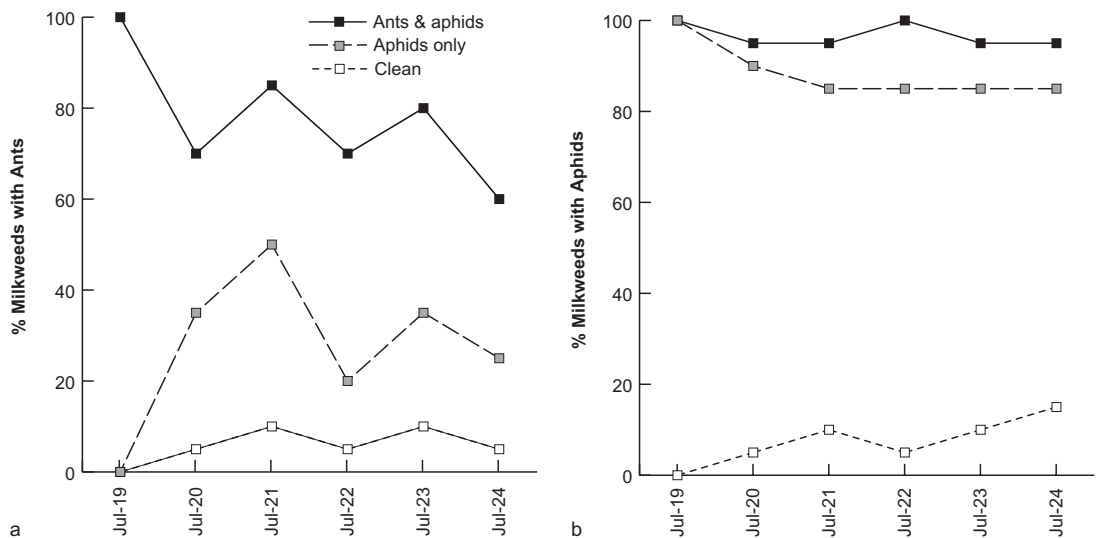


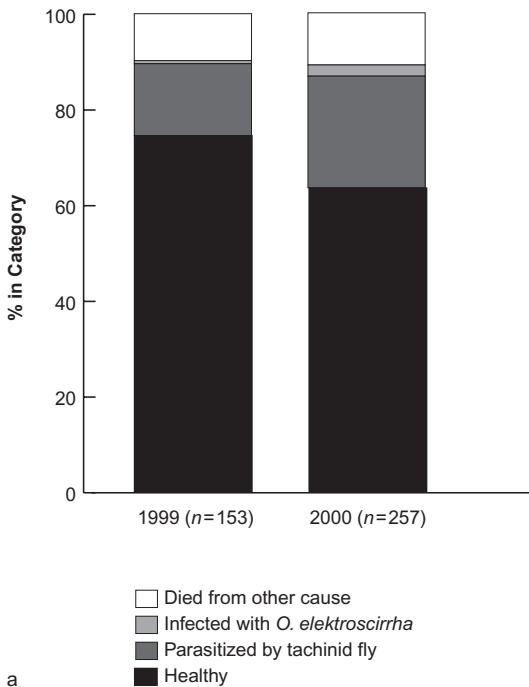
Figure 4.3. Percentage of milkweed ramets (out of 20) in the three initial categories with (a) ants present and (b) aphids present. Plant categories were assigned on the basis of ant and aphid presence on the first day of the experiment, so that all of the ramets in the ants and aphids category had both ants and aphids on day 1, although they did not all continue to have both throughout the experiment. Similarly, no ramets in the clean category had ants or aphids on day 1, but some ramets were subsequently colonized.

Table 4.4 shows the results of comparisons of the three treatment categories. The clean and aphids-only categories are both significantly different from the ants and aphids category, but not from each other. Therefore, ant presence reduces the probability of monarch survival, but aphid presence has no effect.

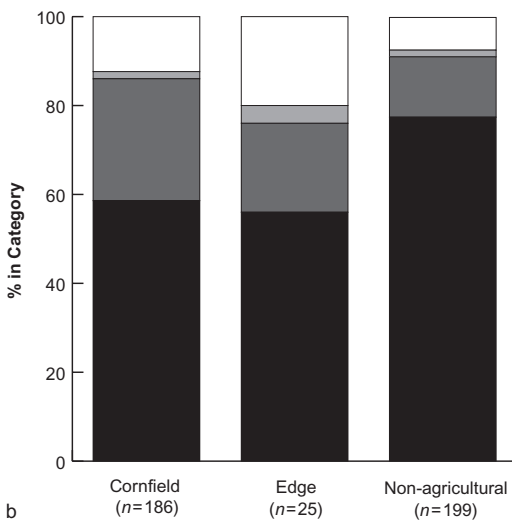
Because both ants and aphids are mobile, the experimental plants did not necessarily remain in their initial categories throughout the experiment. There was fluctuation in the number of plants with ants and aphids on them for the three initial categories (figure 4.3).

