Entomology Publications   Entomology

2020

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Keywords

Disciplines

Behavior and Ethology | Ecology and Evolutionary Biology | Entomology | Natural Resources and Conservation | Population Biology

Comments

ORIGINAL PAPER

Estimates of common milkweed (*Asclepias syriaca*) utilization by monarch larvae (*Danaus plexippus*) and the significance of larval movement

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Abstract

The population of monarch butterflies east of the Rocky Mountains has noticeably declined over the past two decades. The decline is due, in part, to loss of breeding and forage habitat in the Southern and Midwestern USA. To support a resilient overwintering population of six hectares of occupied forest canopy, approximately 1.6–1.8 billion additional ramets of milkweed are needed in the summer breeding range. Milkweed establishment that facilitates natural behavior of monarchs is necessary for effective conservation restoration. This study explored the effect of milkweed ramet density on larval search behavior, milkweed utilization, and survival without predation, parasitism, or competition. Under our experimental greenhouse conditions, monarch larvae abandoned their natal ramet, and subsequent ramets, prior to the pre-pupal wandering stage and before all available leaf biomass on a ramet was consumed. This is consistent with previous field observations. Larvae consumed biomass from three or four milkweed ramets that totaled the approximate biomass of single 10–35 cm ramet. Movement behavior suggests that isolated ramets may not support development through pupation, even though an isolated ramet could provide enough biomass. Our results suggest milkweed patches containing at least two to four ramets of closely-spaced common milkweed would provide sufficient biomass for development and increase the likelihood that larvae moving in random directions would encounter non-natal ramets to support development. Larval movement behavior and biomass requirements are critical aspects of monarch larval biology that should be considered in habitat restoration and maintenance plans, monitoring survey designs and protocols, and population modeling.

Keywords Monarch · *Danaus plexippus* · Common milkweed · *Asclepias syriaca* · Larval movement behavior · Larval host plant abandonment · Survival

Introduction

Monarch butterflies (*Danaus plexippus*) east of the Rocky Mountains are an iconic species known for their annual, tri-country migration. The hectares (ha) of forest surface area occupied by congregations of butterflies is an indirect measure of monarch overwintering population (Garcia-Serrano et al. 2004). From the winter of 2003–2004 through 2018–2019, the average overwintering population was 3.5 ha, which is below an estimated resilient population size of 6 ha (Brower et al. 2012; Oberhauser et al. 2017). Current conditions pose a quasi-extinction risk to the annual migration phenomenon (Semmens et al. 2016). Causes of the decline include loss of overwintering habitat in Mexico, loss of breeding and forage habitat in the Southern and Midwestern USA, and extreme weather conditions (Brower et al. 2012; Inamine et al. 2016; Thogmartin et al. 2017).

Breeding success, and therefore the size of the overwintering population, is dependent on the status of milkweed species (*Asclepias* sp.), in particular common milkweed (*Asclepias syriaca*) (Malcolm et al. 1993; Geest et al. 2019), which are obligate host plants for oviposition and larval development. Because of urbanization and conversion of

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grasslands to row crop agriculture with more efficient weed management technologies, common milkweed abundance has declined in the Midwest USA (Hartzler 2010; Pleasants and Oberhauser 2013; Pleasants 2017). Approximately 1.6–1.8 billion additional milkweed ramets are needed to support an overwintering population goal of 6 ha of occupied forest canopy (Thogmartin et al. 2017). The spatial arrangement and density of new milkweed habitat patches that align with monarch behavior are necessary for effective conservation. The adult female monarch is vagile and simulation studies indicate higher egg densities in the landscape are expected with uniform establishment of small habitat patches, as compared to larger aggregates of habitat widely dispersed (Zalucki and Lammers 2010; Zalucki et al. 2016; Grant et al. 2018). The extent to which increased realized fecundity will result in increased population size depends on larval movement and survival within habitat patches with varying milkweed ramet densities (Zalucki et al. 2016; Zalucki and Kitching 1982c).

Current understanding of larval Lepidoptera dispersal and movement behavior comes largely from observations of species that lay eggs in clusters and whose larvae are polyphagous or oligophagous. With these species, neonates can undergo undirected, long distance dispersals to move away from their siblings by "ballooning" (a behavior where the neonate hangs from the host plant by a strand of silk and is blown downwind; Zalucki et al. 2002; Goldstein et al. 2010; Razze et al 2011; Razze and Mason 2012). Movement by larger larvae occurs by walking, because their weight negates their ability to balloon (Zalucki et al. 2002). For example, both 4-day and 7-day old tobacco budworm (*Heliothis virescens*) were observed to abandon their natal transgenic cotton plant and move to adjacent plants (Parker and Luttrell 1999). Over 15 days, western bean cutworm (*Striacosta albicosta*) larvae traveled up to 3.54 m to a new corn plant (Pannuti et al. 2016). In general, dispersal behavior is observed with species capable of rejecting their natal host plant in search of more suitable or higher quality plant species (Zalucki et al. 2002); e.g., the bay checkerspot butterfly (*Euphydryas editha bayensis*) switches host species based on weather and plant conditions (Hellman 2002).

The extent to which observations of larval movement and natal plant abandonment with poly/oligophagous species, which lay eggs in clusters, can be extrapolated to the monarch, a monophagous species that lays individual eggs on its host plant, is unclear. Long-range monarch neonate dispersal has not been reported. Numerous field studies suggest third, fourth, and early fifth instars commonly abandon their natal common milkweed ramet and move to co-located milkweed (Urquhart 1960; Rawlins and Lederhouse 1981; Borkin 1982; Zalucki and Rochester 2004; De Anda and Oberhauser 2015). The extent to which movement observed in the field is due to top–down or bottom–up drivers is unknown. In addition, the interplay of ramet density, larval movement behavior, leaf consumption rates, developmental rates, and survival rates have not been examined experimentally.