Parasitism rate observations

In 1999, 15% of the fourth and fifth instar monarch larvae collected were parasitized by tachinids; the rate was 23% in 2000 (figure 4.4). Three of the tachinid flies were identified as *L. archippivora* (Riley) (identified by N. E. Woodley, Systematic Entomology Laboratory, Agricultural Research Service, USDA). The other flies were similar in phenotype but were not identified to species. In 1999 and 2000, I observed hyperparasitoids in a total of four of the tachinid fly individuals and these were identified as *Perilampus hyalinus*



a



b

Figure 4.4. Percentage of larvae collected resulting in four outcomes: healthy adults, parasitized by tachinid flies, infected with *Ophryocystis elektroscirrha*, or died from another cause (including bacterial and viral diseases). All larvae were fourth or fifth instars when collected. Figure 4.4a shows the results divided by year; figure 4.4b shows the results divided by habitat.

(Say), though this group is now under revision (identified by E. E. Grissell, Systematic Entomology Laboratory, Agricultural Research Service, USDA). Approximately 10% of larvae collected each year died of other causes, including viral and bacterial

diseases, developmental problems, and accidental deaths. A small percentage of larvae collected (0.6% in 1999, 2% in 2000) were infected with the protozoan parasite *O. elektroscirrha*. All monarchs with *O. elektroscirrha* were highly infected, with more than 1000 spores/cm² (Altizer and Oberhauser 2000).

A chi-square test showed no association between year and parasitism outcome ($\chi^2 = 6.68$, $df = 4$, $p > 0.10$), so I combined the data for both years to analyze the association between habitat type and outcome (figure 4.4), which was significant ($\chi^2 = 19.91$, $df = 6$, $p < 0.005$). More larvae than expected from the cornfields were parasitized, and more larvae than expected from the nonagricultural habitats were healthy.

Figure 4.5 shows parasitism rates for additional sites monitored by Monarch Larva Monitoring Project volunteers. Parasitism rates varied from 0% to 55%. Species were not identified.

DISCUSSION

Effects of aerial and terrestrial predators on monarch survival

The results of the predator exclusion experiment clearly show an increase in monarch survival when terrestrial (nonflying) invertebrates are excluded. Potential predators observed on the milkweed ramets during the experiment included ants, spiders, and red velvet mites, with ants the most common (observed on half of the control ramets at some point during the experiment).

Most of the mortality difference between the control and exclusion treatments occurred within the first 48 h of the experiment, though the differences in mortality widened slightly near the end of the 7-day experimental period. Monarchs on ramets excluding terrestrial predators suffered almost no mortality after eggs began hatching, while those on control ramets suffered mortality during the larval stage. Odds of survival were significantly higher for monarchs on ramets excluding both aerial and terrestrial predators than for those on ramets excluding only terrestrial predators. However, it is difficult to distinguish these differences from a cage effect. Monarchs in the cages were more protected from the elements, particularly during the two large storms that occurred during the experiment, and were probably less likely to fall off of the milkweeds.

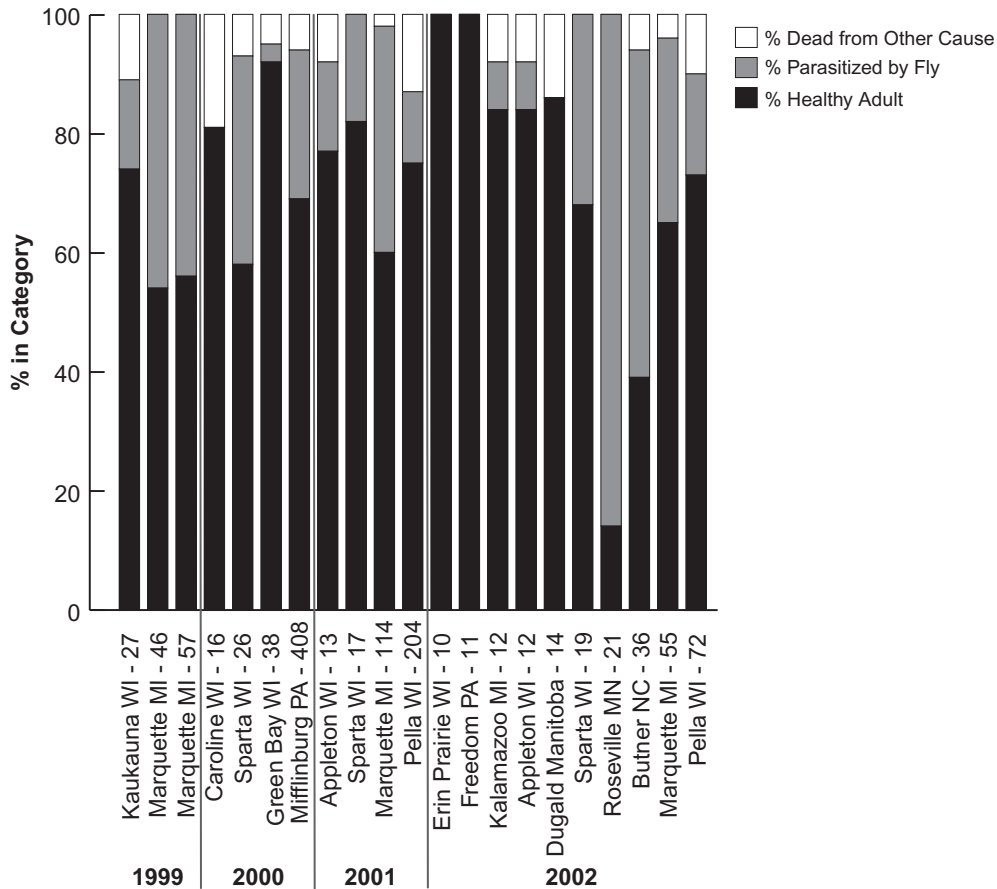


Figure 4.5. Percentage of larvae collected that were parasitized, not parasitized, or dead from another cause. Data from all Monarch Larva Monitoring Project (MLMP) sites with more than 10 larvae collected are shown. MLMP volunteers did not measure infection by *O. elektroscirra*. Data are from 2000, unless otherwise noted. The Mifflinburg, Pennsylvania, site includes parasitism by both tachinid flies and an unknown hymenopteran.

Overall, 65% of the monarchs on the ramets excluding aerial and terrestrial predators were missing or dead at the end of the experiment. Some of this mortality could be due to experimental error or natural sources of mortality other than predators. Experimental errors include predators within enclosures, ineffectiveness of the latex glue technique (causing eggs to desiccate or be lost), accidental removal of eggs or larvae by observers, and larvae mired in the Insect Trap Coating paste. The latter two sources of experimental error are the only sources that can be directly quantified; together, they account for 3 of the 52 nonsurviving monarchs on the ramets excluding aerial and terrestrial predators. Natural mortality sources other than predators include larvae mired in the milkweed latex, larvae killed by the toxic cardenolides in the milkweed, eggs

washed off of the plant in the rainstorms, larvae wandering off of the plant, and infertile eggs that did not hatch. I did not observe any larvae mired in the latex, and larvae are unlikely to be killed by the low cardenolide concentrations in *A. syriaca* (Malcolm and Zalucki 1996). Eggs usually withstand rain events, but most monarch eggs are laid on the undersides of leaves and are perhaps more protected than the eggs in this study (pers. observ.). The percentage of infertile eggs produced by female monarchs is variable, but can be as high as 55% for some females (M. Solensky, pers. comm.).

While it is clear that predator enclosures lead to higher survival rates, more studies are needed to further quantify the effects of predation in natural monarch populations. Data on how these effects vary temporally, spatially, and among different

immature stages would provide a useful complement to this study. To reduce experimental error, future studies using the hole-punched leaf disk technique should use either older eggs that will hatch sooner or a stronger adhesive to reduce the likelihood of eggs falling off the plants. Attaching the leaf disks to the undersides of the milkweed leaves, rather than the upper sides, may also reduce experimental error. In addition, more stable predator enclosures would prevent failure during storms.

Effects of ant predators on monarchs

Based on both field surveys of milkweed and the enclosure experiment, ants (particularly *F. montana*) appear to be a common potential monarch predator, at least in this field site. Ants were found on 30% of milkweed ramets sampled just prior to the experiment and were significantly associated with milkweed ramets colonized by aphids. Surveys in southern Wisconsin by Hendersen and Jeanne (1992) also indicate a significant presence of *F. montana* on *A. syriaca*, with up to 20% of the ramets harboring ants tending aphids. In addition, Hendersen and Jeanne (1992) found *F. montana* on *A. syriaca* from late May until late August, nearly the entire breeding season of monarchs in the Upper Midwest (Prysky and Oberhauser, this volume).