The present study was designed to improve understanding of monarch larvae utilization of common milkweed and movement between milkweed ramets in a greenhouse setting without predation, parasitism, and intra- or interspecies competition. In a series of experiments, we quantified biomass consumed, number of leaves with feeding, and plants visited through development when larvae were provided two, three, or four ramets of common milkweed. We hypothesized that prior to natal ramet abandonment, there would be no differences in larval behavior, consumption of biomass, and number of leaves with feeding injury, despite the number of available ramets. Assuming that larval movement is a correlated random walk, we further hypothesized that larvae would find new host plants more successfully, have a higher cumulative survival rate from neonate to pupation, and shorter developmental times when larvae had access to an increasing number of milkweed ramets. During these experiments, we also collected ancillary data to inform potential motivation for ramet abandonment.

Methods

This study assessed the relationship between milkweed ramet availability and monarch larval behavior and survival. In a preliminary study conducted in 2016, 33 neonates were placed in cages with single stems of common milkweed and observed until pupation. When larvae were observed off their milkweed ramet, they were placed on a new ramet to simulate finding a new plant. All 33 larvae abandoned milkweed ramets 2–3 times during development. Given these consistent observations with a single ramet, the present study employed an experimental design with 2, 3, or 4 caged milkweed ramets to assess if there is an effect of milkweed density on larval survival, movement, and milkweed biomass consumption.

Plants

Common milkweed seed was collected from plants around Ames, Iowa USA in 2016 and 2017. Seeds were stratified by placing a mix of cleaned seed with sand, vermiculite, and water in a cold room (0:24 L:D; 4 °C) for at least 6 weeks. Seeds were planted in 128-cell plug trays (Landmark Plastics, Akron, OH) containing soil (Sungro Professional Growing Mix SS#1-F1P, Agawam, MA) and time-release fertilizer (Osmocote Pro 19-5-8, Dublin, OH). When seedlings had 4–6 leaves, they were transplanted into 8.9 cm square deep perennial pots (Kord, Ontario, Canada). Seedlings and plants were maintained in a greenhouse (sodium light-augmented

16:8 L:D cycle, approximately 56% RH, windows opened when temperatures exceed 21 °C). Plants were watered twice per day. Experiments were initiated approximately 8 weeks after planting when ramet height was between 10 and 35 cm, consistent with monarch oviposition preferences (Urquhart 1960; Bergstrom et al. 1995; Fischer et al. 2015).

Insects

The United States Department of Agriculture (USDA), Agriculture Research Service (ARS), Corn Insects and Crop Genetics Research Unit (CICGRU) in Ames, IA maintains multiple colonies of monarch butterflies. Each year a new colony is established from field collected eggs. Neonates from the colony established the year prior to experimentation were used for each trial to reduce effects of colony inbreeding (i.e., 2016 or 2017 colonies were using in 2017 and 2018 experiments). Experiments were initiated with neonates acquired within five hours of hatching.

Experimental design

Two, three, or four individually-potted common milkweed ramets were placed at least 16 cm from each other in cages created from mesh pop-up laundry baskets (57 × 37 × 55 cm; Honey-Can-Do HMP-03891 Mesh Hamper with Handles, Walmart, Rogers, AK) and "no-see-em" netting (Arrowhead Fabric Outlet, Duluth, MN) under greenhouse conditions (Fig. 1). Ramets were labeled to document larval movement among plants. Milkweed ramets were measured from soil surface to new leaf growth and fully extended leaves were counted (median = 14 leaves per ramet). Leaves with signs of injury or disease were removed prior to initiation of an experiment. Pots were buried in potting soil to provide a uniform surface at the base of the ramets.

Three treatments consisted of cages with two, three or four milkweed ramets. Cages were distributed in a random

complete block design, where each block (rows on greenhouse benches) contained three replicates of each treatment. In 2017, four trials with three blocks were conducted (108 total cages). In 2018, three trials were conducted. Because of plant availability, the first trial contained three blocks, the second trial contained nine blocks, and the third trial contained four blocks (144 total cages).

Monarch neonates were added to cages in random order. One neonate was randomly placed on the top portion of one ramet in each cage; this ramet was termed the "natal ramet". Twice daily (at approximately 0600 h and 1600 h), cages were observed for larvae. If a larva was found dead, the cage was terminated. If a larva could not be found, the cage was monitored during the next two observation periods. A larva missing a total of three consecutive observation periods, or 1.5 days, was assumed dead and the cage terminated.

Trials in 2017 focused on larval ramet abandonment behavior. Ramet occupied, instar, and molting status were recorded until pupation. Twenty-four hours after pupation, pupae were collected and weighed. Day of ramet abandonment was defined as the day when the larva was first observed off the ramet.

Trials in 2018 quantified monarch plant utilization and biomass consumed. Twice daily (at approximately 0600 h and 1600 h), ramet occupied, portion of the ramet occupied (top, middle, or bottom third), surface of the leaf occupied (new leaf growth, top side of leaf, underside of leaf), instar, and molting status were recorded until larvae moved from their natal ramet. When larvae were first observed off the natal ramet, the condition of each plant in the cage was ranked based on the percent of leaves consumed (see Table 1) and the height and number of full leaves were recorded. All leaves with signs of feeding were removed from the ramet, photographed with a Kodak Easyshare camera (model # C1550), and stored in a drying oven (30 °C, 0% RH). If 90% of the leaf or more was consumed, a leaf from a non-experimental milkweed ramet of comparable size to the missing leaf was used to estimate surface area consumed and mass of the consumed leaf (base of the leaf with feeding was matched to the base of a non-experimental full leaf without feeding). Based on results from the 2017 study, there was typically insufficient biomass remaining in cages with 2 or
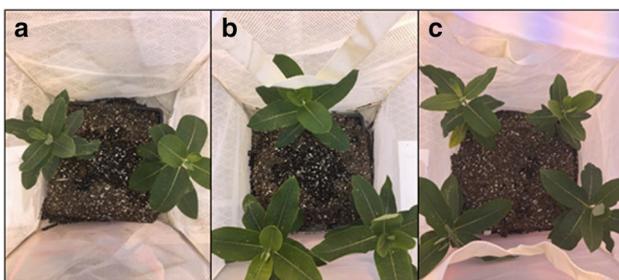

Fig. 1 Experimental cages with common milkweed ramets. **a** Two ramets of common milkweed. **b** Three ramets of common milkweed. **c** Four ramets of common milkweed. Potted ramets were placed approximately 16 cm apart and covered with potting soil to provide a single surface

Table 1 Description of milkweed ramet condition ranking

Rank	Ramet condition
5	No feeding
4	Between 0 and 25% of leaf material consumed
3	Between 25 and 50% of leaf material consumed
2	Between 50 and 75% of leaf material consumed
1	Between 75 and 100% of leaf material consumed
0	All leaves consumed

3 plants after leaves with evidence of feeding were removed. Therefore, after abandonment of a natal ramet, cages containing two or three ramets were terminated. Larvae in cages with four ramets were monitored until pupation. Every time a larva abandoned a ramet, the height of plants in the cage were measured and number of full leaves were recorded. Leaves with feeding were removed, photographed, and dried. Some fifth instars consumed entire leaves and it was not possible to determine the size of missing leaves. These leaves were reported as consumed, but biomass was not estimated. Twenty-four hours after pupation, pupae were collected and weighed and the height of milkweed ramets measured and fully extended leaves counted. Leaves collected with evidence of feeding were dried at room temperature for at least one week. Leaf material was subsequently weighed to estimate dry leaf mass (mg) not consumed per leaf.

Biomass consumed was calculated using ImageJ Software (Rasband 2018; Schindelin et al. 2012) to estimate leaf area consumed (cm^2). Monarch larvae feed on milkweed leaves in predictable patterns (leaf tips, notches out of the side of a leaf, holes in the center of a leaf) and all milkweed leaves have generally the same shape, therefore the area consumed was estimated by measuring the area to fill in the leaf. Dry mass per cm^2 was calculated for each leaf based on the dry leaf mass and the computed leaf area not consumed. An estimate of dry biomass consumed per leaf was determined by multiplying the mass per cm^2 by the total area consumed calculated with ImageJ.

Statistical analysis

Characteristics of natal ramet abandonment for individuals that survived to natal abandonment in 2017 and 2018 were analyzed with generalized linear models in RStudio version 1.0.153 (RStudio Team 2016) using the package emmeans. Variables to test our additional hypotheses (time observed on plant material, survival rates, pupal weights, and developmental times) were analyzed similarly with data collected in 2017. Generalized linear models accounted for trial (with year embedded), block, and treatment; trial, block, and their interactions showed no effect at a 0.05 level of significance.

In all analyses, data residuals were normally distributed and appropriately dispersed. Differences in instar survival rates were evaluated with a chi-square analysis in RStudio version 1.0.153 (RStudio Team 2016).

Ramet abandonment was explored with all individuals that survived to pupation in 2017. Number of plants visited through complete development and total number of ramets abandoned, including the natal abandonment, were summarized. Generalized linear models were used to understand the effect of trial, block, and ramet treatments on these characteristics. Timing of movements (day vs. night) were analyzed with movement data from 2017 and 2018 using a Wilcoxan sign rank test in RStudio 1.0.153 (RStudio Team 2016).

With individuals from 2018, estimates of ramet utilization were quantified. Portion of ramet utilized (top, middle, or bottom third), portion of leaf utilized (top, underside, new growth, or stem), biomass consumed prior to natal ramet abandonment, number of leaves with feeding prior to natal abandonment, and plant condition rank (Table 1) at abandonment were quantified. Biomass consumption was analyzed by instar at time of abandonment using generalized linear models. For larvae provided four plants in 2018, number of plants with signs of feeding and number of leaves with feeding were summed within a cage. Biomass consumption estimates through complete development were made for nine larvae. Two sample t tests assuming equal variance were conducted in RStudio 1.0.153 (RStudio Team 2016) to determine if larvae consumed more leaves and biomass from their natal ramet or from subsequently visited ramets.