Survival was seven times higher for monarchs on the clean ramets and ramets with aphids only than on the ramets with both ants and aphids (35% vs. 5%). Half of the monarchs on the ramets with ants and aphids died or disappeared within the first 24 h of the experiment. This pattern is consistent with my pilot study observations, in which *F. montana* individuals found and destroyed monarch eggs within minutes. Mortality on the clean ramets was likely due either to ants or other predators foraging on those plants during the study or to leaf disks falling off of the ramets. The latter experimental error probably accounted for less mortality in this experiment than the 1998 enclosure experiment because there were no storms and I periodically reattached loose leaf disks during the study. One clean ramet appeared to have been eaten by a deer or other herbivore during the experiment, and that monarch was reported as dead, since it had been in the egg stage. The survival rate of the monarchs on the clean ramets was higher than the survival rate of the monarchs on the control plants in the 1998 experiment, and was approximately the same as the survival rate

observed by Malcolm and Zalucki (1996) on *A. syriaca* over a similar time period.

Given that ant presence is so strongly associated with aphid presence, I had expected to see a lower survival rate on the aphids-only ramets than on the clean ramets. These two categories, however, had virtually the same results (see figure 4.2, table 4.4). There are two possible factors influencing this result. First, ramets in the aphids-only category tended to have fewer aphids than the ramets in the ants and aphids category (see table 4.3). When I set up the experiment, it was unusual to see ramets that had high numbers of aphids, but no ants. Aphid density is likely to influence ant density and foraging (Hendersen and Jeanne 1992). Future studies should examine the effect of aphid density as well as aphid presence on monarch survival. Second, ramets in the aphids-only category may have recruited fewer ants during the experiment than ramets in the ants and aphids category, as evidenced by the differences in ant numbers on the last day of the experiment. While I addressed only the effects of ant presence and not the effects of different ant densities in this experiment, there is likely to be a relationship between ant density and monarch survival.

Given the results of this field study, a more controlled field or lab study to investigate the effects of *F. montana* and other ant species on monarch survival and behavior would be worthwhile. Future studies could examine the effects of ant density, and monarch stage on monarch survival. Spatially explicit studies accounting for the distribution of ant mounds and milkweed ramets may also yield interesting results. Finally, given the severe impact ants can have on monarch survival, experiments on the female monarch's choice of location for oviposition are an important next step. At least one other butterfly avoids host plants with ants (Bernays and Chapman 1994), and similar studies in monarchs would broaden our understanding of how and why monarchs choose specific host plant individuals.

Extent of tachinid fly parasitism in natural monarch populations

The high rates of tachinid fly parasitism observed in both 1999 and 2000 and in multiple locations demonstrate that parasitoids are a very significant source of mortality in monarchs. The difference in parasitism rates among the three habitats surveyed is intriguing. One possible explanation is that *L.*

archippivora demonstrates a density dependence in relation to its monarch host; per ramet monarch densities were significantly higher in cornfields than in the nonagricultural habitats (Oberhauser et al. 2001). The pattern also could be related to the use of alternative hosts by *L. archippivora*. This tachinid species is a generalist, attacking 13 different Lepidoptera families (Etchegaray and Nishida 1975a). Differences in the densities of these alternative hosts in the different habitats may affect the pattern observed. Alternatively, the differences could be due to the behavior of individual flies. Perhaps hosts are easier to find in cornfield habitats, or some other aspect of that habitat attracts them, such as the density or array of the host plants. In Australia, Zalucki (1981) found that milkweed patch size strongly influenced rates of parasitism in monarchs by another tachinid species. Parasitism rates were highest for larger patches of milkweed and lowest on isolated single ramets.

There are not enough data at this point to look for temporal or geographic trends in tachinid fly parasitism in monarchs. However, there is considerable variation within just the Upper Midwest and Great Lakes region (see figure 4.4). Parasitism levels in the Upper Midwest appear to be lower than that observed in nonmigratory populations of monarchs in Mexico (E. Montesinos, pers. comm.).

SYNTHESIS

The results of these studies demonstrate that natural enemies are important to monarchs. Both predators and parasitoids can significantly lower monarch survival, and these effects can occur as early as the egg stage (in the case of ant predators) or as late as the fifth instar or pupa (in the case of tachinid fly parasitoids). Although monarch eggs do contain some cardenolide toxins (Brower 1984), these toxins do not appear to afford them much protection against invertebrate natural enemies.

While these studies lay a basic groundwork for studying natural enemies in the monarch system, they also open many new questions. For example, are natural enemies a regulating factor for monarch populations (Dempster 1984)? How does the effect of natural enemies compare to other factors, such as host plant quality and availability and abiotic factors, and how does this effect vary in space and

time? Do synergistic or antagonistic effects exist between natural enemies and other ecological factors? Previous theoretical and empirical studies indicated that natural enemies, though an important mortality source, do not play a role in the regulation of lepidopteran populations because they are not adequately density dependent (Dempster 1984). The natural enemies studied here, *F. montana* and *L. archippivora*, are likely to be too polyphagous to act as density-dependent monarch population regulators, but this system certainly needs more study to arrive at a definitive conclusion. The answers to these questions are important both for monarch conservation and for improving our understanding of insect herbivore population growth.

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