Results

Survival and developmental endpoints

Number of ramets had no significant effect on the number of individuals surviving to pupation (F = 2.494; df = 2, 155; P = 0.0826). Across both years, 67% (105/156) of individuals survived to pupation (74% in 2017; 52% in 2018 with four ramet cages; see Table 2). Of the 51 individuals that did not survive to pupation, 41 were observed as dead bodies

Table 2 Number (and percent) of individuals that survived to developmental stages by year, number of ramets, and pooled across years and number of ramets

| Developmental stage | Number of individuals | | | | |
	2017 2 Ramets	2017 3 Ramets	2017 4 Ramets	2018 4 Ramets	Pooled
1st Instar	35 (97%)	32 (89%)	33 (92%)	40 (83%)	140 (90%)
2nd Instar	34 (94%)	30 (83%)	31 (86%)	32 (67%)	127 (81%)
3rd Instar	34 (94%)	29 (80%)	29 (80%)	28 (58%)	120 (77%)
4th Instar	32 (89%)	24 (67%)	28 (78%)	26 (54%)	110 (70%)
5th Instar	30 (83%)	22 (61%)	28 (78%)	25 (52%)	105 (67%)
Total	36	36	36	48	156

and 10 were assumed dead because they were missing for three consecutive observation periods. In both cases, mortality was assigned to the instar stage when last observed alive. Mortality rates generally declined with developmental stage, but were not significantly different (X-squared = 0.88198; df = 4; P = 0.9271); 10% died in the first instar, 8.3% died in the second instar, 4.5% died in the third instar, 6.4% died in the fourth instar, and 3.2% died in the fifth instar. Similar trends were noted when data were examined by year and number of ramets per cage (see Table 2).

Based on the experiments conducted in 2017, number of ramets present in the cage had no effect on neonate to pupation development time (F = 0.096; df = 2, 76; P = 0.9080), pupal weight (F = 0.031; df = 2, 75; P = 0.9693), length of time in the pupal stage (F = 0.053; df = 2, 74; P = 0.9485), or the developmental time from neonate to adult eclosion (F = 0.113; df = 2, 74; P = 0.8929). Development from neonate to pupation took 11.6 ± 1.60 (sd) days. Pupae weighed 1.31 ± 0.188 g and pupal duration lasted 9.65 ± 1.21 days. Time from neonate to eclosion was 21.3 ± 2.44 days.

Natal ramet abandonment

As noted previously, in the 2016 preliminary study all 33 instars abandoned their natal ramets. Across 2017 and 2018 trials, all larvae that did not die (n = 162 out of 252) abandoned their natal ramet. There was no significant effect of number of ramets on number of days elapsed prior to natal ramet abandonment (F = 0.201; df = 2, 161; P = 0.8179) or on instar at ramet abandonment (F = 0.008; df = 2, 161; P = 0.9921). Larvae abandoned the natal ramet after an average of 6.73 ± 2.04 (sd) days, 6.33 ± 2.06 days, and 6.62 ± 2.53 days for cages with two, three, or four ramets, respectively. Abandonment occurred most frequently in the fourth instar (mode = 4; mean = 4; sd = 1; max = 5, min = 1). At natal ramet abandonment in 2018, the average ramet condition rank was 2.35 ± 1.36 (min = 0, max = 4); i.e., on average approximately 25–50% of the leaf material remained at natal ramet abandonment.

Larval movement behavior

Larvae were observed more often on the top portion of the ramet than on the middle or the bottom (Fig. 2a; Z < − 3.013; df = 2, 254; P < 0.0073). Larvae also were observed more often on the underside of a leaf than on the top side of the leaf, on the stem, or on new growth (Fig. 2b; Z < − 5.037; df = 3, 339; P < 0.0001). After larvae abandoned their natal ramet and successfully found another, they continued to abandon ramets until pupation. The number of ramets had no effect on total ramet abandonments (F = 0.004; df = 2, 76; P = 0.9961); on average larvae abandoned ramets 3.00 ± 1.26 times (min = 1,
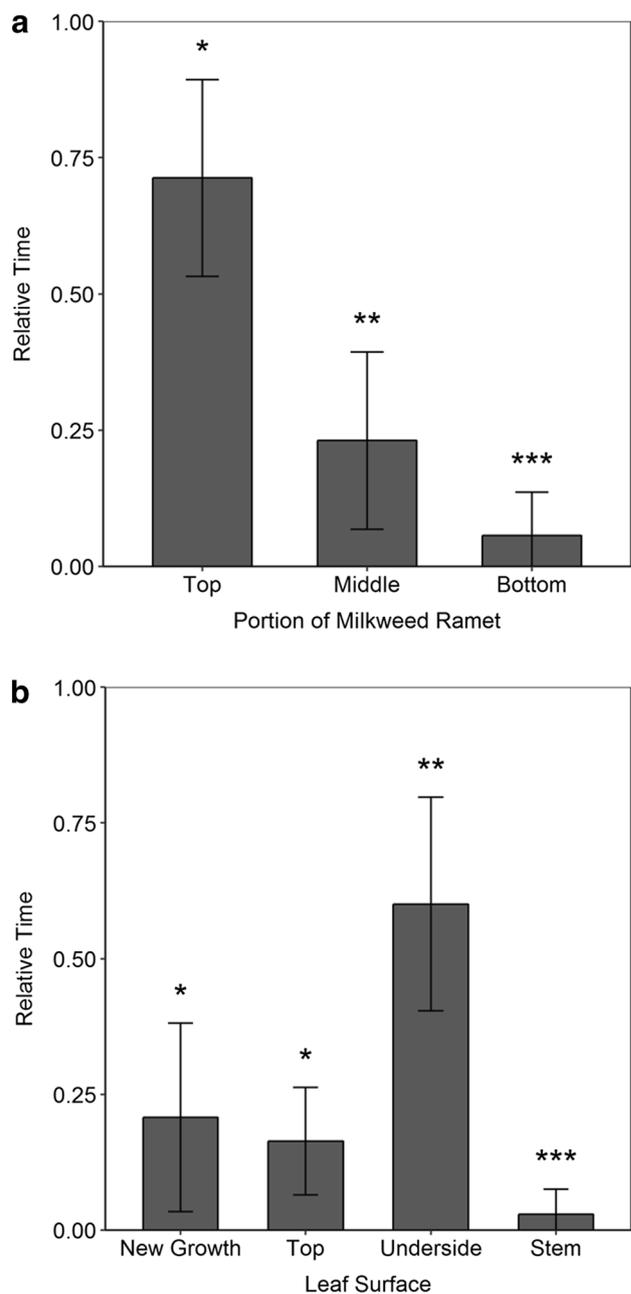

Fig. 2 Relative time monarch larvae were observed on portions of milkweed ramets. Relative time is the number of observations associated with a specific portion or a ramet or leaf surface divided by the total number of observations. **a** Observed location of larva on ramet (top, middle, or bottom third). **b** Surface of leaf where larvae were observed (underside, top, new growth). Bars represent the mean ± one standard deviation. Different number of asterisks (*) above bars represent a significance value < 0.05

max = 6, mode = 2). Most post-natal ramet abandonment occurred during the fifth instar (Fig. 3; Z < − 6.473; df = 4, 384; P < 0.0001), and more of these movements occurred during the day than during the night (mean = 2.37 during day, 1.23 during night; P = 4.275e−07).
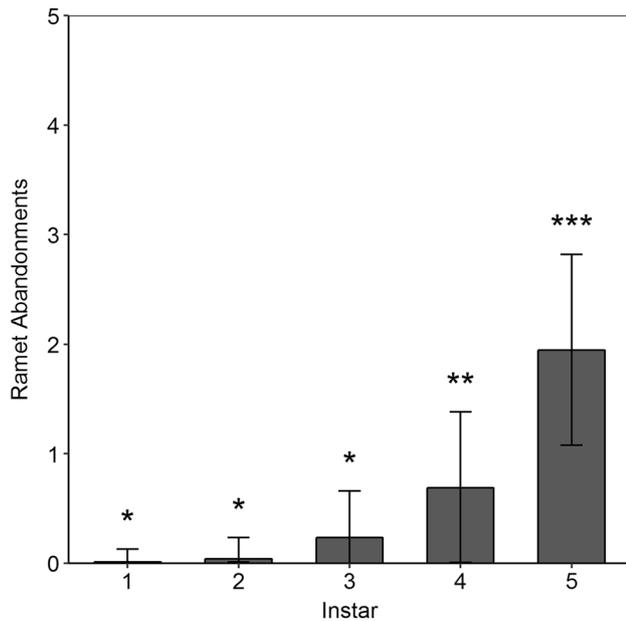
Fig. 3 Average frequency of instar specific ramet abandonments. Bars represent the mean ± one standard deviation. Different number of asterisks (*) above bars represent a significance value < 0.05

Plant utilization

Number of ramets in a cage had no effect on biomass consumption prior to natal ramet abandonment (F = 0.086; df = 2, 73; P = 0.9179) or on the number of leaves with feeding injury prior to ramet abandonment (F = 1.766; df = 2, 78; P = 0.1710). Though larvae abandoned their natal ramet most frequently in the 4th instar, some abandoned at second, third, or fifth instars. The amount of biomass consumed prior to ramet abandonment was dependent on instar stage at abandonment (F = 15.213; df = 3, 73; P < 0.0001). On average, second or third instars that abandoned their natal ramet consumed less than those that abandoned their natal ramet in the fourth or fifth instar (Fig. 4a; Z < 3.338; df = 3, 73; P < 0.0047). Those that abandoned in the fifth instar consumed significantly more than any other instar (Fig. 4a; Z > 3.971; df = 3, 73; P < 0.0004). Those that abandoned their natal ramet in the second, third, fourth or fifth instar consumed 9.03 ± 4.34 mg (sd), 21.7 ± 12.5 mg, 99.6 ± 71.9 mg, and 732 ± 524 mg of dry ramet material, respectively. Similarly, number of leaves on the natal ramet with feeding injury depended on instar stage at abandonment (F = 12.899; df = 3, 78; P < 0.0001). More leaves on the natal ramet had feeding injury when larvae remained on their natal ramet until fifth instar (Fig. 4b; Z > 3.882; df = 3, 78; P < 0.0006). Larvae that abandoned the natal ramet in the second, third, fourth, and fifth instar fed from 3.75 ± 1.49 leaves (sd), 3.71 ± 1.86 leaves, 5.87 ± 2.14 leaves, and 10.7 ± 5.02 leaves on the natal ramet, respectively.
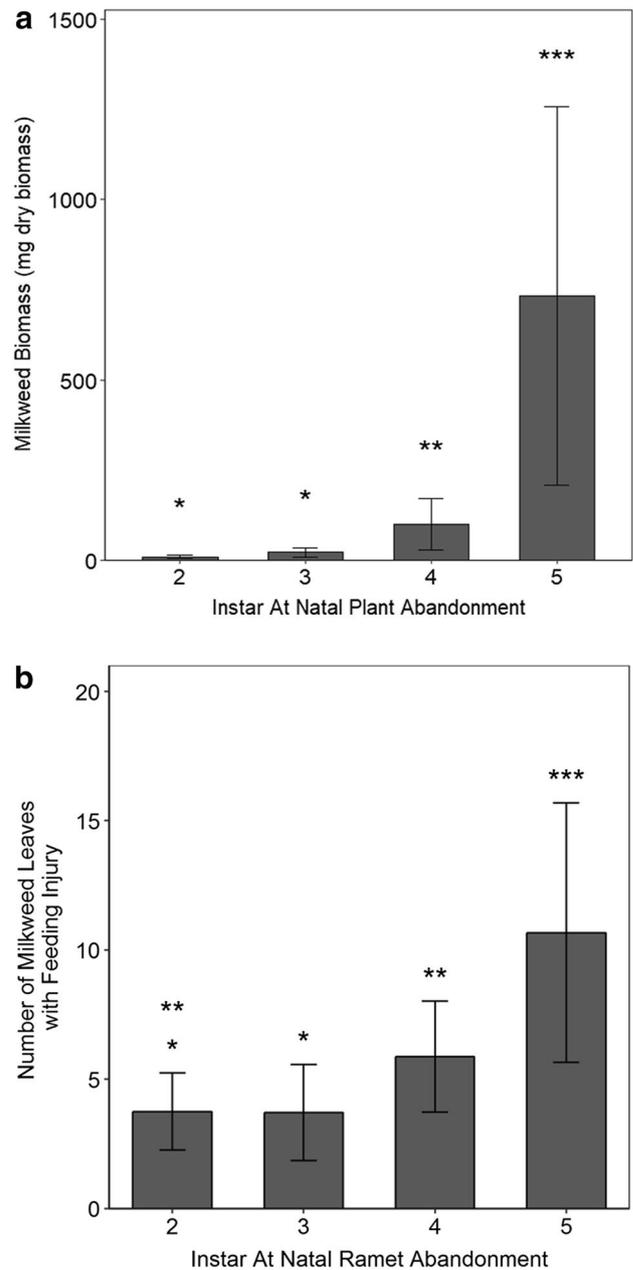

Fig. 4 Larval milkweed consumption at natal ramet abandonment. **a** Estimated natal milkweed ramet consumed by instar. **b** Number of natal ramet leaves with feeding injury by instar. Bars represent the mean ± one standard deviation. Different number of asterisks (*) above bars represent a significance value < 0.05

In 2018, neonates placed in cages with four ramets that survived to pupation consumed 1209.61 ± 412.30 mg of total dry plant material (n = 9; note 21 larvae survived to pupation, but consumed entire leaves, which precluded the means of estimating consumed plant material). Biomass was consumed from 17.11 ± 5.09 leaves. Most total biomass consumed through development (t = − 6.7208; df = 10.626; P = 3.891e−05) and leaves with feeding through

development (t = − 3.4293; df = 14.942; P = 0.003786) were from non-natal ramets. Larvae consumed 119 ± 165 mg of biomass from 5.78 ± 2.94 leaves of their natal ramet and 1090 ± 401 mg from 11.33 ± 3.87 leaves on ramets visited after they abandoned their natal ramet.

In 2017, larvae provided 2, 3, or 4 ramets visited an average or 1.96, 2.33, and 2.93 ramets, respectively; larvae visited more ramets when more ramets were provided, but did not always visit all ramets provided. On average, larvae returned to an already abandoned ramet 0.86 ± 0.90 times. Those that were provided four ramets in 2018 fed on an average of 3.22 ± 0.44 ramets (max = 4, min = 3). When larvae were presented with four ramets, they were more frequently observed on plant material in comparison to "non-plant material" (side of cage, soil, plant labels) than when larvae were provided two ramets (Fig. 5; Z = − 3.417; df = 2, 155; P = 0.0018).

Discussion

The present study explored the effect of milkweed ramet availability on monarch larval survival and behavior. We observed no effect of milkweed ramet availability on survival rates; 67% of individuals survived to pupation. Monarch larval survival has been estimated in several field studies. Nail et al. (2015) estimated 7–10% survival from egg to fifth instar while Geest et al. (2019) estimated 14–30%

Fig. 5 Proportion of larval observations on milkweed ramet (number of observations on a ramet divided by number of observations on ramets, soil, and cage surface). Bars represent the mean ± one standard deviation. Different number of asterisks (*) above bars represent a significance value < 0.05

survival from egg to adult. The higher survival rate in our study is likely attributed to the exclusion of predators and parasitoids. Our instar specific survival was similar to that observed by Malcolm (1994) in outdoor natural enemy exclusion experiments.

Larval milkweed abandonment is documented in several field studies (Urquhart 1960; Rawlins and Lederhouse 1981; Borkin 1982; Zalucki and Rochester 2004; De Anda and Oberhouser 2015). In our 2016 preliminary greenhouse study, we observed natal ramet abandonment through larval development with each of 33 larvae individually placed on isolated milkweed ramets. We hypothesized that larvae would abandon their natal ramet regardless of the number of co-located ramets. Results of the 2017 and 2018 experiments, with 2, 3, or 4 ramets in a cage indicated all larvae abandoned their natal ramet prior to completion of larval development. On average, larvae abandoned their natal ramet during the fourth instar, i.e., on the sixth or seventh day of development.

Natal ramet abandonment did not occur because all available biomass was consumed; approximately 25–50% of the leaf material remained at the time of natal ramet abandonment. Natal ramet biomass consumption rates were similar regardless of the number of ramets in the cage; however, those instars that remained on their natal ramet later in development consumed more biomass from more leaves than larvae that abandoned the natal ramet earlier in development. Early instars fed from four leaves while later instars fed from as many as 11 leaves.

Larvae that abandoned a ramet were likely in search of a new milkweed ramet; however, they probably are not able to detect the presence of surrounding milkweed. Larval perceptual range is estimated to be less than 5 cm (Urquhart 1960). Previous reports suggest that monarch larvae move without orientation (Urquhart 1960; Rawlins and Lederhouse 1981; Borkin 1982). Our study further supports a random walk behavior. If movement was directed, larvae would detect the presence of, and move directly to, a new ramet after abandonment. In contrast, we observed an association between number of ramets present in the cage and number of times monarch larvae were observed on a ramet. Larvae were observed more frequently on plant material when more ramets were provided, presumably because there were more opportunities to randomly find a host plant. Likewise, larvae returned to milkweed ramets that were previously abandoned, suggesting that the movement was not directed.

Consequently, we hypothesized that larvae with access to more ramets would have higher survival rates and pupal weights with shorter developmental times because they would spend more time on plant material. There was, however, no significant effect of ramet number, which suggests even with a random walk larvae were likely not off a ramet for a sufficient time to show any effect of reduced feeding.
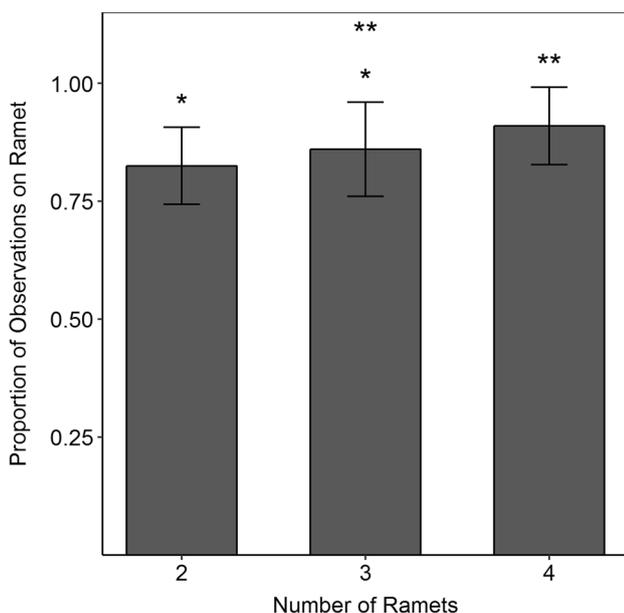
This observation may be due to the distance between ramets (16 cm) in our experimental design. Distances monarch larvae are capable of moving when searching for a new ramet remains uncertain with only Borkin's (1982) observation of a larva that traveled 2.25 m before finding a new ramet. If our experimental design was repeated using larger cages with a greater distance between ramets, we hypothesize that survival rates and pupal weights would increase and/or developmental times would decrease as the number of ramets increased.

After larvae successfully found a new ramet, they continued to abandon milkweed ramets up to five more times. Larvae typically returned to an already abandoned ramet once during development. These movements were not an artifact of the wandering stage prior to pupal formation (Truman and Riddiford 1974) because larvae continued feeding when they arrived on new ramets. Most of the total biomass and leaves consumed through development were from ramets visited after natal ramet abandonment. Therefore, subsequent ramets may be more important biomass for development. Larvae visited more ramets when more were provided, but did not always visit all that were available. We estimated that a larva consumed approximately the same mass as one 11.0–34.1 cm milkweed ramet (1726 mg dried weight ± 829 mg), but they acquired this mass from three or four separate ramets.

Field observations suggest monarch females can 'dump' eggs on isolated ramets of milkweed. Isolated milkweed ramets can contain up to ten monarch eggs at one time (Zalucki and Kitching 1982b). Larvae on isolated stems are also thought to have greater survival rates because of reduced predation (Zalucki and Kitching 1982a; Nail et al. 2015). Based on our observations of natal ramet abandonment, it is unlikely that multiple eggs on one ramet would develop to multiple adult butterflies unless additional ramets were within close proximity. Even if larvae did not abandon their natal ramet, there would likely be insufficient biomass with spring plants to support more than one larva, assuming no inter-species competition or predation, and the size and age of milkweed are similar to those used in the present study. Interactions among multiple larvae on an isolated ramet have yet to be explored. Studies in progress are assessing the effect of intra-species competition on characteristics of larval movement, survival, growth, and development.

While the causes of observed larval movement in field studies may be due to intra- or inter-specific competition, avoidance of predators and/or parasitoids, and/or insufficient forage, these environmental factors were not present in our experiments. We consistently observed natal ramet abandonment over three years of experiments with 1, 2, 3, or 4 ramets and three different monarch cohorts. Our findings suggest abandonment of the natal ramet is an innate behavior; it occurs consistently, predictably and without

prior larval experience. What environmental cue(s) could be responsible for triggering this behavior?

Reduced plant quality due to feeding could cause a negative taxis response. Leaf quality changes as leaves mature. Younger leaves may be more readily digested than older leaves and have higher concentrations of nitrogen and water. Herbivorous insects that feed on young leaves have higher survival, faster development, and larger mass than those that feed on older leaves (Stamp and Bowers 1990; Jordano and Gomariz 1994 and references therein). Reports also suggest monarch females prefer to lay eggs on young milkweed plants with new leaves (Urquhart 1987; Bergstrom et al. 1995; Fischer et al. 2015). Our findings suggest that monarch larvae may prefer new growth over older leaf material; larvae may have abandoned their natal ramet in search of plants with new growth. Larvae were observed significantly more times on the top third of the milkweed ramet, where new growth is present, than on the middle or bottom third of the ramet. Often, leaves from the top third of a ramet were consumed at larval abandonment, with the older leaves remaining on the bottom of the plant (Fig. 6).

Monarch larvae perform best on milkweed species that contain intermediate cardenolide concentrations (Agrawal et al. 2014; Agrawal 2017). Some cardenolides are constitutive and others can be induced within five days of monarch feeding (Malcolm 1994; Malcolm and Zalucki 1996; Van Zandt and Agrawal 2004; Rasmann et al. 2009; Agrawal et al. 2012, 2014). In our study, monarch larvae abandoned their natal ramet after six to seven days, aligning with the time to induce cardenolide production. Though increased milkweed leaf cardenolide concentrations due to herbivory will not cause increased mortality rates (Agrawal et al. 2014), feeding on these leaves could have a fitness cost.

Fig. 6 Representative common milkweed ramet after a 4th instar abandonment. Leaves on the top of the ramet are totally consumed, while leaves on the middle and bottom have no evidence of feeding

Milkweed species with high cardenolide concentrations produce smaller pupae than species low in cardenolides (Agrawal 2017). In our studies, it is possible induced cardenolides reached levels that invoked negative chemotaxis. Future studies assessing larval movement on and between milkweed plants, with and without prior herbivory and including measurements of cardenolide concentrations, would help determine if there is a threshold concentration that elicits natal ramet abandonment.

Alternatively, the consistent observations of natal ramet abandonment could indicate negative chemotaxis to "call for help" compounds released by the milkweed in response to herbivory. While we are not aware of any studies documenting release of compounds by milkweed to attract predators and parasites in response to feeding damage, this response has been reported with other plants (e.g., see Turlings et al 1995; Pare and Tumlinson 1999; Gershenzon 2007; War et al. 2012; Alijbory and Chen 2018). Monarch larvae may also be capable of detecting these secondary plant compounds, which in turn elicits natal ramet abandonment.

Consistent natal ramet abandonment could also indicate negative tactile taxis due to reduced leaf cover with increased herbivory. Also noted previously, 100% of the larvae in the current study abandoned their natal ramet, which ranged between 10 and 35 cm in height. Abandonment of the natal ramet occurred after 25 to 50% of the leaves had been consumed. Larvae also were observed most frequently on the underside of the leaf. Similar observations have been reported by Rawlins and Lederhouse (1981). Loss of leaf material results in less cover 'to hide' from predators and parasitoids. Consequently, with less foliage for camouflage, larvae could be more easily detected by predators/parasitoids because these species often search for frass or feeding injury (Price et al 1980; De Moraes et al. 1998; Oberhauser et al. 2015). In response to predation/parasitism, natural selection may have favored natal ramet abandonment. Leaving a milkweed ramet is risky. However, abandoning a milkweed and successfully finding another, may be associated with lower rates of predation/parasitism and a greater probability of development to a reproductively viable adult, as compared to rates of mortality for larvae remaining on the natal ramet. Future studies with larger plants and more leaves could assess the extent to which abandonment is correlated with a decreased amount of leaf cover.

Regardless of the mechanism(s) that invoke this seemingly innate movement behavior, it has implications for larval monitoring projects. Larvae in our studies abandoned plants an average of three times through development and this movement occurred most often during the day. Most monitoring protocols are based on observing milkweed leaves and ramets for eggs and larvae during the day (Prysby and Oberhauser 2004; De Anda and Oberhauser 2015; Nail et al. 2015; Geest et al. 2019). In these protocols, when larvae are expected in a subsequent sampling period and not found, they are presumed to be dead (Prysby and Oberhauser 2004; Nail et al. 2015; Geest et al. 2019). When a larva is observed but there was no detection in the previous sampling period, data records are adjusted under the assumption of missed detection in the prior sampling period (De Anda and Oberhauser 2015; Nail et al. 2015; Geest et al. 2019). Although these studies account for failed detection when new larvae are observed, they did not account for larvae moving out of the patch. Survey design should account for imperfect larval detection, which is often disregarded when studying invertebrates because of their general abundance in comparison to vertebrates (Kellner and Swihart 2014). For larval monarch surveys, our results suggest current monitoring protocols may over estimate field mortality and do not account for larval movement into and out of habitat patches. Monitoring the ground and vegetation surrounding milkweed ramets as well as searching ramets for standardized durations of time during observation periods may reduce bias in survey results.

Larval movement behavior and biomass requirements are critical aspects of monarch larval biology that should be incorporated in habitat restoration and maintenance practices. Because the eastern North American monarch population grows exponentially over the summer prior to fall migration, it is important that the first generation in the Upper Midwest is productive (Flockhart et al. 2015; Oberhauser et al. 2017). Under our greenhouse conditions and consistent with previous field studies, monarch larvae unfailingly abandon their natal ramet of common milkweed, and subsequent ramets, before all of the available leaf biomass on a ramet is consumed and prior to the pre-pupal wandering stage. In the spring, when monarchs first arrive in the Upper Midwest, milkweed are 10–35 cm in height and likely provide sufficient milkweed biomass to support development of a single egg to pupation. However, because larvae abandon their natal ramets, if the natal ramet is isolated from other milkweed plants the larvae would likely die before finding another plant during a random walk.

In conclusion, while previous modeling studies indicate that isolated ramets in the matrix can increase the number of eggs laid in the landscape (Zalucki et al. 2016), our findings suggest that eggs or larvae observed on isolated ramets will have low survival. Patches containing at least two to four ramets of closely-spaced common milkweed provide sufficient biomass for development and would increase the likelihood that larvae moving in random directions would encounter non-natal milkweed ramets to support development through pupation.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This work is original and has not been submitted to any other journal for consideration. This is a complete study and has not been split into several parts to increase quantity of submissions. Results are presented to the best of our knowledge without fabrication, falsification, or inappropriate data manipulation. No data, text, or theories by others were presented as if they are our own work; proper acknowledgements were given. All work here is well intended. The author list is complete and will not change. Raw data or documents will be provided upon request.

Research involving human and animal participants This research was not conducted on human subjects and was consistent with the United States Animal Welfare Act.

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